Characterization of kidney disease in sub-Saharan Africa

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Statement of own work

I, Robert Kalyesubula, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed: [redacted]

Date: 9th September2021

Thesis Abstract

Background: Chronic kidney disease (CKD) is an established cause of morbidity and mortality worldwide. While methods to determine kidney function are well established in Western populations, there are good reasons to believe these methods may not translate well to sub-Saharan Africa (sSA), meaning the overall burden of CKD is uncertain. The aim of this thesis was to determine the prevalence and mortality associated with renal impairment using conventional estimated glomerular filtration rate (eGFR) approaches, we also compared performance of measured GFR (mGFR) to eGFR and used mGFR based model to impute CKD prevalence in sSA.

Methods: We determined the prevalence and all-cause mortality from impaired renal function [defined as eGFR) <60mls/min/1.73m^2 within a population-based cohort among adults of 18 years and above in rural Uganda. Working with the African Research on Kidney Disease Network (ARK) a consortium of three community-based cohorts from Malawi, Uganda and South Africa, we stratified participants by level of renal function. We intravenously injected 5millilitres bolus of exogenous iohexol and drew venous samples from the contralateral arm at 5, 120, 180 and 240- minute time points to determine the mGFR. We compared the performance of existing equations to mGFR and used a model to impute kidney function based on mGFR.

Results: In Uganda, among 5,979 participants, we found an overall prevalence of eGFR <60 ml/min per 1.73 m^2 of 1.6% (95% CI 1.34–1.99) with up to 1,089 (18.2%) having an eGFR <90 ml/min per 1.73 m^2 in a predominantly young population. Older age, hypertension and anemia were independently associated with impaired renal function. In adjusted analyses, participants with baseline eGFR ≤45mls/min/1.73m^2 had six-fold higher mortality compared to those with eGFR ≥90mls/min/1.73m^2 (HR 6.12 (95% CI 2.27-16.45)) with strong evidence of a linear trend for risk of mortality as renal function declined (p<0.001). Among the 2,578 participants with mGFR and 2433 with cystatin C eGFR from the ARK study, we found that that all eGFR equations overestimate GFR compared to mGFR or cystatin C across the three countries and this was worsened by use of ethnicity coefficient. Using a model to impute kidney function based on mGFR, we estimated CKD prevalence to be two to three-fold higher compared to creatinine-based estimates in populations across six countries in sSA.

Conclusion: Based on existing creatinine-based methods to estimate GFR, we found a relatively low prevalence of impaired renal function in the general population. We also demonstrated that eGFR <45mls/min/1.73m^2 are associated with an increased all-cause mortality. However, using iohexol clearance, we showed that these creatinine-based measures over-estimate GFR and under-estimate CKD. This means a substantial proportion of people with kidney disease are missed by current eGFR equations which may have adverse effects on the health and care of patients with CKD in sSA.

Acknowledgement

I would like to appreciate the GlaxoSmithKline Africa Open Lab for funding both the PhD as well the studies conducted. I would also like to thank the MRC/UVRI & LSHTM Research unit Entebbe for their support through the various stages of the PhD.

I am deeply indebted to my PhD supervisors/mentors; Laurie Tomlinson, Liam Smeeth and Robert Newton who have been exceptional in guiding me and supporting me through the years of my studies. They have been with me every step of the way and I am very grateful to them.

I will also like to thank my advisory committee consisting of Gavin Dryer and Dorothea Nitsch as well my Upgrade team of Kathryn Mansfield, Hamish Dobbie chaired by Krishnan Bhaskaran for their insightful comments and guidance to streamline the research work. I would like to thank Jenny Fleming and Lauren Dalton for administrative support given to me during the course of the studies.

This work would not have been possible without the support of the research teams from Malawi, South Africa and Uganda who are part of the African Research on Kidney Disease Network (ARK) as well as the administrative and support teams at MRC/UVRI & LSHTM Research unit. Their hard work, commitment and dedication helped us to pull off our collaborative efforts that have led to wonderful discoveries on how to best measure kidney function in sSA.

My dear wife Estherloy and our children Leon, Lindsay and Lamson who were very patient with me while I was buried in books during the COVID-19 pandemic. I appreciate other family members and friends for all the social support and encouragement through this

journey. I dedicate this PhD to Mrs. Robinah Lubwama who has been a great pillar in my life despite the challenges she has gone through herself.

I thank God for all of you.

Contents

Tables, Figures and Box summaries

Tables

Figures

Summary boxes

Abbreviations

ACR Albumin Creatinine Ratio

ARK African Research on Kidney Diseases Network

ART Antiretroviral therapy

BSA Body surface area

CKD Chronic kidney disease

CKD-EPI Chronic Kidney Disease-Epidemiology collaboration

DCCT Diabetes Control and Complications Trial

DTPA Technetium-99 m diethylenetriamine penta-acetic acid

ECG Electrocardiogram

EDTA Chromium-51 ethylene diamine tetra-acetic acid

EGFR Estimated glomerular filtration rate

ESKD End Stage Kidney Disease

FAS Full-Age Spectrum

IDMS Isotope dilution mass spectrometry

KDIGO Kidney Disease: Improving Global Outcomes

MDRD Modification of Diet in Renal Disease

MTA Materials Transfer Agreement

NCDs Non-Communicable Diseases

PRISM Preferred Reporting Items for Systematic Reviews and Meta-Analyses

SOPs Standard Operating Procedures

sSA sub-Saharan Africa

UKPDS UK Prospective Diabetes Study

1.0 Thesis design and structure

1.1 Thesis design

This PhD thesis is part of a much bigger study funded by the GlaxoSmithKline Africa NCD Open Lab Programme, which aimed to establish a new equation for measuring glomerular filtration rate in sub-Saharan Africa using cohorts from Malawi, South Africa and Uganda. I used the data from ARK for which I have contributed greatly as the principal investigator for my PhD work.

The thesis was designed to use the existing baseline data collected in census rounds 22 (2011/2012) and 24 (2014/2015) performed on the general population cohort (GPC) in Kyamulibwa a semi-rural village 120 kilometers from Kampala the capital city of Uganda to ensure that we understand the nature of renal abnormalities.

The GPC was set up in 1988 to study the life course of HIV-AIDS but for round 22 (2011-2012) and 24 (2014-2015) the focus was on non-communicable diseases. Blood samples are routinely stored for future studies and participants fully consent for future use of the samples. We used the stored samples to test for creatinine and then linked up the creatinine levels to other collected data. Using this information, we were able to address the objective of prevalence and associated factors.

We used the baseline creatinine and the annually collected information on mortality to determine the all-cause mortality from impaired creatinine over a period from 2012 to 2019.

As part of the PhD, I planned to do a systematic review on the available data in sub-Saharan Africa. However, we later learned that our partners in South Africa had already registered a

similar systematic review with PROSPERO data base. I decided to join their team and participated in the systematic review for the study.

For the third objective of measuring glomerular filtration rate, I selected all patients with eGFR <60mls/min/1.73m^2 since they were few and then stratified the other patients by sex, age and GFR level to have a good representation of the general population as well as different stages of renal impairment. These were used to determine the measured GFR and then generate information on GFR in this population. We used a shared protocol which was jointly developed with the teams from South Africa and Malawi to collect data so that we could later pool the data to address the final objective of determining the best way to estimate GFR in sub-Saharan Africa.

1.2 Thesis structure

The thesis is made up of seven chapters which have been written in a research style format. It includes four research papers which have been associated with the PhD project. Each of the four research papers is a stand-alone paper which was prepared in its own right. This inevitably means that some information on methods, research definitions and settings will be repeated from one paper to another. Each paper is presented in a way that the thesis has a coherent flow and is not necessarily put in the temporal order of publication. Because of the current challenges of travel restrictions because of the COVID-19 pandemic, accessing resources like fast internet, software and printing services that are readily available at the London School of Hygiene and Tropical Medicine, has been a big problem. I have therefore made a few adjustments in the way the PhD chapters are presented. For example, I have included all the appendices at the end of each chapter to ensure that the readers have access to all the materials associated with each chapter in one place. For the same reasons, I have also

included the references at the end of each chapter instead of having them at the end of the thesis.

Two of the published papers were an offshoot of this work but I did not play the leading role in their publication though I contributed substantially. These include the paper on Social Sciences and the systematic review on how to measure GFR in sub-Saharan Africa. I have included brief parts of these where they fit best and included them in the appendix.

Chapter 1: The thesis design and structure show the lay out of the PhD thesis and gives guidance on what is expected in the different chapters and the reasons for the way it is arranged.

Chapter 2: Introduction presents an introduction to the epidemiology of CKD in sub-Saharan Africa. In this chapter I explored the way GFR is estimated using the different equations and went into details of how GFR is measured worldwide and briefly shared what we found in our systematic review. I discuss the limitations of current studies and briefly explain how kidney disease is currently being managed in sub-Saharan Africa. I additionally explored studies looking at mortality from CKD.

Chapter 3: The aims and methods section summarize the thesis aims, objectives and rational. It clearly lays out the study settings and details of how information was collected, stored and analyzed to address each of the objectives. More details are provided for the Iohexol measurement paper that were largely not included in the manuscript for publication due to word limits and new methods of analysis that we learned along the way. The manuscript on 'How to estimate glomerular filtration rate in sub-Saharan Africa: design and methods of the African Research on Kidney Diseases (ARK) Network; is included in this section.

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Chapter 4: Addresses objective 1: determining prevalence of impaired renal function in a rural Ugandan population cohort.

Chapter 5: Addresses objective 2: Impaired renal function as a predictor of mortality among rural Ugandans: results of a general population cohort study.

Chapter 6: Addresses objective 3 of determining the best way to measure kidney function in Uganda, Malawi and South Africa

Chapter 7: The discussion is a summary of key findings from the PhD, how these relate to the existing literature, the implications of the findings to the field of nephrology in sub-Saharan Africa, strengths, limitations, suggestions for future work and conclusions.

The Appendices consist of key documents such as consents, questionnaires, ethics approvals, conference papers and two extra manuscripts published in the course of the PhD but not included in the main thesis.

1.3 Student's contribution

Since I joined MRC/UVRI and LSHTM Research unit in 2015, I contributed substantially to the setting up of the GSK-CKD Open Lab study and the African Research on Kidney Disease (ARK) Network which is a collaboration between the London School of Hygiene and Tropical Medicine in the UK, the MRC/UVRI & LSHTM and LSHTM Research Unit in Uganda, Malawi Epidemiological and Intervention Research Unit (MEIRU) in Malawi and the University of Witwatersrand in South Africa.

1.4 The GSK-CKD Open Lab study and ARK study

In 2015 I joined the Medical Research Council/ Uganda Virus Research Institute and the London School of Hygiene and Tropical Medicine (MRC/UVRI & LSHTM) Research unit as a nephrologist and coordinator for the GlaxoSmithKline-Chronic Kidney Disease (GSK-

CKD) Open Lab study. As the coordinator of the study, I refined the grant application into a protocol and developed the consent forms and questionnaires for the study. I obtained ethical approvals from the ethical review board and the Uganda National Council for Science and Technology. In 2016 I was appointed as a senior scientist and principal investigator (PI) for the GSK-CKD Open Lab study. As the PI I oversaw the running of the study, developed standard operating procedures (SOPs) for each aspect of the study, trained research team members, ensured proper recruitment and follow-up of patients for each component of the study. I was responsible for the proper conduct of the study including submission of renewals, amendments, report writing for the sponsors and the regulatory authorities as well as all the administrative aspects of the study. I led the set-up of the iohexol study lab which required establishment of a close monitoring unit where we kept patients for 5 hours while taking off blood samples and ensured proper collection of 24-hour urine samples, ambulatory blood pressure measurements, electrocardiograms (ECGs) and bio-impedance measurements per protocol. I also worked with my supervisors and the teams from Malawi and South Africa to set up the ARK consortium. I played a key role in development of the materials transfer agreements (MTAs) and oversaw the transfer of samples between the collaborating institutions. In 2019, I hosted a 3-day conference that finalized the data sharing agreements, set the grounds for data analysis and future directions for the ARK consortium in Kampala, Uganda as part of leadership training in my PhD.

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1.5 PhD work, associated publications and my contributions

1. Systematic review: June Fabian, Jaya A George, Harriet R Etheredge, Manuel van Deventer, **Robert Kalyesubula**, Alisha N Wade, Laurie A Tomlinson, Stephen Tollman, Saraladevi Naicker, on behalf of the African Research in Kidney Disease (ARK) Working Group, Methods and reporting of kidney function: a systematic review of studies from sub-Saharan Africa, *Clinical Kidney Journal*, Volume 12, Issue 6, December 2019, Pages 778–787.

I participated in:

1. the retrieval of the systematic review of papers
2. review of full-text articles for the manuscript and scored them accordingly and discussed data inconsistences with the team.
3. the analysis and interpretation of the data
4. the draft of the manuscript and responded to reviewer comments for the final manuscript.

2. Baseline creatinine paper: **Kalyesubula R**, Hau JP, Asiki G, Billy Ssebunya , Sylvia Kusemererwa, Janet Seeley, Liam Smeeth, Laurie Tomlinson, Robert Newton. Impaired renal function in a rural Ugandan population cohort. *Wellcome Open Res* 2019, **3**:149.

I led:

1. the study planning and design
2. the data collection
3. the data management
4. I participated in data analysis

5. I led the interpretation of the analysis
6. I led the writing of the first manuscript and subsequent editing
7. I submitted the final manuscript and did all correspondence with the editors and reviewers.

3. Mortality Paper: **Robert Kalyesubula,** Isaac Sekitoleko, Keith Tomlin, Christian Hansen, Billy Ssebunya, Ronald Makanga, Janet Seeley, Liam Smeeth, Robert Newton, Laurie Tomlinson on behalf of the ARK study. Association of impaired kidney function with mortality among rural Africans: results of a general population cohort study

1. I led the study planning and design
2. I did extensive data management and cleaning, which included generating and resolving data queries and updating databases and review of case report forms (CRF) copies, merging datasets and generating data bases for mortality outcomes.
3. I participated in data analysis
4. I led the interpretation of the analysis
5. I led the writing of the first manuscript and subsequent editing with feedback from my supervisors and co-authors.
6. I submitted the final manuscript and will handle all correspondence with the editors and reviewers.

4. Iohexol methods paper: **Kalyesubula R,** Fabian J, Nakanga W, Newton R, Ssebunnya B, Prynn J, George J, Wade AN, Seeley J, Nitsch D, Hansen C, Nyirenda M, Smeeth L, Naicker S, Crampin AC, Tomlinson LA. How to estimate glomerular filtration rate in sub-Saharan

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Africa: design and methods of the African Research into Kidney Diseases (ARK) study.

BMC Nephrol. 2020 Jan 15;21(1):20.

I led:

1. the study planning and design
2. the interpretation
3. the writing of the first manuscript
4. the subsequent editing of the manuscript and
5. I submitted the final manuscript and did all correspondence with the editors and reviewers.

5. Social Science study on understandings and perceptions of a diagnosis of kidney dysfunction: Seeley J, Kabunga E, Ssembatya J, A Tomlinson L, Fabian J, Smeeth L, Nyirenda M, Newton R, **Kalyesubula R**, Bukenya D; ARK Consortium. Understanding kidney disease in rural central Uganda - Findings from a qualitative study. Glob Public Health. 2020 Apr 30:1-12.

I participated in the:

1. study planning and design and oversaw the data collection.
2. data management
3. data analysis.
4. interpretation of the analysis.
5. writing of the first manuscript and subsequent editing.
6. correspondence with the editors and reviewers.

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6. Measuring kidney function in sub-Saharan Africa: June Fabian, **Robert Kalyesubula (co-first author),** Joseph Mkandawire, Christian Holm Hansen, Dorothea Nitsch, Eustasius Musenge, Wisdom P Nakanga, Josephine E Prynn, Gavin Dreyer, Tracy Snyman, Billy Ssebunnya, Michele Ramsay, Liam Smeeth, Stephen Tollman, Saraladevi Naicker, Amelia Crampin, Robert Newton, Jaya A George, Laurie Tomlinson on behalf of the African Research on Kidney Disease (ARK) Consortium

1. I led the study planning and design for Ugandan part of ARK
2. I led the data collection for Ugandan part of ARK
3. I participated in extensive data management and cleaning, which included generating and resolving data queries and updating databases and review of case report forms (CRF) copies and generating data bases from Uganda.
4. I participated in the development of the data analysis plan
5. I held regular face-to-face and later online meetings (for most of the COVID-19 era) with my supervisors and co-authors for regular feedback.
6. I participated in data analysis.
7. I participated in the interpretation of the analysis.
8. I participated in the writing of the first manuscript and subsequent editing.
9. I participated in correspondence with the editors and reviewers.

1.6 Courses attended

January 2019, Course in Statistic Methods in Epidemiology (SME), London School of Hygiene and Tropical Medicine

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April 2019 Course in Advanced Statistic Methods in Epidemiology (ASME), London School of Hygiene and Tropical Medicine

November 2020, Fall 2020 Epidemiologic Methods (EPI 203), University of California San Francisco, USA

1.7 Conferences and workshop presentations

May 2016, I presented study proposals and protocols at the Collaborators meeting in Johannesburg South Africa.

Feb 2017, I made an oral presentation, NCD consortium, Characterization of chronic kidney disease in Uganda, Kampala, Uganda

June 2018; Support supervisory visit Malawi

April 2019, presented poster on how to estimate GFR in sub-Saharan Africa, Melbourne, Australia.

July 2019, Oral Presentation, Johns Hopkins University, Chronic Kidney Disease of Unknown origin

August, 2019. Conference attendance- Cape Town, South Africa

December, 2019, I organized data sharing workshop in Kampala, Uganda

Jan 2020, Invited Lecture on How to measure Kidney function in Sub-Saharan Africa, Yale school of Medicine, USA. Please refer to **appendix of abstracts** for more details.

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1.8 Statement of impact by COVID-19

1.8.1 Activities affected by COVID-19

I was rotating in London for the final write up with access to fast internet, seclusion from other distractions that occur at home. My rotation in London was cut short and I had to return to Uganda 2 months earlier than planned in March 2020. As a result of this I was put in quarantine for 3 weeks which was quite unstructured and very traumatizing at the time when very little was known about COVID-19. We were guarded by 15 soldiers and cared for by one clinical officer and were not allowed to go outside of our hotel rooms which we paid for out of pocket. We were not allowed to see any relatives at that time and we were actually stigmatized by the public as well as the media. This took a great toll on my progress because I could not concentrate on the PhD work while trying to survive this ordeal. When we were finally accepted to go home, things never remained the same.

I was requested to be part of the task force for care of patients affected by kidney disease and COVID-19 which took a lot of time. As a leading expert of nephrology in the country, I am often called from time to time to support care of critically ill patients with kidney disease and COVID-19 as well as contributing to development of management protocols. This is often impromptu and takes from the academic time.

After leaving London, I could no longer hold regular face to face meetings with my supervisors which had been scheduled to be on a weekly basis for three months to finalize the PhD work. I no longer had access to both human and other resources at the LSHTM and UK as a whole nor interact with other doctoral students for peer support and discussions.

My children that would normally be in school were now at home and I had to provide home schooling which took quite a chunk of time and required a learning curve.

Other problems were restrictions to resources in country due to rampant lock downs and restrictions on movement within the night and lack of access to premises like MRC/UVRI&LSHTM Research unit resources.

At one point we had internet and social media interference during election times that further deprived me of time to focus on PhD work. Poor access to statistical programs for analysis of the research work, write up of the thesis and layout support made this issue more acute.

We also had a lot of unrest during the presidential campaigns from September 2020 to January 2021 with a lot of uncertainties about the future that disrupted concentration and learning.

While we had some peace and quiet from January to April 2021, the second wave of the pandemic hit us at the end of May through June 2021. This has been more devastating affecting young people as well as requiring more attention for clinical support to the emergency units. Our country virtually run out of oxygen and patients begun to have cylinders of oxygen administered from the waiting areas and some from the back of their cars as medical personnel tried to cope with the pandemic effects. With restrictions in movement across districts, restricted access to the internet and Information and Technology (IT) services, rampant power cuts as well as medical care for patients and relatives who have fallen sick from COVID-19; the pandemic has had a great toll on my concentration and ability to perform some of the work required for the thesis write up as explained earlier.

1.8.2 Remedial actions taken for COVID-19

I blocked out regular times for PhD

I held regular online meetings with supervisors which was such a great commitment from their side.

I purchased large data packages to help with internet access while staying at home.

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I took leave from in-country duties as much as would be acceptable for me to create time for the PhD work.

I also shared duties for home schooling with my wife which was a great relief.

2.0 Introduction

2.0 Introduction

2.1 Background

Non-communicable diseases (NCDs) are increasing in sub-Saharan Africa (sSA) and will become the leading cause of death in Africa by 2030[1]. Chronic kidney disease (CKD) is one of the NCDs that has received little attention over the last decade despite being both a consequence and cause of other NCDs. Moreover, CKD prevalence in sSA is projected to increase[2]. Despite this, there remains a paucity of data describing the current prevalence, risk factors and the best way to estimate glomerular filtration rate (eGFR) among people from sSA. This has impeded the development of appropriate prevention and treatment strategies.

The treatment of chronic kidney diseases depends on the stage of the kidney disease as well as the cause of kidney disease when established. For the early stages of kidney disease including stage one to stage two without any additional abnormalities of urine and kidney structure, no active interventions are recommended beyond the treatment of concurrent risk factors and cause of kidney disease. As the kidney disease progresses to stage three which is a GFR < 60mls/min/1.73 m^2, active interventions to delay kidney disease are employed. Among the established methods of slowing kidney disease are interventions that target the causes of kidney disease. The commonest cause of kidney disease world over is diabetes mellitus and adequate control of blood sugar has been shown to delay diabetic nephropathy according to the Diabetes Control and Complications Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS) trials [3, 4]. Management of diabetic kidney disease has its peculiarities and the selection of drugs needs to be looked at critically because some drugs like metformin may be contraindicated in severe kidney dysfunction while others like insulin can cause more episodes of hypoglycemia and therefore need dose adjustments. In addition, treatment of kidney disease involves appropriate blood pressure control. The care of patients with kidney disease in sSA cannot be complete without addressing the management and care

for patients with HIV which causes kidney disease directly or indirectly through opportunistic infections and the drugs used to treat it [5]. While access to antiretroviral therapy (ART) has improved in sSA, patients are living longer and are prone to developing CKD which may progress to end stage kidney disease (ESKD). Irrespective of the cause once the patient reaches ESKD, they will require either dialysis or kidney transplantation. Access to dialysis in most African countries is very limited and most of those that have it require patients to pay out of pocket. Moreover, the home managed form of peritoneal dialysis is not readily available and patients often only have the choice of hemodialysis which is available in only limited centers, largely capital cities. Kidney transplant, which is the ultimate solution for end stage kidney failure is only available in 8 out of the 56 countries in Africa[2]. The rest of the countries have to refer patients for kidney transplant at very prohibitive costs. Often less than 1% of patients who need kidney transplant have access to it[6].

It is therefore very important that proper ways to diagnose and treat CKD are delineated and clear guidelines developed for management of CKD with particular attention to the specific situation of sSA.

2.2 Kidney function and GFR

The kidneys are two bean shaped organs located in the retroperitoneal space of the abdomen. Though small in size in comparison to other organs in the body like the lungs, the kidneys are no less important. The kidneys are responsible for maintaining the internal environment of the body constant, help in regulating blood pressure through the renin-angiotensin-aldosterone system, supporting hemoglobin production through production of erythropoietin, regulating acid base balance as well as ensuring electrolyte balance and production of vitamin D. The kidney is also responsible for excretion of the by-products of metabolism through production of urine. The kidney clears up to 180 liters of blood per day of toxic products [7].

2.0 Introduction

This brings us to the concept of glomerular filtration rate (GFR). The glomerular filtration rate is used to measure the excretory function of the kidney and does not directly measure renal blood flow. Glomerular filtration rate is used as one of the ways to estimate kidney function[8]. We cannot directly measure GFR in humans. It is assessed from clearance measurements or serum levels of filtration markers. These markers are either endogenous or exogenous solutes that are mainly eliminated by glomerular filtration.

Clearance of a substance is defined as the volume of plasma cleared of a marker per unit time. Clearance of substance y is the sum of the urinary and extrarenal clearance. Indeed, substances that are eliminated by both renal and extrarenal routes, have higher plasma clearance than their urinary clearance.

2.3 How to measure GFR – measured GFR

Measured glomerular filtration rate (mGFR) remains the gold standard for determining kidney function [9, 10]. The big question is, what is the best marker for doing this? Inulin has been recognized for many years as one of the best markers[11]. However, because of the complex nature of the methods required for inulin, its cost and lack of availability, other methods have been proposed and evaluated through a systematic review [12]. Central to this debate, is that the ideal marker to measure GFR should have certain characteristics to provide accurate results. It should be freely filtered at the glomeruli; not be metabolized; not be bound to plasma proteins and be non-toxic. Additionally, the ideal marker should be excreted only by the kidneys and should neither be reabsorbed nor secreted by the renal tubules. The marker should easily be measured and should be stable in both blood and urine [13]. Thus, finding an ideal marker is complicated.

2.0 Introduction

The proposed methods used to determine measured GFR in addition to inulin include: measurement of clearance of iothalamate, iohexol, Diethylenetriamine Pentaacetic Acid (DTPA), Ethylenediaminetetraacetic acid (EDTA) and 24-hour urinary creatinine collection for estimation of creatinine clearance [13, 14]. However, all of these have their challenges. DTPA and EDTA, like inulin, are expensive and not readily available in most African countries. 24-hour urinary collection for creatinine clearance is often inaccurate due to difficulty in ensuring a complete collection as well as tubular creatinine secretion which leads to overestimates of measured GFR, especially at lower GFR levels [13-16]. Iohexol on the other hand presents a very good option that may address most of the issues faced with other markers. Its advantages include ready availability, low cost, good safety profile, low protein binding, low levels of toxicity at the dose used for measuring GFR, stability at room temperature, and being able to provide an accurate measure of glomerular filtration [13, 14].

2.4 Evidence for iohexol as a gold standard for measured GFR

We elected to use serum iohexol to measure GFR and determine the optimal method to estimate GFR. This selection is backed by evidence showing that iohexol meets the criteria to be a gold standard for measured GFR as has been elaborated in a recent review by Delanaye[14]. Results from two studies comparing serum iohexol and inulin have shown good correlation between the two methods [17, 18]. Iohexol has also been compared with other markers: several studies showed excellent agreement between 51Cr-EDTA and serum iohexol methods[19-22]; Iohexol outperformed 99Tc-DTPA[23] and Iothalamate[20, 24]. Iothalamate overestimates GFR because it is secreted by the tubules.

2.5 Creatinine and cystatin C

Creatinine is an endogenous biomarker that results from the break-down of creatine phosphate in muscle tissues. It has a molecular weight of 113.13g/mol. The normal range of

creatinine in males varies from 0.6 - 1.2 mg/dl (53-106 µmol/L) and 0.5-1.1 mg/dl (44-97 µmol/L) in females using the kinetic (enzymatic) method[25]. Creatinine like other physiological markers is not only affected by the GFR but by other factors often referred to as non-GFR determinants. These include the rate of generation of creatinine, its reabsorption and secretion by the tubules of the kidney as well as its elimination from other parts of the body (extra-renal elimination)[26]. These physiological processes are not routinely measured which brings in errors in creatinine measurements.

Creatinine therefore falls short of an ideal biomarker because it is secreted by the tubules and is affected by other factors which are not related to GFR [27]. There are also significant inaccuracies with creatinine measurement as an assay especially when measured by the Jaffe method [28]. Serum creatinine levels vary substantially with ethnicity, age, sex, physical activity and nutritional status [9, 27]. Several sources of variability in estimating GFR have been described including biological variability in serum creatinine, laboratory induced errors in creatinine measurement and choice of estimating equation [29, 30]. The imprecision of creatinine measurement is more marked at values near the normal range where it is most critical to determine earlier stages of CKD [29, 31, 32].

To overcome some of the challenges of using creatinine as a marker for eGFR, the National Kidney Disease Education Program of the USA published comprehensive guidelines to regulate creatinine measurement and reporting [33]. Key among these is that the creatinine methods should be to a reference method for creatinine that is IDMS (isotope dilution mass spectrometry) traceable. Secondly, after recalibration to IDMS, the total error allowable for creatinine should be fixed at less than 10% error in eGFR [34].

The other biomarker used in measurement of kidney function is cystatin C. Cystatin C is a protein with a molecular weight 13 Kilo Daltons and is expressed by all nucleated cells in the

body. The normal range of serum cystatin is 0.6 to 1 mg/l. Cystatin C is freely filtered by the glomerulus and fully reabsorbed and metabolized by the renal tubules for recycling within the body. It is less affected by diet and muscle mass than creatinine. Cystatin C levels have been noted to correlate better with GFR [35, 36]. However, Cystatin C levels are affected by smoking and male sex and the levels may be raised in people with active inflammation and high basal metabolic index [37].

2.6 Creatinine and estimation of GFR

Estimating equations use easily measurable major determinants of creatinine such as demographic factors including age, sex and the black ethnicity as surrogate markers for non eGFR determinants in combination with creatinine to calculate the GFR[26]. Creatinine has been widely used to estimate GFR because it is easier to measure compared to other markers such as cystatin C. There are several equations used to estimate GFR from serum creatinine, correcting for factors such as age, sex, weight in use worldwide [38-43]. Because of earlier studies done in African Americans, a correction coefficient is incorporated into some of the equations to address the issue of ethnicity and creatinine variability[39].

The first equation to be developed was the Cockcroft-Gault (CG) equation largely based on factors that can easily be determined including; age, gender, weight and body surface area (BSA). The equation is expressed as:

Cockcroft-Gault equation: *eGFR (mL/min/1.73m^2) = [140-age (years) x weight (kg) x (0.85 if female) x 1.73m^2)] / [S-Cr x BSA (m^2)] (Where SCr- stands for serum creatinine)*

The CG equation was used in many drug studies and most of the dosing schedules in CKD are based on it. The fact that it is easy to calculate also lends it to easy use by clinicians from developing countries where laboratories are not yet mandated to report eGFR [44]. The challenge with CG is that it needs to be corrected for the body surface area, making direct

laboratory reporting impossible, and it underestimates GFR [45]. This prompted researchers to seek for alternatives [13, 46]. This led to the development of the Modification of Diet in Renal Disease (MDRD) study equation[39]. This is a 4-variable equation adjusted for age, sex, ethnicity and creatinine level:

***IDMS traceable 4-v MDRD equation**: eGFR (mL/min/1.73m^2) = 175 x S-Cr$^{-1.154}$ x age (years)$^{-0.203}$ x (0.742 if female) x (1.1212 if African American)[39] (where IDMS-isotope dilution mass spectrometry)*

However, the development of the MDRD equation was based on patients who had glomerular filtration rates <60 mls/min/1.73m^2 and thus performs poorly in the general population when the creatinine is normal or near normal levels. To address this issue Levey and his colleagues devised the CKD-EPI equation, which determines GFR with more accuracy in patients who have near normal kidney function [40].

***CKD-EPI equation for creatinine**:* eGFR (mL/min/1.73m^2) = 141 × min(S-Cr/κ, 1)$^{\alpha}$ × max(S-Cr/κ, 1)$^{-1.209}$ × 0.993^{age} × 1.018 [if female] × 1.159 [if black]

There is evidence from several studies suggesting that cystatin C measurements either alone or in combination with creatinine greatly improve the accuracy of eGFR and the prediction of death, ESRD and cardiovascular disease [47, 48]. These have been endorsed by the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) CKD guidelines to be used alone or in combination with creatinine when eGFR accuracy is of high importance.[10] The recommended CKD-EPI creatinine-cystatin C equation is shown below:

***CKD-EPI equation for creatinine and cystatin C**: eGFR (ml/min/1.73m^2) = 135 x min(SCr/ κ,1)$^{\alpha}$ x max(SCr/ κ,1)$^{-0.601}$ x min(SCysC/0.8, 1)$^{-0.375}$ x max(SCysC/0.8, 1)$^{-0.711}$ x 0.995^{age} x 0.969 [if female] x 1.08 [if black]*

where; SCr is serum creatinine, SCysC is serum cystatin C, κ is 0.7 for females and 0.9 for males, α is -0.248 for females and -0.207 for males, min(SCr/k,1) indicates the minimum of SCr/κ or 1, and max(SCr/κ,1) indicates the maximum of SCr/k or 1, min(SCysC/0.8,1) indicates the minimum of SCysC/0.8 or 1 and max(SCysC/0.8,1) indicates the maximum of SCysC/0.8 or 1[10].

Other equations have been developed in Europe and these include:

The Revised Lund-Malmö equation: $_eX–0.0158\times Age+0.438\times ln(Age)$

Where Female Cr < 150 µmol/L: X = 2.50+0.0121 × (150–Cr); Female Cr ≥ 150 µmol/L: X = 2.50–0.926 × ln(Cr/150); Male Cr < 180 µmol/L: X = 2.56+0.00968 × (180–Cr); Male Cr ≥ 180 µmol/L: X = 2.56–0.926 × ln(Cr/180)[42] where Cr is serum creatinine

Full-Age Spectrum FAS (creatinine) Equation FAS-GFR: 107.3 /Creat/Q x $0.988^{(Age\ -40)}$ if >40 years

where Q-values are the mean or median SCr value for age-/sex-specific healthy populations[43]

2.7 Overview of performance of GFR estimating equations

The performance of GFR estimating equations has evolved over time and adaptations are being made to ensure that early diagnosis of kidney disease is made and decisions can be made to delay progression as well as initiate appropriate therapies to affected individuals[26]. Creatinine variability and imprecisions in measurements makes its isolated use a poor predictor of kidney function. According to the international guidelines it is recommended that reporting eGFR should be done along with the creatinine level once the eGFR is <60mls/min/1.73m^2 because the true GFR level is more precisely estimated by eGFR at this level[10]. The performance of GFR estimating equations varies according to the population

under investigation as well as the purpose and accuracy required for its use. CKD-Epi or more accurate equations are recommended for use by KDIGO. Each of the equations has its strength and limitations largely emanating from the population in which it was developed as well as what has been done so far to validate its use in other populations. Below I outline the estimating equations uses and limitations.

The CG equation has been used for the longest time and remains relevant because most of the drug dosing studies were done using this equation [38, 49]. The CG uses four parameters of age, weight, gender and creatinine serum level, it has to be standardized for 1.73m^2, which is a major limitation factor for its determination in the laboratory. Most of the studies using the CG are also old and used the Jaffe method of creatinine measurement which may have errors of overestimation [50].

The MDRD equation can be used as 4-variable equation (black race, gender, serum creatinine and age) or as a 6-variable equation (gender, race, age, serum creatinine, blood urea nitrogen and albumin). The 4-variable equation is used most and was developed using a population of 1,648 patients with previously diagnosed CKD[39]. The MDRD equation has been shown to underestimate GFR among patients with preserved renal function [30, 51].

The African American ethnicity coefficient is included for all individuals of a black race when estimating GFR using the MDRD and CKD-EPI equations. This is based on the MDRD study where individuals of African origin had higher creatinine levels for the same value of measured GFR compared to their non-black counterparts[39]. This was further confirmed in the African American Study of Kidney Disease (AASK)[52]. However, there are growing concerns on the use of the ethnicity coefficient factor as a form of discrimination against individuals of black color further aggravating disparities in access to kidney care services. The opponents argue that race is a dynamic characteristic and is not a reliable proxy for

biological characteristics. Because of this and other issues there is a big call to drop the use of the ethnicity coefficient when determining eGFR [53, 54].

The CKD-Epi was developed to address the shortcomings of the MDRD equation by including participants with normal kidney function. This equation was developed in a population of 8,254 individuals with a mean GFR of 68ml/min/1.73m^2 based on iothalamate clearance. It is also based on four factors including ethnicity, age, sex and serum creatinine level (CKD-Epi$_{cr}$) or serum cystatin C (CKD-Epi$_{cys}$)[40]. The CKD-Epi has been found to be more accurate in determining GFR. This was confirmed in a meta-analysis of 11 general population studies (90,750 participants) and 5 cohort studies of CKD patients (2960 participants) [55]. Sadly, all the studies used in the meta-analysis were from Europe and North America and may not reflect the true picture among people in Africa. Moreover, some of the cohorts used in the studies had variable methods of creatinine measurement and the measurement of cystatin C was not standardized across the cohorts. Researchers note that, eGFR$_{cys}$ is not more accurate than eGFR$_{cr}$ but the combination eGFR$_{cr\text{-}cys}$ is more accurate than either of the equations alone[26, 56].

The Lund-Malmo is a relatively newly developed equation that depends on three factors (gender, age and serum creatinine). In a study by Nyman among 2,847 adult Swedish patients, the revised Lund-Malmo equation performed better than both the CKD-EPI and the MDRD equations. It was noted that MDRD and CKD-EPI overestimated Iohexol measured GFR in patients with decreased kidney function, young adults and elderly[42]. The Lund-Malmo equation has never been studied among people from Sub-Saharan Africa.

The FAS equation has the advantage of being developed in both children and adults. It was developed from a European cohort to address the challenge of the transition between 16 to 18 years of age [43]. The modified FAS equation draws from the FAS and CKD-Epi equations

with 11,251 participants from 7 studies for development and internal validation and 8,378 participants from 6 studies for external validation. The advantage is that it covers individuals from two to ninety years with a wide range of serum creatinine (0.45 -5.54 mg/dL) and seems to outperform the CKD-Epi$_{Cr}$ equation. The major limitation is that it included individuals of European origin and had no black patients, nor inclusion of other minority groups [57].

2.8 Performance of eGFR equations in Sub-Saharan Africa

We undertook a systematic review with a purpose of establishing current literature on the methods of assessment of kidney function in sSA (see appendix for publication). For the purposes of this thesis, I will focus on the literature available to compare the accuracy of eGFR to measured GFR (mGFR), overall and with reference to the impact of adjustments for the ethnicity coefficient on estimating kidney function. We conducted the systematic review in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. The period selected for review included all original research studies published from 31/01/2008 to 31/ 9/2018. We searched online databases of Pubmed, African Journals Online (AJOL) and Web of Science using the relevant medical subject headings. Based on the title and abstract, all studies from the sSA region that assessed kidney function in adults were evaluated according to inclusion and exclusion criteria agreed upon.

In summary, we identified ten studies that measured GFR using a gold standard reference method, four from South Africa and one each from Ivory Coast, Congo, Ghana, Kenya, Seychelles and Sudan [58-63]. The Kenyan study and one study from South Africa focused on adult participants with HIV infection [60, 64]. This was particularly relevant for the sSA region because of widespread use of tenofovir-containing antiretroviral therapy regimens as first-line treatment. The methods used to measure GFR included iohexol on dried blood spots, iohexol plasma clearance, technetium-99m diethylenetriamine penta-acetic acid

(DTPA), chromium-51 ethylenediamine tetra-acetic acid (EDTA), inulin and 24-hour urinary creatinine clearance. Six studies compared the performance of currently recommended eGFR equations to a reference mGFR method.

The impact of using the ethnicity coefficient for the 4-v MDRD and CKD-EPI equations was also evaluated. Overall, most studies demonstrated that when compared to the mGFR method, inclusion of the ethnicity coefficient resulted in overestimation of eGFR in Africans.

The key finding from the review is that we still have few studies looking at measured GFR from sSA. All of the studies used very small sample sizes of less than 150. In as much as the studies followed the standard guidelines for measuring creatinine, which was considered for estimating the values for eGFR; the majority excluded participants with CKD. It is therefore not surprising that these studies showed low bias and good accuracy ranging from 73% to 93% (accuracy within 30% of the mGFR) when plasma iohexol clearance was used as the gold standard. This is a reassurance that iohexol is good marker for mGFR in the general population and can practically be used in sSA.

Moving on from this review, what our study will add is greater power as well as well as including patients with chronic kidney disease or impaired renal function, and use of Cystatin C in addition to creatinine. These were excluded from most of the studies outlined above. In addition, all our creatinine measurements were standardized using an IDMS-traceable assay to a standard reference material.

2.9 Impaired renal function and chronic kidney disease definitions and classification

Chronic kidney disease (CKD) has been defined by KDIGO as abnormalities of kidney structure or function, present for more than 3 months [10].

The diagnosis requires the presence of either of the following for more than 3 months:

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- Decreased GFR: GFR <60 ml/min/1.73 m^2

AND/OR

- Markers of kidney damage (one or more):
  - Albuminuria [Albumin Excretion Ratio (AER) >30mg/24 hours; Albumin Creatinine Ratio (ACR) >30mg/g or >3 mg/mmol]
  - Urine sediment abnormalities
  - Electrolyte and other abnormalities due to tubular disorders
  - Abnormalities detected by histology
  - Structural abnormalities detected by imaging
  - History of kidney transplantation

Chronic kidney disease is classified by cause, level of GFR and albuminuria. It has been staged according to level of GFR into five stages as shown in table 1 below recommended by KDIGO.

Table 1: KDIGO staging of kidney disease

eGFR (mls/min/1.73m^2) & Albuminuria (mg/g) Classification[65]	
Normal or high	G1≥ 90
Mildly decreased	G2 60–89
Mild to moderately decreased	G3a 45–59
Moderately to severely decreased	G3b 30–44
Severely decreased	G4 15–29
Kidney failure	G5 <15
Albuminuria categories ACR (mg/g) and related terms: A1 <30 (normal to mildly increased); A2 30–300 (moderately increased); A3 >300 (severely increased)	

Table 1. Abbreviations G- grade, A –Albuminuria, ACR- albumin creatinine ratio; eGFR- estimated glomerular filtration rate

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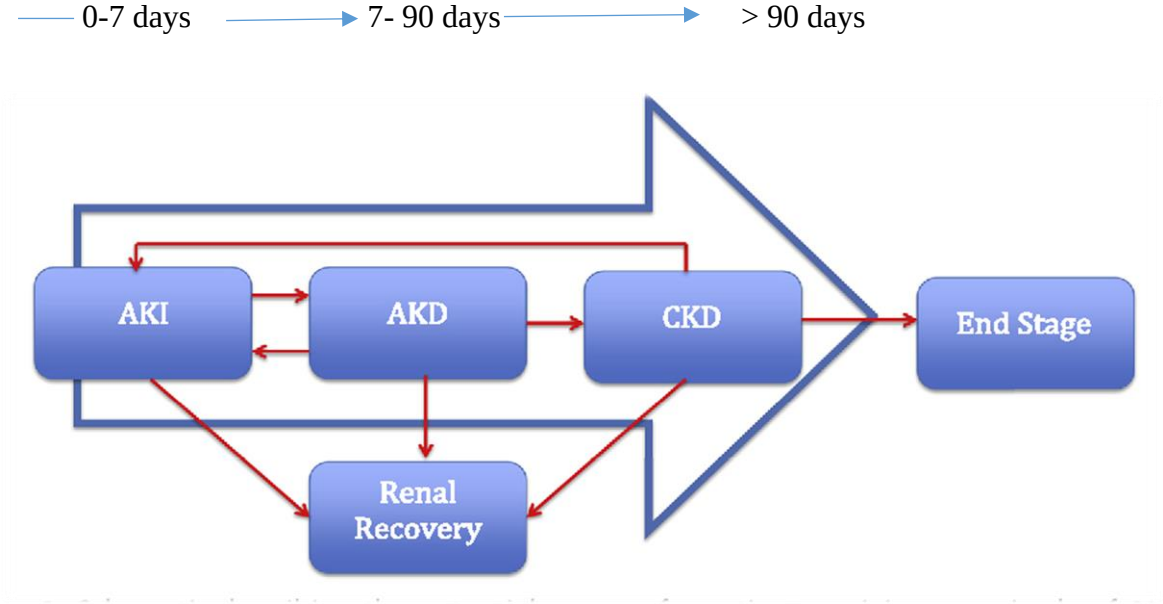
On the other hand, impaired renal function is defined as any abnormality in kidney function that has not been measured to be present for more than three months. This includes acute kidney injury (2-7 days) and acute kidney diseases and disorders, which encompasses the all disorders with reduced kidney function or kidney damage up to three months see **Figure 1** [66, 67]. It is important to note that acute kidney injury can be transient and reversible.

Figure 1: Acute kidney injury, acute kidney disease and chronic kidney disease classification



Definitions: AKI- Acute kidney injury; AKD-Acute kidney disease, CKD-chronic kidney disease; ESRD-End stage kidney disease (Adopted from Migizuki KA, 2018)

Diagnosis of impaired renal function also involves measurement of creatinine, urine volume, microalbuminuria or other markers of kidney damage as above and can also be graded in accordance with the KDIGO guidelines.

Measurement of urinary protein excretion is also an important marker of kidney disease.

The following measurements have been proposed for initial testing of proteinuria (in descending order of preference (KDIGO)[10];

1. Urine albumin-to-creatinine ratio (ACR)

2. Urine protein-to-creatinine ratio (PCR)

3. Reagent strip urinalysis for total protein with automated reading

4. Reagent strip urinalysis for total protein with manual reading

For all tests, an early morning urine is preferred. An ACR of 30 mg/g or greater (≥3mg/mmol) on a random untimed urine should be confirmed with a subsequent early morning urine sample.

2.10. How common is CKD in sSA?

Several studies have looked at the prevalence of kidney disease in Africa. These have largely been cross-sectional in nature and the majority of them in high-risk populations. Community and hospital-based studies have given differing prevalence rates of CKD in sSA ranging from 0.7% to 41.3% depending on the methods and population used [44, 68-73]. Most of the studies are low quality and use inconsistent methods for assessing and defining kidney disease [74].

Two systematic reviews have noted a prevalence of CKD of 4.6% to 13.9% [74, 75]. The outstanding issues in the reported studies were around the quality of the data generated. Many of the studies from sSA do not meet the KDIGO criteria of measuring creatinine at least 3 months apart, including other abnormalities such as albumin creatinine ratios, proteinuria or structural abnormalities of the kidney. In a systematic review by Stanifer, only 3 out of 90, (3.3%) studies were population based. Of the 21 studies included in the meta-analysis only three (14%) were considered to be of high quality according to KDIGO criteria [10, 74]. Many of the studies used either a single creatinine based eGFR and or proteinuria alone to define CKD. This is likely to overestimate the prevalence of CKD because it includes acute cases and does not exclude cases of proteinuria or elevated albumin: creatinine ratios due to non-renal causes [74, 76]. One of the robust studies; the Africa Wits-International Network for the Demographic Evaluation of Populations and their Health Partnership for Genomic

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Studies (AWI-Gen), recruited participants from four rural communities (Burkina Faso, Ghana and two from South Africa) and two urban communities (Nairobi, Kenya and Soweto, South Africa). They defined kidney disease as either having an eGFR of <=60mls/min/1.73 m^2 and or albuminuria (urine albumin creatinine ratio of >3mg/mmol). Among the 8110 participants enrolled in the study, the mean age was 49.9 years and the age-standardized GFR <=60mls/min/1.73 m^2 was 2.4%; 9.6% for albuminuria and 10.7% for both low GFR and albuminuria (which they defined as CKD). They reported a higher prevalence in the South African sites compared to the West African sites. The key risk factors for kidney dysfunction were older age, hypertension, diabetes mellitus and HIV-infection among the participants while male sex was found to be protective. This study was based on populations from the four countries and illustrates some of the key challenges faced in determining kidney disease prevalence in sSA. As an example, the participants from Soweto, South Africa were missing the ACR measurements and were therefore excluded from the analysis for risk factors. Furthermore, all the participants in this study were aged restricted as 40-60 years in accordance with the study protocols. Even in these well-established cohorts, up to 2592 participants out of 10,702 (24.2%) had missing data on crucial variables for the study. There was no second measurement of neither creatinine nor albuminuria and serum creatinine was measured according to the Jaffe's kinetic method which is less robust than the enzymatic method due to interfering substances [77, 78]. Due to poor validation of all existing GFR estimating equations; the authors reported their findings based on both MDRD and CKD-Epi equations with and without the ethnicity coefficient adjustments.

The utility, feasibility and additional value of a second creatinine measurement along with the additional diagnostic and prognostic value of proteinuria or albumin creatinine ratio (ACR) has not been well evaluated in sSA. However, there are indications that we may be overestimating the prevalence of CKD. In a study from the general population in Morocco

with a follow up of participants with baseline abnormal kidney function at three, six and twelve months, a decrease of close to 50% in the prevalence of CKD was noted when confirmation of "proteinuria/hematuria and chronicity" of eGFR were included in accordance with the KDIGO guidelines[79]. Conversely, there was an underdiagnoses (false negatives) of CKD in younger individuals with an eGFR >60 ml/min/1.73 m^2. This has major implications for sSA where the majority of the population is less than 45 years of age. There is conflicting information on the leading risk factors for kidney disease in sSA with some studies such as AWI-Gen and systematic review on global burden of disease emphasizing the leading role of the traditional risk factors such as hypertension, diabetes mellitus and HIV-infection [80] while other studies note that there may be other key drivers of kidney disease in sSA. In the studies from East Africa, between 49.1% and 66% of kidney disease is not explained by hypertension, HIV-infection or diabetes mellitus [44, 81, 82] implying that there may be other key drivers of kidney disease in this population. Given the limited understanding of the causes and factors for progression of kidney disease, other factors (which are less common in high-income countries) such as sickle cell disease, herbal and traditional medicine use, glomerulonephritis and infections such as schistosomiasis and malaria may play a major role in the causation and progression of CKD in sub-Saharan Africa [83-85]. Overall, CKD is associated with poorer health status in LMICs and end stage kidney disease (ESRD) has a greater negative health impact in those affected [86].

2.11 Mortality associated with chronic kidney disease

The leading causes of death among patients with chronic kidney diseases include cardiovascular complications, infections and end stage kidney disease [87-89]. In developed countries, end stage kidney disease (ESKD) is a chronic manageable disease often treated with either dialysis or kidney transplant. There is a paucity of data on kidney disease and mortality in general populations in sub-Saharan Africa. A review of the literature from 2012

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(GPC mortality study inception) to 2019 from PubMed, African Journals online and Scopus using the term impaired renal function AND sub-Saharan Africa (sSA) AND mortality without language restriction identified 144 articles. Our review noted that all the studies were medium to low quality with the majority hospital based 133 (92%), cross sectional (90%) and with prospective studies largely in children with an average follow up of 18 months. The few prospective studies in adults are largely hospital based, with substantial loss to follow up or were largely in disease specific populations which may not be applicable to the general population in sSA [90-94].

There is low inclusion of kidney disease patients in the few cohorts that exist on the African continent [1, 80, 95]. Most of the current studies have had a single measure of creatinine and or microalbuminuria and follow up has not been achieved [74, 96, 97]. In a study among HIV-infected individuals in Zambia baseline renal dysfunction was associated with increased risk of mortality with an adjusted hazard ratio of up to 3.6 (95% CI, 2.4-5.5) after 90 days as compared to those with normal renal function[70]. Among patients with acute heart failure kidney dysfunction (low EGFR or high blood urea nitrogen) with or without proteinuria has been associated with increased risk of mortality [91, 93, 98]. For example, in a study of 558 adults from Tanzania, patients discharged with heart failure and combination of low eGFR (<60mls/min/1.73m^2)/proteinuria (+ dipstick) died within 12months of follow up compared to those without renal abnormality where the mortality rate was lower at 50% [93]. These studies demonstrate that among patients with pre-existing conditions, renal dysfunction is associated with increased risk of mortality. What has not been clearly established in most of Africa is what proportion of patients with abnormal kidney function die as compared to those with normal kidney function in the general population.

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2.0 Introduction

Appendix for chapter 2

Appendix 1a Methods and reporting of kidney function: a systematic review of studies from sub-Saharan Africa
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CKJ Review

CKJ REVIEW

Methods and reporting of kidney function: a systematic review of studies from sub-Saharan Africa

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ABSTRACT

Globally, chronic kidney disease (CKD) is an emerging public health challenge but accurate data on its true prevalence are scarce, particularly in poorly resourced regions such as sub-Saharan Africa (SSA). Limited funding for population-based studies, poor laboratory infrastructure and the absence of a validated estimating equation for kidney function in Africans are contributing factors. Consequently, most available studies used to estimate population prevalence are hospital-based, with small samples of participants who are at high risk for kidney disease. While serum creatinine is most commonly used to estimate glomerular filtration, there is considerable potential bias in the measurement of creatinine that might lead to inaccurate estimates of kidney disease at individual and population level. To address this, the Laboratory Working Group of the National Kidney Disease Education Program published recommendations in 2006 to standardize the laboratory measurement of creatinine. The primary objective of this review was to appraise implementation of these recommendations in studies conducted in SSA after 2006. Secondary objectives were to assess bias relating to choice of estimating equations for assessing glomerular function in Africans and to evaluate use of recommended diagnostic criteria for CKD. This study was registered with Prospero (CRD42017068151), and using PubMed, *African Journals Online* and Web of Science, 5845 abstracts were reviewed and 252 full-text articles included for narrative analysis. Overall, two-thirds of studies

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did not report laboratory methods for creatinine measurement and just over 80% did not report whether their creatinine measurement was isotope dilution mass spectroscopy (IDMS) traceable. For those reporting a method, Jaffe was the most common (93%). The four-variable Modification of Diet in Renal Disease (4-v MDRD) equation was most frequently used (42%), followed by the CKD Epidemiology Collaboration (CKD-EPI) equation for creatinine (26%). For the 4-v MDRD equation and CKD-EPI equations, respectively, one-third to one half of studies clarified use of the coefficient for African-American (AA) ethnicity. When reporting CKD prevalence, <15% of studies fulfilled Kidney Disease: Improving Global Outcomes criteria and even fewer used a population-based sample. Six studies compared performance of estimating equations to measured glomerular filtration rate (GFR) demonstrating that coefficients for AA ethnicity used in the 4-v MDRD and the CKD-EPI equations overestimated GFR in Africans. To improve on reporting in future studies, we propose an 'easy to use' checklist that will standardize reporting of kidney function and improve the quality of studies in the region. This research contributes some understanding of the factors requiring attention to ensure accurate assessment of the burden of kidney disease in SSA. Many of these factors are difficult to address and extend beyond individual researchers to health systems and governmental policy, but understanding the burden of kidney disease is a critical first step to informing an integrated public health response that would provide appropriate screening, prevention and management of kidney disease in countries from SSA. This is particularly relevant as CKD is a common pathway in both infectious and non-communicable diseases, and multimorbidity is now commonplace, and even more so when those living with severe kidney disease have limited or no access to renal replacement therapy.

Keywords: albuminuria, chronic kidney disease, creatinine, estimated and measured glomerular filtration rate, prevalence, systematic review

INTRODUCTION

In sub-Saharan Africa (SSA), a double burden of infectious and non-communicable diseases contributes to a potentially high risk for chronic kidney disease (CKD). However, the true prevalence of CKD remains difficult to quantify [1, 2]. A systematic review of the epidemiology of CKD in SSA concluded that most studies were of medium to low quality due to poor sampling methods and unreliable laboratory measurements of kidney function [1]. Many of these studies were conducted in urban hospitals with participants who had multiple risk factors for CKD, and proteinuria was the most common measure of kidney function [1]. Given that convenience sampling may lead to overestimating the burden of CKD at population level, and that the diagnostic criteria used to define CKD were sub-optimal in many of the studies from the review, the reasons for this low research quality merit consideration.

First, the funding and infrastructure required for population-based CKD prevalence studies is substantial and, even in the most well-resourced environments, conducting these studies is challenging [2]. Second, in the absence of validated, affordable, point of care diagnostics for kidney disease, for studies to conform to the internationally accepted Kidney Disease: Improving Global Outcomes (KDIGO) guidelines requires (i) laboratory measurement of serum creatinine using an isotope dilution mass spectroscopy (IDMS)-traceable standard reference material for creatinine; (ii) calculation of the glomerular filtration rate (GFR) using the serum creatinine; (iii) measurement of urine protein or albumin from a spot urine sample, preferably laboratory quantified as an albumin:creatinine ratio; and (iv) in the absence of clinical evidence that confirms chronicity, repeating these serum and urine measurements after a minimum period of 3 months [3, 4]. These requirements impose substantial logistic, infrastructural and financial hurdles for clinical researchers, particularly in resource-limited settings where the burden of CKD is projected to be highest.

For any study using KDIGO criteria, access to accurate diagnostic laboratory services is essential as even small variations in creatinine measurement can impact on population prevalence estimates [2]. This is underappreciated by clinician scientists and may reflect poor interdisciplinary collaboration with chemical pathologists. For example, the older colorimetric picric acid (Jaffe) method is less accurate but cheaper than the recently developed enzymatic method. While the enzymatic method is recommended, in SSA most laboratories use the Jaffe method, for which a correction factor should be applied [1]. To reduce inter-laboratory measurement bias, the Laboratory Working Group of the National Kidney Disease Education Program (NKDEP) in the USA published guidelines in 2006 that recommended use of an IDMS-traceable standard reference material for creatinine measurement [5]. Consequently, the four-variable Modification of Diet in Renal Disease (4-v MDRD) equation was re-expressed for IDMS-traceability and the CKD Epidemiology Collaboration (CKD-EPI) equations are based on the use of an IDMS-traceable method [6, 7]. Adherence to standard internal and external quality assurance procedures are additional obligatory steps that mitigate laboratory-induced bias. In SSA and other low and middle income settings, reliable diagnostic laboratory infrastructure cannot be assumed. The lack of study-specific standardization in sampling and measurement of creatinine is considered the greatest obstacle to determining the global prevalence of CKD [2, 8].

Further bias may be introduced through the choice of estimating equation for GFR. Initially, the National Kidney Foundation guidelines recommended the 4-v MDRD equation for estimating GFR [3]. This equation was derived from a relatively small study sample in the USA with a coefficient that corrected for a higher creatinine observed in African-Americans (AAs). A similar correction coefficient for AA ethnicity was derived for use with the CKD-EPI equation, which is now the preferred equation in the revised KDIGO guidelines for 2014 [4]. In studies from SSA, when the coefficients for AA ethnicity are used for either of these two equations, they consistently overestimate GFR [9–15]. In the absence of appropriate, validated estimating equations for African populations, some studies have omitted the coefficients for AA ethnicity when using the 4-v MDRD and CKD-EPI equations [16, 17]. Researchers need to be cognizant of the limitations imposed by these equations and the bias that could be introduced into their results.

The primary objective of this review was to appraise reporting of laboratory methods for creatinine measurement in studies utilizing creatinine to assess kidney function in SSA. The secondary objectives were to assess bias relating to: choice of

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equations used to estimate GFR; use of coefficients for AA ethnicity for the 4-v MDRD and CKD-EPI equations; criteria used to diagnose CKD; and study design and sampling strategies.

MATERIALS AND METHODS

Search strategy and selection criteria

This narrative systematic review was registered with the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42017068151) and completed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) together with the revised quality assessment of diagnostic accuracy studies (QUADAS-2) guidelines [18–20]. The period selected for review included all original research studies published from 31 January 2008 to 31 December 2018. This was based on the assumption that after the NKDEP guidelines were published in 2006, this would set the standard for the widespread implementation of IDMS-traceable creatinine assays in diagnostic laboratories by 2008—and we would see a similar trend in studies from SSA [5]. For studies that determined the prevalence of CKD, the recommended criteria for diagnosis of CKD were first published in 2002 and subsequently updated in 2012 [3, 4]. Likewise, we anticipated that these guidelines would inform the choice of criteria for clinical studies.

The online databases for PubMed, *African Journals Online* and Web of Science were searched using the relevant medical subject headings (Supplementary data, Appendix S1). Based on the title and abstract, all studies from the SSA region that assessed kidney function in adults were evaluated according to inclusion and exclusion criteria agreed upon by the team conducting the systematic review (Supplementary data, Appendix S1). Only those abstracts with studies with full-text articles, available in English, were selected for the final analysis.

Quality assessment and data extraction

The online database search generated abstracts for screening. J.A.G. and M.v.D. independently screened abstracts from *African Journals Online* and Web of Science. There were no additional references identified through bibliography searches. Results were compared and differences of opinion referred to J.F. or H.R.E. Similarly, J.F. and H.R.E. independently screened abstracts from PubMed and followed the same process with differences referred to J.A.G. and M.v.D. Duplicates were removed and the team agreed on a list of eligible abstracts for which full-text articles were sourced by J.A.G. and H.R.E. For each full-text article, data were extracted by two authors who worked independently (J.F., J.A.G., H.R.E., M.v.D. and R.K.). Each author then compared their data extraction to the second author and discrepancies were resolved or referred to the remaining authors for review. If needed, J.F. contacted authors via email for clarification of study-specific information and incorporated the feedback for those who responded.

The QUADAS-2 assessment sheet (Supplementary data, Appendix S2) was used as a template by the team to pilot the data extraction process. After conducting the pilot, a revised form, adapted for the specific needs of this study, was generated and accepted by the group for use. This was formulated into a study-specific data extraction sheet (Supplementary data, Appendix S3). In summary, where appropriate for each full-text study, the data were abstracted as follows: (i) was GFR measured or estimated, and by which method; (ii) was creatinine measured, and by which method, and was the method IDMS-traceable to a standard reference material for creatinine; (iii) was cystatin C measured; (iv) did the study determine CKD prevalence—if so, by which criteria, was chronicity confirmed with repeat measurements; and (v) study design, sample selection and sample size. The estimating equations for creatinine, cystatin and creatinine-cystatin, and CKD criteria are defined in Supplementary data, Appendix S4. Data were analyzed using simple descriptive statistics including frequency and percentage tabulation for continuous variables. A narrative analysis was considered more appropriate for the aim of this systematic review and a meta-analysis was therefore not conducted.

Performance of eGFR equations

For those studies with measured GFR (mGFR) and comparisons of performance of the different estimated GFR (eGFR) equations, the relative performance of these equations was assessed using: (i) bias: median of difference between estimated and mGFR; 95% confidence interval (CI), when available; and (ii) P30: percentage of eGFR values within 30% of the (gold standard) mGFR; 95% CI, when available.

RESULTS

From the online database searches, there were 5845 records published during the review period. The procedure followed for study identification and selection is summarized in Figure 1. The final number of full-text articles assessed as eligible for inclusion into this systematic review totaled 252 (Supplementary data, Appendix S5). The results are presented as follows: (i) laboratory method for creatinine measurement; (ii) estimating equations for eGFR; (iii) mGFR using a gold standard method, comparison of the performance of eGFR equations in relation to mGFR and the impact of coefficients for AA ethnicity; (iv) diagnostic criteria for CKD; and (v) quality of the studies.

Laboratory method for creatinine measurement

The laboratory method for creatinine measurement was not reported in two-thirds of studies (159/252; 63.1%). For those that included a method, the Jaffe method was by far the most common (80/86; 93.0%). Only six studies described an enzymatic method. Most studies (206/252; 81.7%) did not state whether their laboratory was using an IDMS-traceable standard reference material for creatinine measurement. When stipulated, IDMS-traceable assays were more common (34/39; 87.2%) than non-IDMS traceable assays, of which there were only five (Table 1).

Estimating equations for GFR

Most studies used an estimating equation to assess kidney function (231/252; 91.6%) and some used more than one, so that eGFR equations were used in 363 instances. Of the available equations, the 4-v MDRD was most frequently used (146/363; 40.2%), followed by the CKD-EPI equation for creatinine (94/363; 25.9%) and Cockcroft–Gault (85/363; 23.4%). When the 4-v MDRD equation was used, one-third used the 4-v MDRD re-expressed for IDMS traceability (46/146; 31.5%), half did not stipulate which version was used (74/146; 50.7%) and the remainder used the original 4-v MDRD (prior to the introduction of an IDMS-traceable standard reference material for creatinine). The AA coefficient was used for a third of the 4-v MDRD equations (45/146; 30.8%) and almost half (72/146; 49.3%) did not stipulate if this was used. For the CKD-EPI creatinine equation, more studies used the AA coefficient (39/94; 41.5%) and a quarter did not clarify if this was used (23/94; 24.5%) (Table 1 and Supplementary data, Appendix S4).

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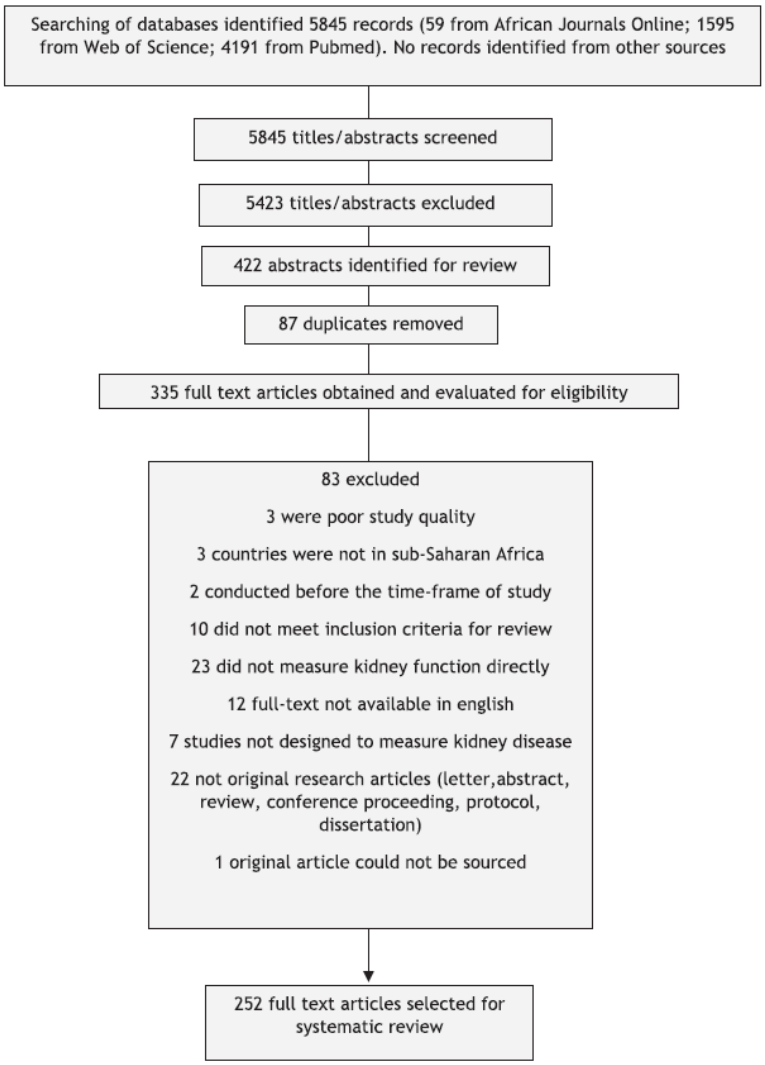

FIGURE 1: Flowchart for study identification and selection.

mGFR using a gold standard method, comparison of the performance of eGFR equations in relation to mGFR and the impact of coefficients for AA ethnicity

There were 10 studies that measured GFR using a gold standard reference method, 4 from South Africa and 1 each from the Democratic Republic of the Congo, Ghana, Ivory Coast, Kenya, Seychelles and Sudan [9–13, 15, 22–25]. The Kenyan study and one study from South Africa focused on adult participants with HIV infection [12, 13]. This was particularly relevant for the SSA region because of widespread use of tenofovir-containing antiretroviral therapy regimens as first-line treatment. The methods used to measure GFR included iohexol on dried blood spots, iohexol plasma excretion, technetium-99m diethylenetriamine penta-acetic acid (DTPA), chromium-51 ethylenediamine tetra-acetic acid (EDTA), inulin and 24-h urinary creatinine clearance. Six studies compared the performance of currently recommended eGFR equations to a reference mGFR method. The impact of using the coefficient for AA ethnicity for the 4-v MDRD and CKD-EPI equations was also evaluated. Overall, most studies demonstrated that when compared with the mGFR method that was used, inclusion of the coefficient for AA ethnicity resulted in overestimation of eGFR in Africans (Table 2). Some studies compared the relative performance of one or more eGFR equations without reference to a gold standard mGFR. Since the scientific validity of this practice is unsubstantiated, no further analysis was conducted.

Diagnostic criteria for CKD

Most studies (162/252; 64.5%) defined CKD using a broad range of criteria that have been summarized in Table 3. Fewer than 15% of studies confirmed chronicity by repeating the out of range test for eGFR and/or albuminuria/proteinuria after the minimum recommended period of 3 months, a requirement for diagnosing CKD as per KDIGO guidelines. Notably, most studies that fulfilled the KDIGO requirements were published in the latter half of the period under review [26–38].

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Table 1. Reporting of laboratory creatinine measurement and eGFR equations

Laboratory creatinine measurement ($n = 252$ studies[a])	
Creatinine method	Jaffe/enzymatic 80 (31.7%)/6 (2.4%)
	Not stated 159 (63.1%)
	Not measured 7 (2.8%)
Creatinine method	IDMS-traceable 34 (13.5%)
	Non-IDMS-traceable 5 (2.0%)
	Not stated 206 (81.7%)
	Not measured 7 (2.8%)
eGFR equations[a] ($n = 363$ eGFR equations[b])	
Creatinine clearance ($n = 9$; 2.5%)	BSA[d] normalized (4/9)
	BSA not normalized (3/9)
	BSA not stated (2/9)
Cockcroft–Gault ($n = 85$; 23.4%)	BSA normalized (29/85)
	BSA not normalized (28/85)
	BSA not stated (28/85)
Non-IDMS-traceable 4-v MDRD ($n = 26$; 7.2%)	+ Coefficient for AA ethnicity (14/26)
	− Coefficient for AA ethnicity (5/26)
	Coefficient not stated (7/26)
IDMS-traceable 4-v MDRD ($n = 46$; 12.7%)	+ Coefficient for AA ethnicity (23/46)
	− Coefficient for AA ethnicity (15/46)
	Coefficient not stated (8/46)
4-v MDRD (not stated) ($n = 74$; 20.4%)	+ Coefficient for AA ethnicity (8/74)
	− Coefficient for AA ethnicity (9/74)
	Coefficient not stated (57/74)
CKD-EPI[c] (creatinine) ($n = 94$; 25.9%)	+ Coefficient for AA ethnicity (39/94)
	− Coefficient for AA ethnicity (32/94)
	Coefficient not stated (23/94)
CKD-EPI[c] (creatinine + cystatin C) ($n = 6$; 1.7%)	+ Coefficient for AA ethnicity (3/6)
	− Coefficient for AA ethnicity (3/6)
Other ($n = 23$; 6.3%)	eGFR equation not specified (17/23)
	Different eGFR equation used (6/23)

[a]Percentages rounded to one decimal point and may not sum to 100%.
[b]Of 252 studies, 21 did not use an eGFR method. Of the remaining studies (231), some evaluated more than one eGFR method, thus totaling 363 eGFR equations.
[c]CKD-EPI equation for creatinine alone, or creatinine and cystatin C, or cystatin C alone.
[d]BSA (body surface area), normalized to 1.73 m^2 [21].

Quality of studies

Most study designs were cross-sectional (172/252; 68.3%), fewer were case-controlled (41/252; 16.3%) and the methodology was unclear in the remainder (39/252; 15.4%). Many studies reported 'prevalent' CKD, but this was restricted to hospital- or clinic-based convenience samples and often focused on participants at high risk for kidney disease, for example, those with sickle cell disease, diabetes, hypertension, obesity, HIV and cardiac failure. For true population prevalence of CKD, there were eight randomly sampled population-based studies that determined prevalent CKD [10, 17, 39–44]. None of the population-based studies reported incident CKD.

DISCUSSION

There is scope for improving the quality of studies on kidney function in SSA. This includes aspects of study design and sampling, reporting of laboratory methods for creatinine measurement and IDMS traceability, detailing choices for GFR equations that include coefficients for AA ethnicity, and diagnosing CKD using appropriate criteria. While this is the first review of its kind for SSA, similar findings have been reported in Europe, Mexico, Uruguay and India [8, 45].

There are published NKDEP guidelines for the laboratory reporting of creatinine; however, two-thirds of our studies did not report the method of creatinine measurement and even fewer reported whether the method was IDMS-traceable to a standard reference material for creatinine. This limited insights into the extent of implementation of laboratory standards for creatinine-based testing of kidney function, but our findings reflect a need for better study-specific interdisciplinary collaboration between chemical pathologists, epidemiologists, public health and clinical scientists. Collaboration would deepen the scientific rationale and strengthen the methodological components of studies that characterize kidney disease, particularly at population level.

Many studies applied an estimating equation for GFR, but it is noteworthy that Cockcroft–Gault was still relatively frequently used, despite its omission from clinical practice guidelines since 2002. Perhaps this is due to the long history of this equation and its ease of use in clinical settings. Because of the time period for this review, it would be expected, as we have confirmed, that the 4-v MDRD equation was the most frequently used equation, but most studies did not identify which version of the equation was used in relation to IDMS traceability. Depending on which equation was used [CKD-EPI(serum creatinine, SCr) and/or 4-v MDRD], up to half of studies did not state whether the coefficients for AA ethnicity were used. In this regard, two recent studies from the Democratic Republic of the Congo and Ivory Coast (both using iohexol plasma excretion to measure GFR) deserve mention. The former, supporting prior findings from South and Eastern Africa, confirmed that omitting coefficients for AA ethnicity with CKD-EPI(SCr) and 4-v MDRD equations improved accuracy. Together, these studies highlight the critical importance of validating eGFR equations in populations in which they are recommended for use [9, 12, 13, 15]. The Ivorian study goes a few steps further, where for the first time in West Africans, normal reference ranges for GFR are now available and the performance of the Full Age Spectrum for creatinine (FAScreat) equation was validated. Originally derived from northern Caucasian populations, the FAScreat equation incorporates adjustment for age and gender as a Q value. In the Ivorian study, using Q values derived for West Africans, the performance of FAScreat was better than CKE-EPI(SCr) and requires no adjustment for ethnicity [25, 46]. If the FAScreat equation is validated in other regions of SSA and proven to be superior to the currently recommended CKD-EPI(SCr) equation, this provides strong support for changing current eGFR equations recommended for use in KDIGO clinical practice guidelines [4]. More broadly, remembering that performance of CKD-EPI(SCr) has been questioned in Asia, it is incumbent on policy makers—including KDIGO, to prioritize use of validated population-appropriate eGFR equations particularly when relating to diagnosis of CKD—given the global health implications [46, 47].

The gold standard reference methods used for mGFR also require some reflection due to the potential biases that might arise from their use [49–51]. In Europe, iohexol is the preferred method while in the USA, iothalamate is most commonly used. Recently, it has been suggested that iohexol may be the most practical and accurate measure of GFR for a few reasons: it is stable at a wide range of temperatures and has a very good clinical safety profile; iohexol is more accurate than iothalamate (which can systematically overestimate GFR by 10–15% due to tubular secretion); there is very little difference between

Table 2. Studies from SSA with mGFR comparing performance of eGFR equations with/without coefficients for AA ethnicity

Study	Sample size	Self-reported ethnicity; HIV status	mGFR method	mGFR (mL/min/1.73 m^2)	eGFR equation	Bias with AA ethnicity coefficient % (95% CI)	Bias without AA ethnicity coefficient % (95% CI)	P30 with AA ethnicity coefficient % (95% CI)	P30 without AA ethnicity coefficient % (95% CI)
South Africa 2008 [9]	100	Black African; 20 HIV positive	^{51}Cr-EDTA	61.5	4-v MDRD[a]	27	5	52	74
South Africa 2012 [11]	148	Black African; HIV negative	^{99}mTc-DTPA	38	4-v MDRD[b]	Not reported	Not reported	50	55
Kenya 2013 [12]	99	Black African; HIV positive	Iohexol blood spot	115	4-v MDRD[b]	18	−3	73	83
					CKD-EPI(SCr)[c]	10	−4	82	85
South Africa 2016 [13]	100	Black African; HIV positive	^{51}Cr-EDTA	92.5	4-v MDRD[a]	38.4 (27.5; 49.3)	14.2 (5.2; 23.2)	43.3	59.8
					CKD-EPI(SCr)[c]	33.7 (25.0; 42.4)	15.3 (7.8; 22.8)	41.2	62.9
					CKD-EPI(SCrCys)[d]	11.5 (5.4; 17.6)	2.9 (−2.9; 8.8)	73.0	78.0
Ivory Coast 2018 [24]	237	Black African; HIV negative	Iohexol plasma excretion	103	CKD-EPI(SCr)[c]	NA	NA	NA	NA
					[e]FAScreat	NA	NA	NA	NA
Democratic Republic of the Congo 2018 [15]	93	Black African; HIV status not declared	Iohexol plasma excretion	92.0	4-v MDRD[a]	13.6 (8.0; 19.2)	−4.9 (−9.6; −0.2)	79.6 (71.2; 87.9)	86.0 (78.8; 94.0)
					CKD-EPI(SCr)[c]	17.2 (13.3; 21.2)	2.3 (−1.3; 5.8)	73.1 (63.9; 82.3)	81.7 (73.7; 89.7)
					CKD-EPI(SCrCys)[d]	9.0 (5.9; 12.0)	1.5 (−1.4; 4.4)	87.1 (80.2; 94.0)	92.5 (87.0; 97.9)
					CKD-EPI(Cys)[f]	1.5 (−1.8; 4.7)		91.4 (85.6; 97.2)	

[a]Re-expressed IDMS traceable 4-v MDRD equation.
[b]Original 4-vMDRD equation.
[c]CKD-EPI equation for serum creatinine.
[d]CKD-EPI equation for serum creatinine and serum cystatin C [52].
[e]Full Age Spectrum (FAS) creatinine equation; using Q values derived for age and gender [25, 46].
[f]CKD-EPI equation for serum cystatin C (no adjustment for AA ethnicity) [53].

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Table 3. Studies evaluating CKD in SSA ($n = 162$)

Number of studies[a,b] $n = 162$, n (%)	Parameter used to define CKD	Concordance with KDIGO definition of CKD: Creatinine-based eGFR equation	Urine protein or albumin	Confirmation of chronicity
4 (2.5)	Serum creatinine	☹	☹	☹
1 (0.6)	Serum creatinine + urine protein	☹	☺	☹
67 (41.3)	Serum creatinine-based eGFR	☺	☹	☹
8 (4.9)	Serum creatinine-based eGFR + follow up measurement at ≥3 months	☺	☹	☺
22 (13.6)	Serum creatinine-based eGFR + urine albumin	☺	☺	☹
38 (23.5)	Serum creatinine-based eGFR + urine protein	☺	☺	☹
1 (0.6)	Serum creatinine-based eGFR + urine albumin + urine protein	☺	☺	☹
12 (7.4)	Serum creatinine-based eGFR + urine protein + follow up measurement/s at ≥3 months	☺	☺	☺
3 (1.9)	Urine albumin	☹	☺	☹
5 (3.0)	Urine protein	☹	☺	☹
1 (0.6)	Urine protein + follow up measurement at ≥3 months	☹	☺	☺

[a]One study was excluded as the definition used for CKD was unclear.
[b]Percentages have been rounded to one decimal point and might not sum to 100%.

laboratory techniques for measuring iohexol, for example when comparing high-performance liquid chromatography with ultraviolet detection and liquid chromatography-tandem mass spectrometry, whereas differences have been demonstrated with iothalamate; and there is an external quality control program for iohexol to aid standardizing interlaboratory variation in measurements which does not exist for iothalamate [48]. It is critical to contextualize this for SSA, as the 4-v MDRD and CKD-EPI eGFR equations were all developed using iothalamate and the coefficient for AA ethnicity would further overestimate GFR. For the few studies in this review that compared mGFR with eGFR, the reference methods differed, but none used iothalamate and three used iohexol (two intravenously for measurement of plasma excretion and one with dried blood spots) [12, 15]. While iohexol blood spots may be relatively inferior to intravenous administration of iohexol, the use of dried blood spots was possibly the most pragmatic, on the assumption that access to laboratory support was unlikely in rural Kenya.

This systematic review highlights potential sources of bias inherent in the assessment of kidney function in clinical studies in SSA. This has created the opportunity to increase awareness of the requirements for laboratory-based creatinine assays, the appropriate choice of estimating equations for calculating GFR and the appropriate use of diagnostic criteria for CKD. In response, we propose an 'easy to use' checklist for researchers as a guide for the reporting of kidney function in SSA (Table 4). We hope this will minimize bias and strengthen future studies conducted in the region. In addition, inferring population prevalence of CKD in SSA from small convenience samples of individuals at high risk for developing CKD must be cautioned, as the risk of overestimating CKD is substantial.

While the focus of this systematic review has been clinical research, the implications of our findings have much broader application for the management of kidney disease in SSA. This encompasses individual patient care in the setting of acute and chronic kidney disease, home-based monitoring of CKD as a component of an integrated care model for chronic disease management, and public health policy for screening and prevention of CKD in SSA. None of this can be realized without affordable and accurate diagnostics for kidney disease. To achieve this, validated population-appropriate estimating equations for glomerular function need to be prioritized, as well as innovative approaches to accurate and affordable point of care diagnostics for assessing kidney function and diagnosing CKD.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

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Table 4. Recommendations for reporting kidney function in SSA populations: the African Research of Kidney Disease (ARK) checklist for researchers

mGFR (gold standard reference method)—method and biomarker (^{51}Cr-EDTA; ^{99}mTc-DTPA; inulin, iohexol, iothalamate)
- Urinary clearance of biomarker, state which biomarker; OR
- Plasma clearance of biomarker, state which biomarker

Laboratory creatinine method—include all the following:
- Enzymatic
- Jaffe (alkaline picrate): modified or compensated
- IDMS traceable to a standard reference material
- The external quality control program used by the laboratory for creatinine

Estimating equations for GFR—state which equation was used:
4-v MDRD equation
Original 4-v MDRD equation
$[\text{eGFR (mL/min/1.73 m}^2) = 186 \times \text{SCr}^{-1.154} \times \text{age (years)}^{-0.203} \times (0.742 \text{ if female}) \times (1.1212 \text{ if AA})]$ [53]
Use if laboratory method for creatinine was not IDMS-traceable
State whether the coefficient for AA ethnicity was used
Re-expressed[a] *4-v MDRD equation*
$[\text{eGFR (mL/min/1.73 m}^2) = 175 \times \text{SCr}^{-1.154} \times \text{age (years)}^{-0.203} \times (0.742 \text{ if female}) \times (1.1212 \text{ if AA})]$ [6]
Use if laboratory method for creatinine was IDMS-traceable
State whether the coefficient for AA ethnicity was used

CKD-EPI equation for creatinine
CKD-EPI equation
$[\text{eGFR (mL/min/1.73 m}^2) = 141 \times \min(\text{SCr}/\kappa, 1)^{\alpha} \times \max(\text{SCr}/\kappa, 1)^{-1.209} \times 0.993^{\text{age}} \times 1.018 \text{ (if female)} \times 1.159 \text{ (if black)}]$ [21]
Laboratory method for creatinine measurement must be IDMS-traceable
State whether the coefficient for AA ethnicity was used

Cockcroft–Gault equation
In its original form, this equation does not adjust for body surface area (BSA). To compare this equation to 4-v MDRD or CKD-EPI equations, which are adjusted for BSA, it is necessary to use the duBois formula and adjust for BSA [21]
Cockcroft–Gault equation
$[\text{eGFR (mL/min)} = [140 - \text{age (years)} \times \text{weight (kg)} \times (0.85 \text{ if female})]/\text{S-Cr}]$[54]
Cockcroft–Gault equation with BSA normalized to 1.73 m^2
$[\text{eGFR (mL/min/1.73 m}^2) = [140 - \text{age (years)} \times \text{weight (kg)} \times (0.85 \text{ if female}) \times 1.73 \text{ m}^2]/[\text{S-Cr} \times \text{BSA (m}^2)]]$

Full Age Spectrum (FAS) equation for creatinine
FAScrea = 107.3/(SCr/Qcrea) × {[0.988(Age − 40) when age >40 years]} [25]
Qcrea = derived from reference healthy population

Diagnosis of CKD using KDIGO criteria [4]—include the following:
True prevalence requires a randomized population-based sample: describe the sampling strategy
KDIGO Clinical Practice Guidelines (2012) are recommended for diagnosis of CKD and require testing for:
- Urine albumin/protein—if qualitative, confirm with quantitative test, preferably albumin:creatinine ratio **AND**
- Serum creatinine: use CKD-EPI equation[b] for calculation of eGFR
- In the absence of prior testing or additional supporting evidence[c] that confirms chronicity, demonstrate chronicity with a repeat of the abnormal diagnostic test after a minimum 12 weeks.

Recommendation: In SSA, for CKD-EPI equation—omit coefficient for AA ethnicity [9–13, 24]

[a]Re-expressed using an IDMS-traceable assay to a standard reference material.
[b]Use of 4-v MDRD and Cockcroft–Gault equations not recommended.
[c]Supporting evidence can be findings on renal ultrasound; and/or proof of pre-existing kidney disease from medical records or prior urine and serum test results.

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AUTHORS' CONTRIBUTIONS

J.A.G., M.v.D., H.R.E., R.K. and J.F. reviewed full-text articles and extracted the data. J.F. conducted the narrative analysis, wrote the first draft of the paper and incorporated revisions into the final draft. H.R.E. compiled recommendations for reporting of studies that assess kidney function. H.R.E., J.A.G., R.K., S.N., S.T., L.A.T., M.v.D. and A.N.W. contributed to revising the first draft, editing and review of the final draft of the article.

CONFLICT OF INTEREST STATEMENT

None declared. The results presented in this article have not been published previously in whole or part, except in abstract format.

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3.0 Problem statement, aim, hypothesis, objectives and methods

3.0 Problem statement, aim, hypothesis, objectives and methods

3.1 Introduction

In this chapter I summarize the Statement of the problem, hypothesis, rationale, aim, and methods for the studies in the PhD thesis. The methods paper which was published outlines the details of how we conducted the studies in the three countries (Malawi, Uganda and South Africa) involved in the African Research on Kidney Disease (ARK) study. I have however included an additional section focused on the estimation of GFR from iohexol sampling that was not included in the methods paper. I also use this chapter to explain in detail how data were cleaned up and how errors were minimized as well as the derivation of some of the tables that where eventually published. Most of this additional detailed explanation was not included in the published methods paper. Please also find the approvals for the study attached in the appendix at the end of the chapter.

3.2 Problem Statement

One in ten people have kidney disease worldwide. The worst stage of kidney disease is end stage kidney disease (ESKD) and up to 70% of individuals affected reside in low-income countries such as those in sSA [1]. Proper management of kidney disease requires accurate diagnosis and staging of kidney function. However, all the recommended equations for estimating kidney function based on creatinine and cystatin C originated from high income countries with limited validation in sSA. Moreover, creatinine (the most used marker of kidney function) varies with age, sex, muscle mass, physical activity and possibly ethnicity. This creates a challenge in determining the current burden of kidney disease in this part of the world. There remains a paucity of data describing the current prevalence, risk factors and the best way to estimate glomerular filtration rate (eGFR) among people from sSA. This has impeded the development of appropriate prevention and treatment strategies.

3.0 Problem statement, aim, hypothesis, objectives and methods

3.3 Study Rationale

While methods to measure kidney function are well established in Western populations, there are good reasons to believe these methods may not translate well to sSA, meaning the overall burden of CKD is uncertain, and making it difficult to identify individuals who warrant interventions.

In addition, the published prevalence of kidney disease varies widely across sSA. This is largely attributed to the population under study as well as the way in which kidney disease is defined. There are multiple equations used to determine kidney function across the world, but these have not been well validated in sSA. There are several imprecisions and estimation errors that arise out of the markers used to measure kidney function as well as the ethnicity coefficients that have been used in an attempt to 'correct' for the black race. Since most of these equations were developed in high income countries, their performance in sSA needs to be verified. Therefore, we designed the study to determine the population prevalence of impaired kidney function using creatinine, elucidate on how to measure GFR on a large sample of our cohort and to generate information on the mortality related to kidney function in sub-Saharan Africa. The information generated was planned to guide further use of ethnicity factors in sSA as well as provide valuable insight into the best equations to use when determining GFR among people of sSA.

3.4 Thesis aim

The aim of this thesis was to determine the prevalence and mortality associated with impaired renal function in the general population and assess how to best measure renal function among adults in sub-Saharan Africa (sSA).

3.0 Problem statement, aim, hypothesis, objectives and methods

3.4.1 Research Hypothesis

We hypothesize that there will be substantial imprecision in currently used equations for estimating GFR within the sub-Saharan population.

3.4.2 Thesis objectives

Objective one: To determine the estimated prevalence of, and factors associated with, impaired renal function within the general population cohort (GPC) in Uganda.

Objective two: To determine the association between kidney function and all-cause mortality in the GPC in Uganda.

Objective three: To assess the most accurate way to determine kidney function in sub-Saharan Africa (Uganda, Malawi and South Africa).

3.5 Research Paper 1: Methods paper

The methods paper published in BMC Nephrology gives details on the three cohorts from Malawi, South Africa and Uganda from which the studies were conducted. I explain in detail the study settings, how each site selects participants, calculation of sample size and plans for statistical analysis as well as ethical approvals.

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1604453/REPH	**Title**	Dr.
First Name(s)	Robert		
Surname/Family Name	Kalyesubula		
Thesis Title	Characterization of kidney disease in sub-Saharan Africa		
Primary Supervisor	Dr Laurie Tomlinson		

If the Research Paper has previously been published, please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published? When was the work published? If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	BMC Nephrology January 2020 N/A
Have you retained the copyright for the work?* Was the work subject to academic peer review?	No Yes

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Where is the work intended to be published? Please list the paper's authors in the intended authorship order:	

Stage of publication	

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I collected the data, participated in data analysis, drafted the first manuscript, submitted the manuscript as first author and did all correspondence with the reviewers and the editors.

SECTION E

Student Signature	[redacted]
Date	28 June 2021

Supervisor Signature	[redacted]
Date	28 June 2021

Research paper 1: How to estimate glomerular filtration rate in sub-Saharan Africa: Designs and methods of the African Research into Kidney Diseases (ARK) study.
https://bmcnephrol.biomedcentral.com/track/pdf/10.1186/s12882-020-1688-0.pdf

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BMC Nephrology

STUDY PROTOCOL Open Access

How to estimate glomerular filtration rate in sub-Saharan Africa: design and methods of the African Research into Kidney Diseases (ARK) study

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Abstract

Background: Chronic kidney disease (CKD) is a substantial cause of morbidity and mortality worldwide with disproportionate effects in sub-Saharan Africa (SSA). The optimal methods to estimate glomerular filtration rate (GFR) and therefore to determine the presence of CKD in SSA are uncertain. We plan to measure iohexol excretion to accurately determine GFR in Malawi, South Africa and Uganda. We will then assess the performance of existing equations to estimate GFR and determine whether a modified equation can better improve estimation of GFR in sub-Saharan Africa.

Methods: The African Research on Kidney Disease (ARK) study is a three-country study embedded within existing cohorts. We seek to enrol 3000 adults > 18 years based on baseline serum creatinine. Study procedures include questionnaires on socio-demographics and established risk factors for kidney disease along with anthropometry, body composition, blood pressure, blood chemistry and urine microscopy and albuminuria. We will measure GFR (mGFR) by plasma clearance of iohexol at 120, 180 and 240 min. We will compare eGFR determined by established equations with mGFR using Bland-Altman plots. We will use regression methods to estimate GFR and compare the newly derived model with existing equations.

Discussion: Through the ARK study, we aim to establish the optimal approach to estimate GFR in SSA. The study has the advantage of drawing participants from three countries, which will increase the applicability of the findings across the region. It is also embedded within established cohorts that have longitudinal information and serial measures that can be used to characterize kidney disease over a period of time. This will help to overcome the limitations of previous research, including small numbers, selected population sub-groups, and lack of data on proteinuria.
The ARK collaboration provides an opportunity for close working partnerships across different centres, using standardized protocols and measurements, and shared bio-repositories. We plan to build on the collaboration for this study for future work on kidney disease in sub-Saharan Africa, and welcome additional partners from across the continent.

Keywords: Measurement, Glomerular filtration rate, Chronic kidney disease, Sub-Saharan Africa

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3.0 Problem statement, aim, hypothesis, objectives and methods

Background

Chronic kidney disease (CKD) is a substantial cause of morbidity and mortality worldwide with an estimated prevalence of 8–16% [1]. A recent systematic review estimated the prevalence of CKD in sub-Saharan Africa to be around 14% [2]. However, the optimal equation to accurately estimate glomerular filtration rate (GFR) among sub-Saharan African populations is uncertain. This uncertainty is a major barrier to identifying individuals with CKD and to estimation of disease burden.

Worldwide, several equations have been used to estimate GFR from serum creatinine [3–7]. Unfortunately, most of these have been derived from high-income countries with minimal validation in sub-Saharan African populations. Their accuracy at an individual level is limited, particularly in relation to the adjustments for African-American ethnicity, which might be related to variations in muscle mass. Serum creatinine levels at a given level of renal function vary substantially with ethnicity, age, sex, physical activity and nutritional status [8–14]. Several sources of inaccuracy in estimating GFR have been described, including biological variability in serum creatinine, laboratory induced errors in creatinine measurement and choice of estimating equation [15, 16]. The imprecision of creatinine measurement is more marked at values near the normal range where it is most critical to determine earlier stages of CKD [17–19]. Several methods for direct measurement of GFR are available through measurement of clearance of inulin, iothalamate, iohexol, and radio-active agents such as technetium-99 m diethylenetriamine penta-acetic acid (DTPA) and chromium-51 ethylene diamine tetra-acetic acid (EDTA), and 24-h urinary creatinine collection for estimation of creatinine clearance [20]. However, all of these have their challenges. DTPA, EDTA and inulin are expensive and not readily available in most African countries, while 24-h urinary collection is often inaccurate due to difficulty in ensuring a complete sample, coupled with the additional limitation of tubular creatinine secretion [20–22].

Iohexol is a readily available compound, which can be used to measure the GFR. Its advantages include low cost, excellent safety profile, low protein binding, low levels of toxicity at the dose used for measuring GFR, stability at room temperature (20 to 25 °C), and being able to provide an accurate measure of glomerular filtration [20, 23]. Previous studies using iohexol to measure GFR in sub-Saharan Africa have included few people with CKD [13, 24, 25]. The largest study to date conducted in the Democratic Republic of Congo and Ivory Coast used plasma iohexol to measure GFR in 494 participants. They noted that the African-American race coefficient did not improve the performance of creatinine-based GFR estimates of iohexol GFR. In particular, the Chronic Kidney Disease Epidemiology (CKD-EPI) and Modification of Diet in Renal Disease (MDRD) equations performed better without the race coefficient in participants with GFR ≥60 mL/min/1.73m^2. The authors also evaluated the Full Age Spectrum (FAS) equation and found it to be as accurate as CKD-EPI for GFR ≥60 mL/min/1.73m^2 but better for those with creatinine based GFR < 60 mL/min/1.73m^2. Addition of cystatin C did not improve performance of the equations among this study group [25].

In the proposed study, we will use iohexol excretion in a population sample of 3000 participants with and without CKD to measure GFR and determine the optimal equation to estimate GFR in Uganda, Malawi and South Africa.

Methods

Primary objectives

1. To measure GFR using plasma clearance of iohexol
2. To assess the performance of existing eGFR equations, namely Cockcroft -Gault (CG), Modification of Diet in Renal Disease (4-v MDRD) and Chronic Kidney Disease-Epidemiology collaboration (CKD-EPI) by comparing these to measured GFR, among sub-Saharan Africans.

Secondary objectives

In a Ugandan sub-sample, to assess the feasibility of using dry blood spot collection of iohexol in the determination of GFR.

In a Ugandan sub-sample to explore the current community understanding of kidney disease and available treatments from traditional healers and herbalists.

Study organization and sites

We will have three participant countries across SSA (Fig. 1) and these centres have formed a new collaborative group, African Research on Kidney Disease (ARK) around this initial study.

A working committee will review the protocols for the design, conduct, progress and data collection and analysis plan. The committee will meet in person on several occasions to review protocols and measurement tools and to agree on a minimum dataset. The three centres have unique characteristics that will enrich the study with diversity among participants. The details of each of the different cohorts in which the current study will be nested are described below:

Uganda

In Uganda, the study will be based in the General Population Cohort (GPC), originally established in 1989 [26]. The study area is located in rural south-western Uganda

3.0 Problem statement, aim, hypothesis, objectives and methods

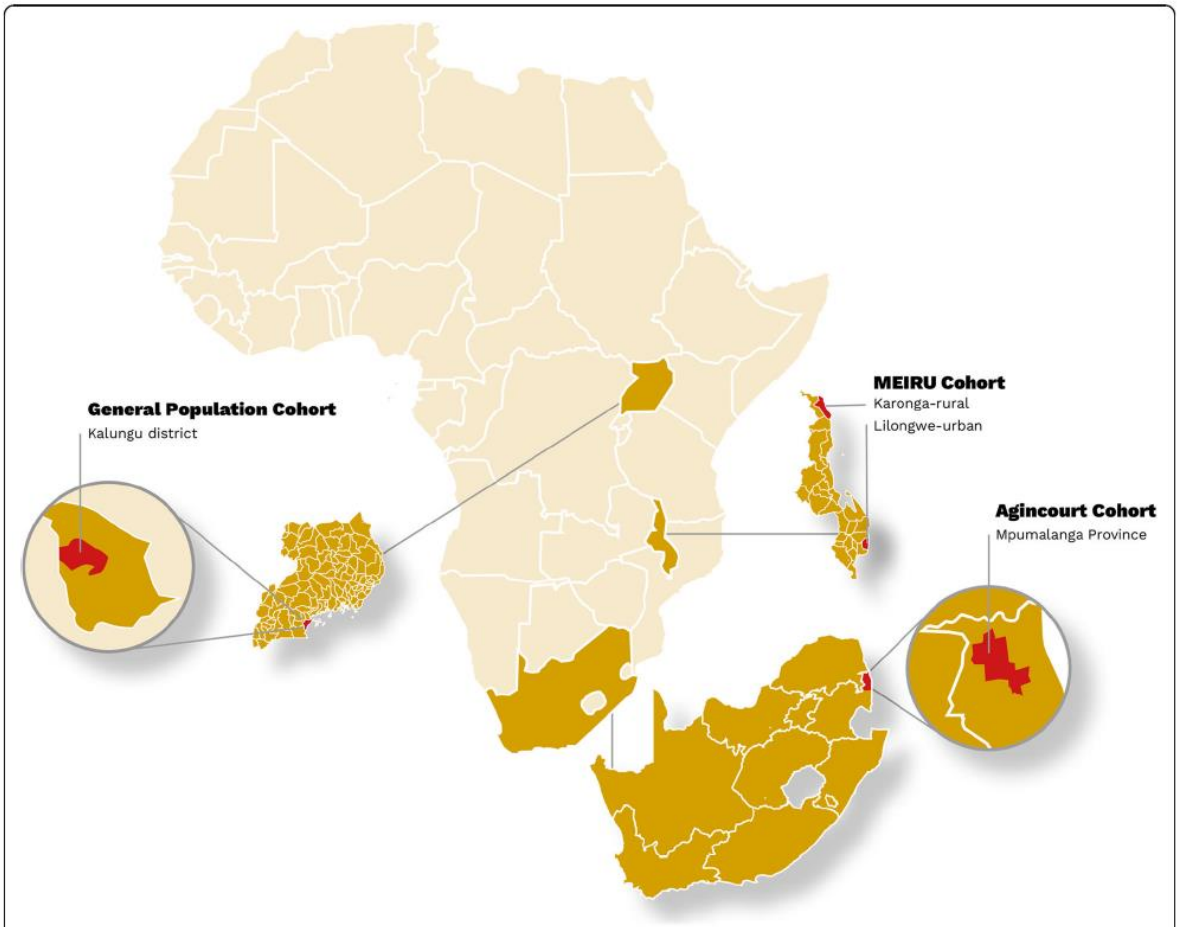

Fig. 1 Map of Africa showing the ARK centres from Malawi, South Africa and Uganda. The general population cohort is run by the Medical Research Council-Uganda virus Institute and the London School of Hygiene and tropical Medicine (MRC-UVRI-LSHTM, Uganda). The Malawi Epidemiology and Intervention Research Unit (MEIRU) runs the MEIRU cohort in Malawi and the Medical Research Council/Wits University Rural Public Health and Health Transitions Research Unit runs the Agincourt Unit in South Africa. Map proposed by Robert Kalyesubula and illustrated by Helmut Kraus

in one sub-county of Kalungu district, approximately 120 km from Entebbe town. The cohort comprises all residents of 25 adjacent villages, approximately 22,000 people with men and women in equal proportions, and 52% older than 13 years. The study population for the general population cohort is recruited through annual house-to-house rounds of the census where study participants are selected. Medical care is available through an established general clinic.

Malawi

The Malawi Epidemiology and Intervention Research Unit (MEIRU) conducts ongoing epidemiological studies based at two sites, which will be used for recruiting participants for this study. The first is Karonga District in rural northern Malawi, predominantly subsistence farmers, fishermen and informal traders. The second site is Area 25 of Lilongwe, the capital city of Malawi. The urban area is socio-economically mixed and includes government and industry employees, traders and those in casual employment. We will enrol participants from both sites, providing a true population-wide sample and enabling urban: rural comparisons.

The stratified study sample was drawn from a pool of over 5000 individuals who were tested for serum creatinine as part of a larger survey (n = 33,000) of chronic conditions. Medical care is available through an established chronic care Non-Communicable Disease (NCD) clinic.

South Africa

In South Africa, the study will be located within the Medical Research Council/University of the Witwatersrand

Rural Public Health and Health Transitions Research Unit in Agincourt, Bushbuckridge sub-district of Mpumalanga province. The area includes a high-functioning health and socio-demographic surveillance system (HDSS) which covers approximately 115,000 people based on an annual update of resident status and vital events. The study setting also serves as a national pilot site for the development of integrated chronic disease care. The population still has gaps in access to electricity, water and tarred roads and unemployment rates are high, leading to high rates of labour migration. Patients will be managed through the primary health care system consisting of six clinics, two health centres and three district hospitals. The HDSS has a central clinic with advanced laboratory capacity, which will be used for this study.

Study design

This will consist of cross-sectional studies embedded within ongoing cohorts in Malawi and Uganda and a longitudinal study in South Africa, all with serial biobanking.

Data collection, sample size and selection of sample

We intend to recruit 3000 participants in total. We have based the power calculation is based on the number of study participants needed to examine the accuracy of GFR estimating equations for 'true' GFR, at each of the three study sites separately. We have specified this as having 90% power to detect whether eGFR is within 5% of the iohexol value at an eGFR of 60mls/min, assuming a standard deviation of 25mls/min [9] and with a two-sided alpha of 0.05. This gives an estimated required sample size of 730 participants. Allowing for participants who do not wish to participate in this element of the study, and technical failures, we intend to recruit 1000 participants in each country.

In order to fulfil the aims of the study, a structured approach is needed. This will differ between South Africa and the other two sites (Malawi and Uganda).

Details of site recruitment

We plan to recruit from each of the sites guided by the previous recruitment protocols. Each of the sites will however, have two phases of recruitment.

For the Uganda GPC, the first phase will consist of measurement of creatinine from stored sera.

Participant selection for this part of the study has been described elsewhere [27]. Briefly, participants were selected from the general population cohort, which is a community-based open cohort study of residents of 25 neighbouring villages. The participants are selected through house-to-house mobilisation and community surveys conducted through village-based hubs. For Uganda, 5979 individuals were identified for baseline creatinine measurement, which included all the adults surveyed in that round. We will use creatinine levels from the stored samples to stratify participants for the second phase of the study. Here, we will select participants according to eGFR cut offs of normal (>90mls/min/1.73m^2); impaired renal function (60-90mls/min/1.73m$^{2)}$ and low GFR (<60mls/min/1.73m^2), all additionally stratified by age and sex. Selected participants will be approached by the community engagement lead (community mobilizer) for each village. Once participants assemble, the study team will take time to explain the details of the study to them, after which individual informed consent will be obtained. Participants will undertake a questionnaire to collect demographic data as well as history of known risk factors for kidney disease. Participants will also undergo detailed biophysical measurements and blood draws as detailed in the next sections. We will give participants an appointment to attend the medical clinic at the central Research Station, where iohexol measurements and other study tests will be performed.

For Malawi, for the first phase of the study, we will use creatinine assays from a previous survey to guide the selection of participants for the second phase of the study using a similar approach to that of Uganda. However, we will recruit participants 18 years and above from Malawi through household visits where similar procedures to those outlined above will be undertaken. We will carry out iohexol testing and other physical tests at a research clinic (phase 2). Details of participant recruitment have been detailed elsewhere [28].

In South Africa, we will determine baseline creatinine and the prevalence of CKD using an age and gender stratified random sample of 2250 members of the rural Agincourt HDSS. The number of participants sampled in each stratum will be determined in order to ensure proportional allocation, based on the population demographic distribution. We will visit participants in their homes for the first phase of the study where the questionnaires, biophysical measurements and take blood draws to determine Human immunodeficiency virus (HIV) status, blood sugar level, lipid profile as well as the baseline creatinine level. We will repeat creatinine and urine albumin measures after a minimum period of 3 months to confirm CKD. For the second phase of the study, we will recruit participants from Phase 1 through household visits and give them a referral date to the clinic for iohexol measurement along with other tests (See Table 1 and Fig. 2 for details).

Inclusion criteria

- Adults aged 18 years and above from the three population cohorts

3.0 Problem statement, aim, hypothesis, objectives and methods

Table 1 Clinical and Laboratory measurements within the ARK study, by country

Type of information	Equipment	Field procedures	Participant type	Definition of variable	Site participation		
					Malawi	S. Africa	Uganda
Demographics	Questionnaire	Face-to-face interview	all	Sex, age, education status, marital status	x	x	x
Chronic disease history and family history	Questionnaire / medical records	Face-to-face interview	all	HTN, DM, CKD, Stroke, heart disease	x	x	x
Treatment history	Patient report/ records	Face-to-face interview	all	HTN, DM, CKD, Stroke, HIV,TB, Heart disease, Cancer, Backache	x	x	x
Health behaviours	Patient report	Face-to-face interview	all	Tobacco use, alcohol consumption, Physical activity, vegetable, fruits, salt and water intake	x		x
Traditional medicine use	Questionnaire	Face-to-face interview	all	Use of herbs and traditional medicines	x	x	x
Physical examination	Omron® SECA® Flexible tape SECA© scales	Clinical Examination in the field, machines calibrated weekly	all	BP (mmHg), waist and hip circumferences (cms), height (m), weight (Kgs)	x	x	x
ABPM	ABPM spacelabs®	24 h BP, 30 min interval day, 1 h interval night	Sample per eGFR quarter	BP wake periods and sleep periods	x		x
BIA	Bodystat®	Tested in the field or clinic with participant, calibration weekly	all	Fat mass (kg), Lean mass (kg), Dry lean mass(kg), Total body water (L), Impedance at 50 KHZ (Ω)	x	x	x
DXA SCAN	Hologic Discovery A. QDR 4500 Series	Whole body scan performed in clinical research clinic during iohexol testing	all	Lean Mass (g), Fat mass (g) Fat % and BMI		x	
Ultra sound scan	Logiq e	Performed at clinic	all	Probe 4c for kidneys and bladder and probe12L CIMT probe		x	
ECG	ECG 300A®/ MAC600®	ECG protocol followed	all	LVH using the Sokolow-Lyon criteria	x	x	x
CBC	BC®	Venous blood draw	all	Total cell count, HB, MCV	x		x
24 HR URINE	Cobas Roche®	urine using small bucket.	Select patients for 24 h proteinuria, salt and feasibility.	Volume (mls) Protein (mg) Creatinine (mmol/L) Na^+ mmol/L			x
Malaria screen	Malaria RDT®	Done in the community	all	Malaria infection	x		x
CRP	Cobas® & BC®	Blood draw	all	CRP	x		x
Hepatitis B	Cobas® ABBOTT (i1000SR)	Blood draw	all	HepB SAg	x	x	x
Hepatitis C	Cobas® (Uganda), HCV antibody rapid test (Malawi)	Blood draw	all	HepC Ab	x		x
ASOT	Cobas® & BC®	Blood draw	All	> 300			x
HIV screen	Alere Dertemine-Stat-pak-Bio line & Abbott Determine	MOH serial testing algorithm	all	HIV status	x	x	x
Schistosomiasis	Microscopy	Examination of centrifuged urine	all	Schistosoma hematobium	x	x	x
Microalbuminuria	CLINITEK® + analyser & Cobas® & BC®	ACR	all	Urine ACR	x	x	x
Urine analysis	Clinitek® & UroColor	Early morning urine	all	Protein, blood, glucose, WBCs, QuickVue Hcg Urine for pregnancy	x	x	x
Lipid profile	Cobas® & BC®	Blood draw	all	Cholesterol, LDL,	x		x

3.0 Problem statement, aim, hypothesis, objectives and methods

Table 1 Clinical and Laboratory measurements within the ARK study, by country *(Continued)*

Type of information	Equipment	Field procedures	Participant type	Definition of variable	Site participation		
					Malawi	S. Africa	Uganda
				HDL, TGs			
RBS	BC®	Point of care testing (Uganda $ SA)	all	Diabetes > 11.1 or on medical treatment	x	x	x
HBA1C	Cobas® & BC®	Blood draw	All	6.5% or on treatment	x		x
Creatinine	Cobas® & BC®	Jaffe and IDMS	all	For eGFR estimation	x	x	x
Cystatin-C	Cobas®		all	For eGFR estimation	x	x	x
Iohexol	Omnipaque 300 mg I/ml Healthcare® HPLC®	Clinic based blood draws at 5, 120, 180 and 240 min after administration	all	Measured GFR	x	x	x
Iohexol, DBS	DBS®	DBS by finger prick at 120 and 240 min	300 selected by eGFR	Validation of measured GFR technique			x
Aldosterone/Renin	Cobas Roche®		Selected population		x		x

All biochemistry was tested using Cobas equipment in Uganda and South Africa and Beckman Coulter equipment in Malawi
Abbreviations: *ABPM* Ambulatory Blood Pressure Monitor, *ACR* Albumin Creatinine Ratio, *ASOT* Antistreptococcal antibody titres, *BC®* Beckman Coulter®, *BIA* Bioimpedance Analysis, *BP* Blood Pressure, *CBC* Cell blood count, *CKD* Chronic Kidney Disease, *CRP* C-Reactive protein, *CIMT* Carotid intima-medial thickness, *DBS* Dry blood sample, *DM* Diabetes Mellitus, *ECG* Electrocardiography, *eGFR* Estimated glomerular filtration rate, *HB* Haemoglobin, *HTN* Hypertension, *HPLC* High liquid pressure chromatography, *IDMS* Isotope dilution mass spectrometry, *LVH* Left ventricular hypertrophy, *MCV* Mean corpuscular volume, *MOH* Ministry of Health, *RBS* Random blood sugar, *SA* South Africa, *TB* Tuberculosis

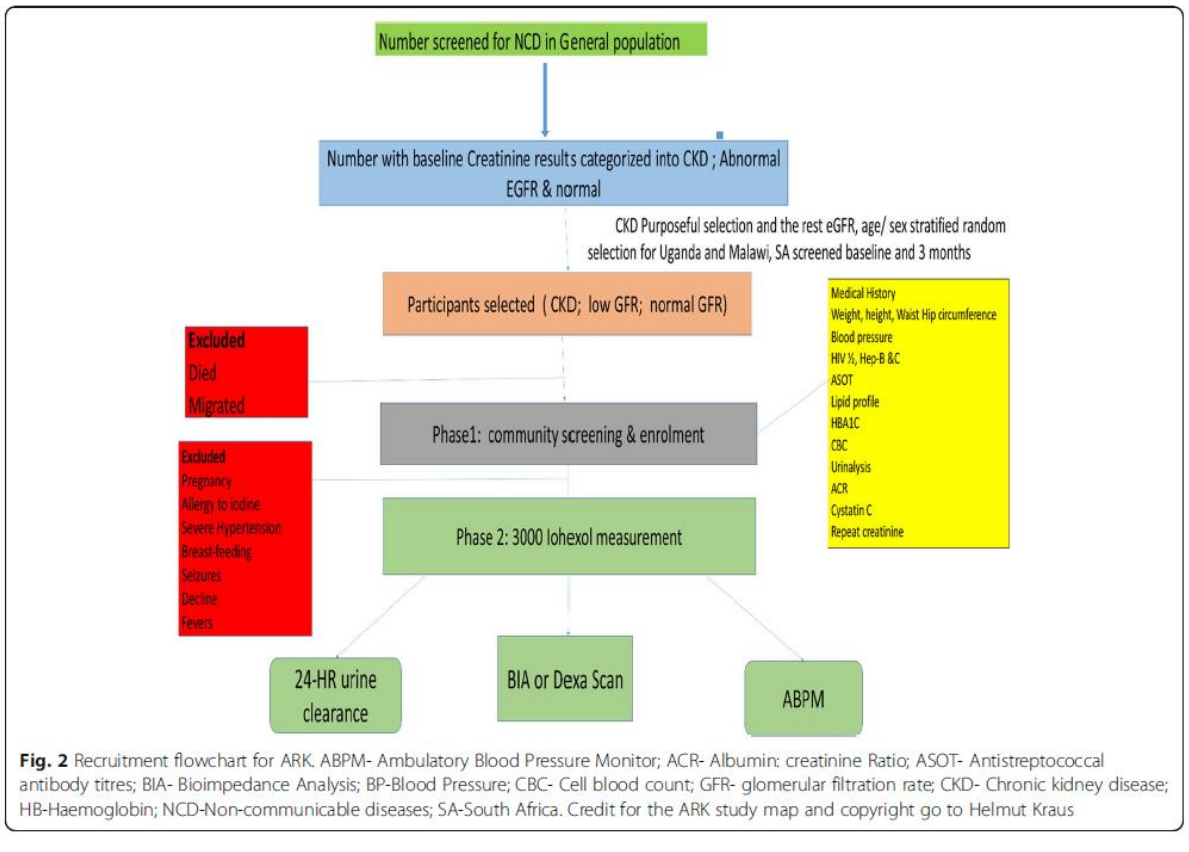

Fig. 2 Recruitment flowchart for ARK. ABPM- Ambulatory Blood Pressure Monitor; ACR- Albumin: creatinine Ratio; ASOT- Antistreptococcal antibody titres; BIA- Bioimpedance Analysis; BP-Blood Pressure; CBC- Cell blood count; GFR- glomerular filtration rate; CKD- Chronic kidney disease; HB-Haemoglobin; NCD-Non-communicable diseases; SA-South Africa. Credit for the ARK study map and copyright go to Helmut Kraus

- Able to give informed consent

Exclusion criteria

- Blood pressure > 180/110mmhg
- Pregnancy
- Breast-feeding mothers
- Known allergy to iodine–containing substances
- Uncontrolled seizures (defined as a seizure within the last 12 months).
- Acute febrile illness

Demographic factors

We will collect data on demographic factors including age, sex, place of residence, education, occupation and livelihood, tobacco use, alcohol use, dietary history, physical activity. We will also take a medical history and treatment for chronic diseases including HIV, diabetes mellitus, hypertension, heart disease and CKD as well as previous and current use of traditional medicine and drugs.

Physical examination

We will measure height, weight, waist circumference and hip circumference and calculate the body mass index (BMI) and the waist hip ratio accordingly. We will classify BMI according to WHO categories (weight/height^2: kg/m^2): underweight (< 18.5 kg/m^2), normal weight (18.5–24.99 kg/m^2), overweight (25.0–29.99 kg/m^2) and obese (> 30.0 kg/m^2).

We will undertake cardiovascular assessment through blood pressure and 24-h ambulatory blood pressure (BP) measurements (ABPM) on a sub-sample of participants (Malawi and Uganda), and electrocardiography (ECG). We will measure BP using Omron® M6 (for small, medium and large participants) and Omron HBP 110 machines (for obese participants). We will measure BP in triplicate after at least 5 min of rest and take the mean of the last two readings as the true blood pressure. We will derive BP classification from the National Institute of Health guidelines: where participants with a systolic BP greater than 120 mmHg but less than 140 mmHg, and/or a diastolic BP greater than 80 mmHg but less than 90 mmHg will be classified as pre-hypertensive. We defined hypertension as having a diastolic BP greater than or equal to 90 mmHg, systolic BP greater than or equal to 140 mmHg or being on treatment for high blood pressure. 24-h ambulatory blood pressure will be undertaken on a selected number of participants with no hypertension, pre-hypertension and hypertension across the spectrum of eGFR ranges and will capture wake and sleep periods. We will use the ECG for assessment of LVH using the Sokolow-Lyon criteria [29].

We will perform bioimpedance analysis (BIA) using the Bodystat® machine to measure body fat in relation to lean body mass for parameters outlined in Table 1. We will also perform a Dual-energy X-ray absorptiometry (DXA) scan for body composition in South Africa and use it to examine validity of the BIA measurements across countries.

Laboratory investigations

The key laboratory measurement for this study will be iohexol (Omnipaque 300 and 350 mg I/mL, GE Healthcare) clearance. The iohexol measured GFR will serve as the gold standard for comparison with other methods.

Using aseptic technique study nurses will insert an IV line into the non-dominant arm of the participant and use the line for subsequent blood draws. We will administer a single dose of 5 ml of iohexol over 30 s through an intravenous cannula inserted in the dominant arm contralateral to the established IV line, followed by a 10 ml normal saline flush. We will weigh the iohexol dose in grams to a specificity of 0.01 g by weighing the syringe before and after administration of the iohexol. The research nurses will draw four milliliters of blood for the iohexol assay again at approximately 5, 120, 180 and 240 min after injection of iohexol, and record the exact time. We will calculate the GFR using a single compartment model based on iohexol clearance between 120 and 240 min.

In Uganda at the time points of 120 and 240 min, we will also collect a capillary sample of dried blood spots to compare to intravenous sampling of iohexol GFR as a modification from previous studies [11, 30]. Should this method prove to be accurate, it will greatly facilitate further studies in resource-limited settings. In order to avoid inter-laboratory variations, all plasma and blood spot samples of iohexol will be measured at the National Health Laboratory, Johannesburg, South Africa by ultra-pressure liquid chromatography-tandem mass spectrometry with identification and quantitation based on MRM transitions. The laboratory is accredited for the International Organization for Standardization (ISO) standard 15,189 and also participates in the internal quality control run at a high and a low level every 20 samples. The laboratory has participated in Equalis external control since 2017.

We will measure creatinine using the enzymatic method in Uganda and Jaffe for South Africa and Malawi standardized against an isotope dilution mass spectrometry (IDMS) method across all sites. We will adopt the recommended estimation and reporting format or measured renal function proposed by Fabian et al [31]. We will measure serum cystatin C to determine the accuracy of the cystatin-based CKD-EPI equations. We will measure Cystatin C using the standardized Roche Gen2 assay, which has been standardized against certified reference material (ERM-DA471/IFCC). To minimise batch variation, samples will be

analysed at the end of the study at one central laboratory at MRC/UCRI&LSHTM for Uganda and Malawi while the samples in South Africa will be analysed at the National Health Laboratory, Johannesburg, South Africa. To understand laboratory differences and enable calibration we will cross-validate and measure 50-paired samples for creatinine in Uganda, Malawi and South Africa, and cystatin between South Africa and Uganda. Additional tests include full blood count (including haemoglobin level), tests for screening of infections (malaria, streptococcal infections, Hepatitis B, Hepatitis C and HIV Infection), C-reactive protein, random blood glucose, HbA1c and the fasting lipid profile as markers of metabolic risk.

We will perform four urine tests. We will take an early morning spot urine sample for quantitative urine albumin: creatinine ratio, an early morning urine dipstick analysis to qualitatively assess haematuria, leucocyturia and proteinuria, and microscopy will be performed on the centrifuged urine to screen for urinary schistosomiasis within 1–2 h of collection. We will take a 24-h urine collection for urinary albumin excretion, sodium and 24-h creatinine clearance in a sample of 300 participants from Uganda during the second phase of the study.

Details of the equipment used for the measurements are outlined in Table 1 below.

Qualitative sub-study in Uganda

The qualitative sub-study in Uganda will have two components: a study of people with abnormal renal function, and an enquiry into medicinal product supply and use sourced from traditional healers and herbalists.

This sub-study of people with abnormal renal function will include up to 50 participants found to have CKD during the recruitment of the 1000 participants for the overarching study. In order to recruit a comparator group for the 50 CKD participants, a further 50 participants without CKD of a similar age living in a neighboring house to each case will be invited to take part. Data from both cases and 'controls' will be collected through two in-depth interviews. These selections were made on an estimated sample to reach saturation.

For the sub-study with traditional healers/herbalists, we will approach ten traditional healers and herbalists and invite them to participate in one or more interviews to discuss the common ailments they treat and the types of herbal and other preparations that they use in their practice.

Bio-banking

Serum, plasma and urine samples will be collected, processed and frozen and transported to the central laboratory for additional tests that cannot be conducted at the originating laboratory. All three centers have quality assurance systems for processing, sample transportation and sample storage management systems [26, 32, 33]. All sites have received ethical approval for testing of specimens for genetic studies and exploration of new markers of CKD.

Data management

Data management will follow local established procedures ensuring quality, confidentiality and proper use of abnormal results to guide patient care when needed. Electronic data capture allows automatic data checks such as double entry of numbers and range checks. We will enter anonymized data into compatible statistical databases from centres in all three countries to for sharing between all investigators.

Quality assurance

All three centres have quality assurance procedures that support the studies at different levels. Study staff are trained and certified according to national and international guidelines governing research. The ARK team holds regular meetings to ensure that study protocols and research standards are adhered to. We will follow standard sample storage protocols for the biorepository.

Statistical and qualitative analysis plan

We will tabulate baseline characteristics stratified by country. We will perform initial analyses in a sample of 1500 participants selected to represent all centres, ages, sex, and eGFR categories for modification of the equation, and allow for subsequent validation using the remaining data. We plan to recombine the training and test sets for fitting a final model. We are also open to alternatives to data-splitting such as re-sampling techniques (cross-validation and bootstrapping), that would allow us to develop the model on the whole dataset and still validate predictive accuracy. Evidence from previous derivation of eGFR equations suggests that errors are multiplicative, so we will do analysis on the log (ioxehol-GFR) scale. To determine the measured GFR (iohexol-GFR); we will calculate the slope from the 3 samples at 120, 180 and 240 min points using the exact time of collection turn these into a GFR normalised to BSA using standard methods [13, 34]. We will also put in the R-value of the fit, which will be used to exclude ones where the fit is particularly poor. In our primary analysis we will evaluate the performance of the CockCroft Gault, 4v-MDRD, CKD-EPIcr, CKD-EPIcyst, CKD-EPIcr/cyst equations with and without ethnic correction factor by calculating the bias, precision and accuracy at 10% (P10) and 30% (P30) compared to iohexol GFR as done by previous studies [13]. Derivation of GFR estimating equations is rapidly developing and we will also consider evaluation against newly developed methods including the full age spectrum (FAS) and the Lund-

Malmo GFR estimating equations [35, 36]. We will assess the performance of the different equations for eGFRs with and without ethnic factors using Lin's Concordance Correlation Coefficient and Spearman correlation coefficients to evaluate the degree to which pairs of observations fall on the 45° line through the origin. We will calculate bias and relative bias. We will evaluate precision by the standard deviation of the bias (random error) and root-mean-square error. We will test the difference in P30 accuracy between eGFRs with the exact McNemar test.

We will use Bland-Altman plots to investigate the measurement error of existing equations of log(MDRD-eGFR), log(CKD-EPI-eGFR), log(CG-eGFR) and log(cystatin C-eGFR) compared to log(ioxehol-GFR). If needed, to develop modified correction factors for the setting, we will carry out regressions to predict log(ioxehol-GFR) as a function of age, gender, and creatinine/cystatin C. Predicted eGFRs will subsequently be compared with measured GFRs in the validation sample. We will investigate what happens if information on weight, height, and bioimpedance measures are added to the regression model and use computed R^2 values to investigate whether anthropometry adds relevant information over and above existing equations.

All statistical analyses will be performed using STATA 15 SE (Stata Corp, Texas, USA).

For the qualitative study, we will record interviews with permission from the participant and transcribe and translate them. We will revise the interview guidance after the initial interviews in order to collect greater depth of data on emerging themes. We will review transcripts for accuracy and enter these into Dedoose, qualitative data analysis software. We will conduct data analysis using an iterative coding process, during the interview period. After data collection, we will perform open coding, and move to more refined codes, subthemes, and then themes. We will analyze themes and code them using a constant comparison method. We will apply the codes to interview transcripts and summary statements with representative quotes developed for each theme.

Ethical considerations

We have obtained ethical approvals from the Uganda Virus Research Institute, Research Ethics Committee (UVRI-REC-#HS 1978) the Uganda National Council for Science and Technology (UNCST-#SS 4283), from the Malawi National Health Sciences Research Committee (#1072) and the University of Witwatersrand Human Research Ethics Committee (#M160938).

We will obtain written informed consent from participants for the collection and analysis of genetic samples as well as iohexol testing and for the use of their clinical records for research purposes. We will seek approval for all study procedures including material transfer agreements from the respective ethical review boards. In case we diagnose medical conditions through study screening, we will refer the participants to appropriate medical facilities. We will refer participants found to have advanced CKD to appropriate nephrology services as directed by the study physicians in each centre.

Discussion

This study aims to establish the optimal approach to estimate GFR in sub-Saharan Africa. Our study has the advantage of drawing participants from three countries and both rural and urban settings within sub-Saharan Africa, which will increase the applicability of the findings across the region. Furthermore, we embedded the ARK study within established cohorts that have background data and longitudinal serial measures that we can use to characterize kidney disease over a period. The study will seek to overcome a number of the limitations of previous research.

There are challenges with measuring iohexol GFR, with the major one being accuracy of measurement of iohexol [23, 37]. Stringent measures, shared standard operating procedures across the centres, and analysis in one certified laboratory will help minimise errors.

Measurement of creatinine is another challenge. Creatinine is influenced by many factors and these can greatly influence the estimation, particularly at low eGFR [19, 37, 38]. It is particularly important that the methods used for creatinine measurements are the same across sites and are validated across the centres. We opted to use the Jaffe method for South Africa and Malawi and enzymatic method in Uganda, standardized against an isotope dilution mass spectrometry method across all sites to improve the accuracy in measurement. We will measure Cystatin C in order to assess whether the addition of cystatin C by itself or in combination with creatinine assays further refines the accuracy of estimating GFR within SSA. Several studies have shown that the addition of cystatin C to the estimation formula improves its accuracy [39, 40] and using CKD-EPI-creatinine/cystatin C improved the accuracy in a study from Congo [13]. Using a unified approach, these issues will be addressed by this study. We also plan to measure bioimpedance to define body composition and its contribution to measured GFR variation across the sites, and to assess the correlation of body composition measured by BIA with the gold standard, DXA – which will be done in South Africa [41]. Bioimpedance measurement was included in this study for measurement of lean mass and total body water and we hope to use it to optimize GFR estimation on an individual patient basis, for example in a patient with significant oedema. While this

has not proven useful in high-income settings, such technologies may be useful in low-resource settings [42]. Bio-impedance will help in incorporating more parameters allowing more flexibility to work with the data to get the best formula.

Assessment of risk factors for CKD in sub-Saharan Africa has previously been hampered by inaccurate measurement of GFR. Although, not exhaustive, we included a number of risk factors for CKD in our study. We will examine the role of traditional cardiovascular risk factors including diabetes mellitus, obesity and lipid profiles along with conventional and ambulatory blood pressure monitoring. In addition we will measure the impact of infections which may be of great importance in this region where infectious diseases are common and may play a major role in disease causation alongside genetic factors [43–49].

However, there are limitations. Prolonged blood sampling for iohexol excretion may help to define GFR in patients with very poor renal function [23]. We chose not to include this in our protocol to minimize participant burden. In addition, we do not include participants from other regions of sub-Saharan Africa, including Central and West Africa but we welcome data-sharing with studies in other regions.

The African Research into Kidney disease (ARK) collaboration will provide definitive information about optimal measurement of renal function in sub-Saharan Africa, and is an opportunity for establishing close working relationships across different centres, using standardized protocols and measurements, and shared biorepositories. We plan to build on the collaboration for this study and for future work on kidney disease in sub-Saharan Africa, and welcome additional partners from across the region including North African and Arab populations.

Abbreviations

ARK: African Research into Kidney Diseases; CKD: Chronic kidney disease; CKD-EPI: Chronic Kidney Disease-Epidemiology collaboration; DTPA: Technetium-99 m diethylenetriamine penta-acetic acid; EDTA: chromium-51 ethylene diamine tetra-acetic acid; EGFR: Estimated glomerular filtration rate; IDMS: Isotope dilution mass spectrometry; MDRD: Modification of Diet in Renal Disease; MGFR: Measured glomerular filtration rate

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Authors' contributions

RK, JN, RN, DN, MN, LS, SN, ACC and LAT conceived the study, designed the study, drafted work and substantially revised the manuscript. CH, WN, BS, JP, JG, ANW, JS designed the study, drafted work and substantially revised the manuscript. All authors have approved submission of the manuscript and subscribe to the contents as accurate.

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Availability of data and materials

Not applicable

Ethics approval and consent to participate

We have obtained ethical approvals from the Uganda Virus Research Institute, Research Ethics Committee (UVRI-REC) the Uganda National Council for Science and Technology (UNCST), from the Malawi National Health Sciences Research Committee and the University of Witwatersrand Human Research Ethics Committee (Medical).
We will obtain written informed consent from participants for the collection and analysis of genetic samples as well as iohexol testing and for the use of their clinical records for research purposes. We will seek approval for all study procedures including material transfer agreements from the respective ethical review boards. In case we diagnose medical conditions through study screening, we will refer the participants to appropriate medical facilities. We will refer participants found to have advanced CKD to appropriate nephrology services as directed by the study physicians in each centre.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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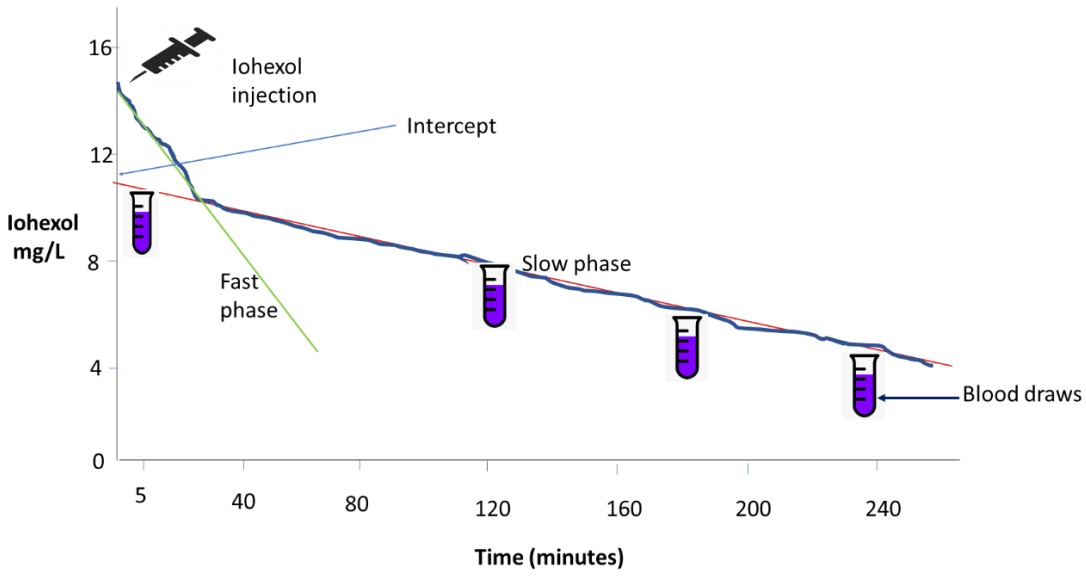
3.0 Problem statement, aim, hypothesis, objectives and methods

3.6 Estimation of GFR from iohexol sampling and making sense of individual results.

To measure GFR we used the excretion of iohexol following intravenous injection. We calculated iohexol plasma clearance as the ratio of the injected amount of the iohexol and the area under the disappearance curve. The total area is the sum of the fast decay due to distribution from the blood space and the slow decay which is related to renal clearance from filtration or tubular secretion. Early blood samples, were taken at 5 minutes to confirm intravascular delivery of iohexol. We used three venous blood samples at 120, 180 and 240 minutes, to compute the slow phase. We recorded the exact time periods at which the blood was drawn in our computations to accurately plot the graph of decline of iohexol level. We assumed a two-compartment model but only directly calculated iohexol excretion in the slow decay phase. The fast phase was estimated by the Brochner-Mortensen equation[2]. The solid blue line represents the iohexol plasma concentration levels over time and the green line represents fast curve while the red line represents the slow decay curves (see **Figure 2** adopted from Levey AS, 2017)[3].

Figure 2: GFR measurement using plasma clearance of iohexol



3.0 Problem statement, aim, hypothesis, objectives and methods

We used the Bland-Altman plots (discussed in more detail below) to evaluate agreement between mGFR and the different GFR estimating equations including; Cockroft Gault (CG), Four variable Modification of Diet for End stage Renal Disease(4-v MDRD): with, and without the ethnicity coefficient; Revised Lund-Malmö, Full-Age Spectrum FAS (creatinine) and CKD-Epi$_{cr}$: with, and without ethnicity coefficient as well as CKD-Epi with cystatin C and a combination of both creatinine and cystatin C (CKD-Epi $_{cr+cysc}$).

We assessed the performance of GFR estimating equations compared to reference mGFR, stratified by stage of CKD, by comparing:

- absolute bias (median/median of the difference between eGFR-mGFR);
- relative bias (median of the difference, expressed as a percent)
- precision (root mean square error, the standard deviation of the residuals)-(IQR mean/median difference)
- accuracy (P_{10}; P_{30}), the proportion of estimates that differ from mGFR by less than 10% and less than 30%.

We also determined the proportion of participants whose CKD staging was incorrectly classified when compared to mGFR.

To determine the robustness of our results to measurement error, we conducted a number of sensitivity analyses. These were (i) R >0.985; all participants with (ii) Volume of distribution (Vd) that fall within sex-specific normal values (11.0-17.0 litres for women); (13.0-20.0 litres for men), and (iii) using both (i) and (ii) above. These parameters were based on recommendations from the British Nuclear Medicine Society (BNMS) guidelines[4]. The volume of distribution range is calculated using +/- 25% of 8 x BSA as the check.

3.0 Problem statement, aim, hypothesis, objectives and methods

3.6.1 Making sense of the results from iohexol testing to mGFR

I will now use the information from an individual in the study, a 42-year-old female, to explain the way the mGFR was determined and what this may mean to the individual patient. I will show how mGFR compares with eGFR calculated from CKD-Epi with and without ethnicity coefficient and cystatin C. I then use the pooled Uganda data to illustrate the use of the Bland-Altman plot and how bias was estimated for all the equations we studied.

Table 2 showing patient results and subsequent calculations based on either real-time or planned time of iohexol sample collection.

Characteristics and measurements		
Sex	Female	
Age (years)	42	
Height (cm)	151.5	
Weight (Kg)	64.0	
Iohexol weight (I+S- Syringe wt)	5.14	
Iohexol 5 (5mins)	639.3	
Iohexol 120 (*actual* 125mins)	154.5	
Iohexol 180 (*actual* 189mins)	116.1	
Iohexol 240 (*actual* 247mins)	94.4	
Calculations for derivation of measured GFR	**Result calculated using correct blood sampling times**	**Result calculated using planned blood sampling times**
Intercept	269.8	264.7
R^2-exp	0.996	0.993
I-rate (lambda)	0.00431	0.00437
Dose of iohexol	3877	3877
Volume of distribution (Vd)	14.36	14.65
Slow phase (SI) GFR	61.89	64.06
BSA (Haycock formula)	1.66	1.66
SI GFR BSA (corrected)	64.49	66.70
Final GFR corrected for phase 1	58.83	60.20
Creatinine (umol/L)	66.67	66.67
Cystatin C	1.04	1.04
CKD-Epi Cr (No ethnicity)	105.21	105.21
CKD-Epi Cr (with ethnicity)	121.94	121.94
CKD-Epi cys	65.64	65.64

The GFR is determined by using the area under the curve for the different plasma concentrations.

- Slow intercept (SI) GFR = Q/AUC

where Q is the volume of iohexol injected in the patient and AUC is the area under the plasma concentration curve.

We used the measured iohexol at the exact times of 125, 189 and 247 minutes to get the intercept for the second phase (phase 2-slow phase).

- Intercept = log (154.5:116.1:94.4,125:189:247),2) = 269.8
- Lambda (Slope/min) estimated from linear regression analysis of $\log_e P_i$ (iohexol plasma concentration) against t_i (time)

  Multiple exponential regression of (154.5:94.4, 94.4,125125:247),2)) = 0.00431

The volume of distribution which is interpreted as the combined volumes of the vascular and extravascular spaces in which the iohexol is diluted is calculated based on intercept and the amount of iohexol administered in litres.

This is critical because the volume of distribution is used to determine the slow intercept GFR. **Table 2** above and **Figure 2** below illustrate the need to record the real time of correction of the sample of iohexol. If not recorded properly, this would lead to the wrong estimation of the intercept points (269.8 vs 264.7), the Vd, and subsequently the measured GFR.

- Slow intercept GFR = Vd(L) x Slope (min^{-1})

SI GFR = 14.36x 0.00431 x 1000 (for conversion to per liter (L))

= 61.89ml/min

Correcting for BSA

It is necessary to correct the SI GFR to body surface area (BSA) before the figure can be compared with reference data GFR as recommended by the BNMS because a larger person would be expected to have a higher GFR than a smaller one due to greater nephron mass. In order to make a fair comparison between different sizes of people we would need to standardize their size. The reference used is the BSA of an average person of 70 kilograms which is 1.73m^2. The BSA correction is done before correcting for the first phase. The SI GFR has to be corrected for the body surface area to be standardized. We corrected for the estimated body surface area of this participant from 1.66m^2 to 1.73 m^2 which is standard-thus

- S1 GFR corr = SI-GFR* (1.73/BSA)
- BSA(Haycock)= $0.024265*\text{Weight(Kg)}^{0.5378}*\text{Height (cm)}^{0.3964}$

$$=0.024265*64^{0.5378}*151.5^{0.3964} = 1.66$$

Therefore, S1 GFR corr = 61.89* (1.73/1.66) = 64.49 mls/min/1.73m^2

Absolute mGFR corrected for rapid phase

Of note, the phase 2 GFR (SI GFR) estimated by using the slope intercept is inaccurate because it ignores the fast exponential contribution to the total AUC. It therefore overestimates the true value of GFR especially for patients with normal GFR[3]. In order to account for the first phase (rapid phase) we used the Brochner-Mortensen (BM) correction for adults, to account for iohexol clearance in the first (rapid) exponential phase as

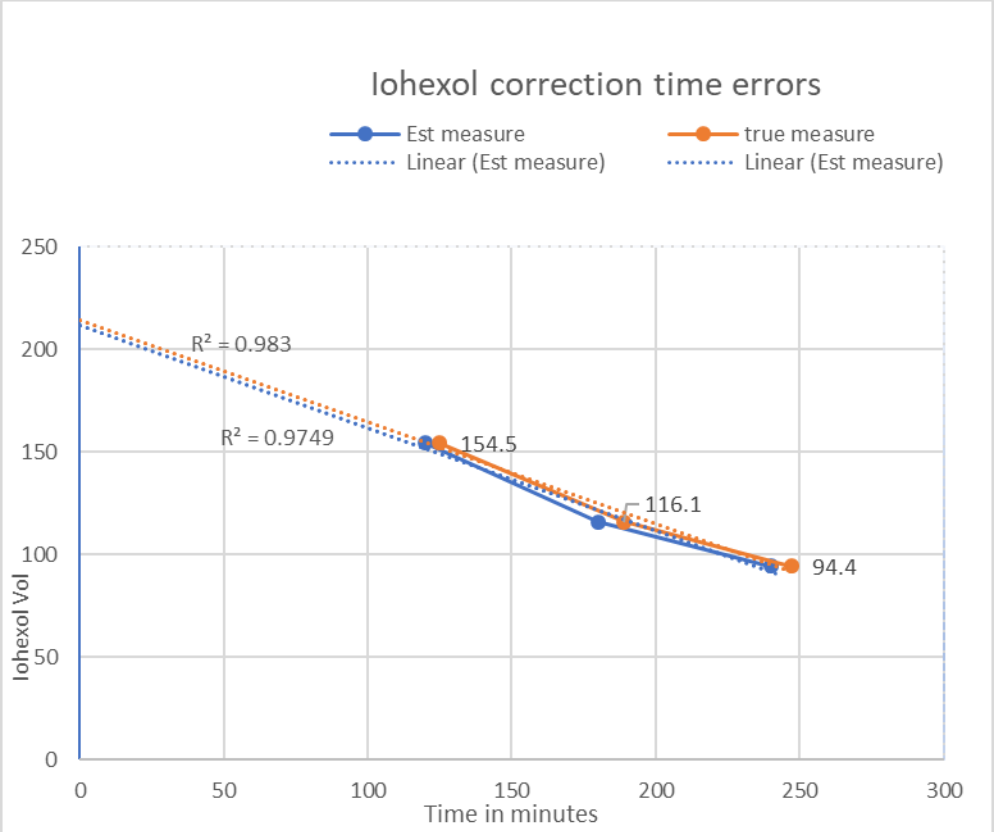
- GFR-BM = $(0.9908*64.49) – (0.001218*64.49^2)\}$[1].

= 58.8mls/min/1.73m^2

In our example the correctly measurement GFR would be 58.8mls/min/1.73m^2 which would place our patient in CKD stage 3 as opposed to incorrectly measured GFR of 60.2mls/min/1.73 m^2 which would be stage 2. From a clinical perspective, the different classifications would lead to different recommendations in the care and follow up of the patient. From a research perspective, if the times of blood sampling are later than planned but not recorded correctly, this would add a degree of inaccuracy: if they were late for multiple participants this would lead to systematic error. We used the exact times to negate this.

Figure 3 is a plot of iohexol levels at both planned accurate times illustrating errors from time of iohexol collection on intercept and r-value.

Figure 3: Graph illustrating errors from time of iohexol collection and influence on intercept and r-value



From **Table 2**, the measured GFR from iohexol is 58.8mls/min/1.73m^2, close to that of cystatin C at 65.64mls/min/1.73m^2 while that of CKD-Epi is 105.2mls/min/1.73m^2 without ethnicity coefficient and 121.94mls/min/1.73m^2 with ethnicity coefficient. The patient met all the criteria that we selected to determine an adequate quality measurement for the iohexol analysis: known syringe weight and therefore volume of iohexol infused, sequential decline in iohexol at subsequent times and an appropriate volume of distribution of 14.6 liters.

3.6.2 Sensitivity analysis

Sensitivity analysis based on volumes of distribution (Vd)

Abnormal volumes of distribution (very large or small) suggest an error in the measurement of the GFR. Unfortunately, we have no validated normal values of Vd for SSA. We performed sensitivity analysis, restricting the population to those within the sex-specific limits suggested in the BNMS guidelines notwithstanding the associated uncertainties of its validity in our study population, and that they are also not validated for people with abnormal kidney function (**Figures 4, 5 and 6**).

Figure 4 shows unrestricted volumes of distribution from Uganda. These anomalous Vds from Uganda appear to have originated from among those without syringe weight (the first 200 trial sets we did). Their intercepts had large values of >800 and were excluded in the main analysis (See highlight in red in the blue histogram)

Figure 4: Histogram for unrestricted volumes of distribution from Uganda

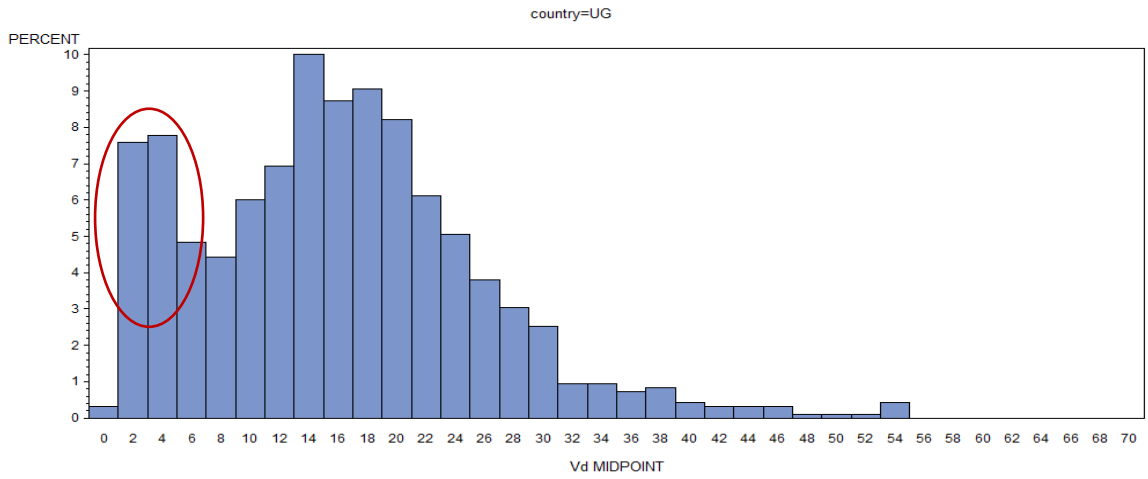


Figure 5 (red bar chart**)** Below shows the sensitivity analysis for all iohexol patients after excluding those without syringe weights before and after iohexol administration. In the early stages of the study, we did not have the before and after weights of the syringes used for iohexol administration. This made it difficult to determine the total amount of iohexol administered.

Figure 5: Histogram for unrestricted volumes excluding participants with no syringe weight

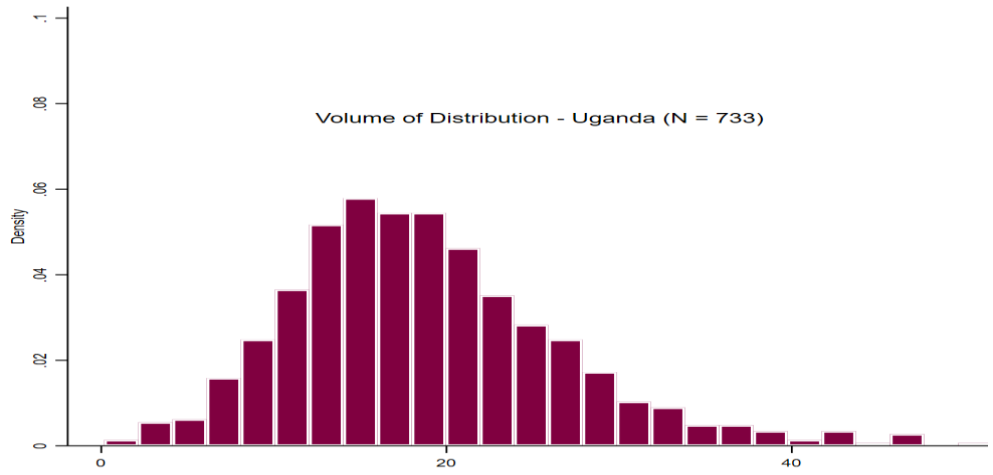
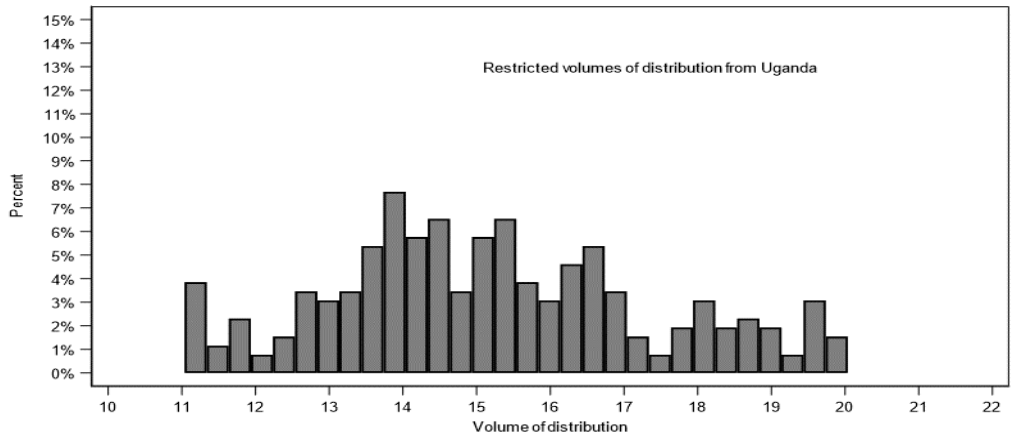


Figure 6 below (grey bar chart) shows sensitivity analysis excluding all females with Vd outside [11; 17] and all males with Vd outside [13; 20] which normalized the volumes of distribution for the Ugandan sub-population.

Figure 6: Histogram for restricted volumes for females and males

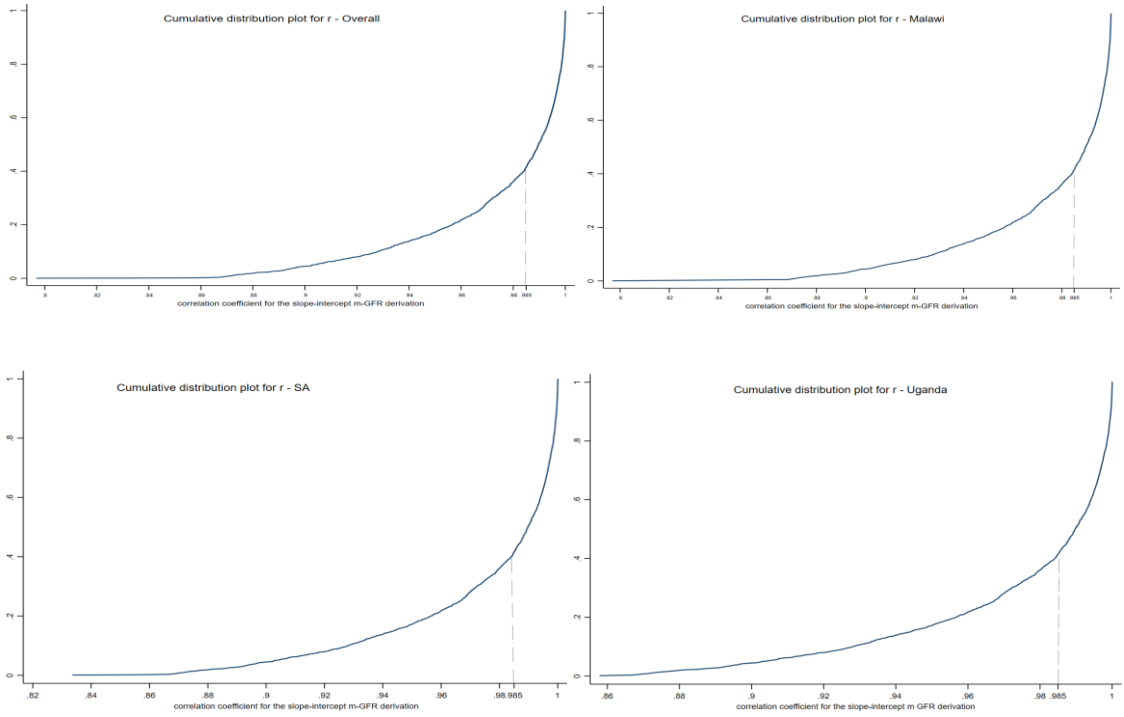


3.0 Problem statement, aim, hypothesis, objectives and methods

Sensitivity analysis based on correlation coefficient-r

Figure 7 below shows the cumulative distribution plot overall and by country for sensitivity analysis based on correlation coefficient r which is the line of best fit for the three iohexol measurements. Notice that about 60% of the population had correlation coefficients (r) above 0.985. The BNMS guideline indicate that r values greater than 0.985 among participants with a normal GFR are likely to indicate a higher quality measurement. There is no clear guidance of R values for those with abnormal GFR.

Figure 7 Cumulative distribution plot: correlation coefficient (r) for the slope-intercept iohexol GFR derivation overall and by country



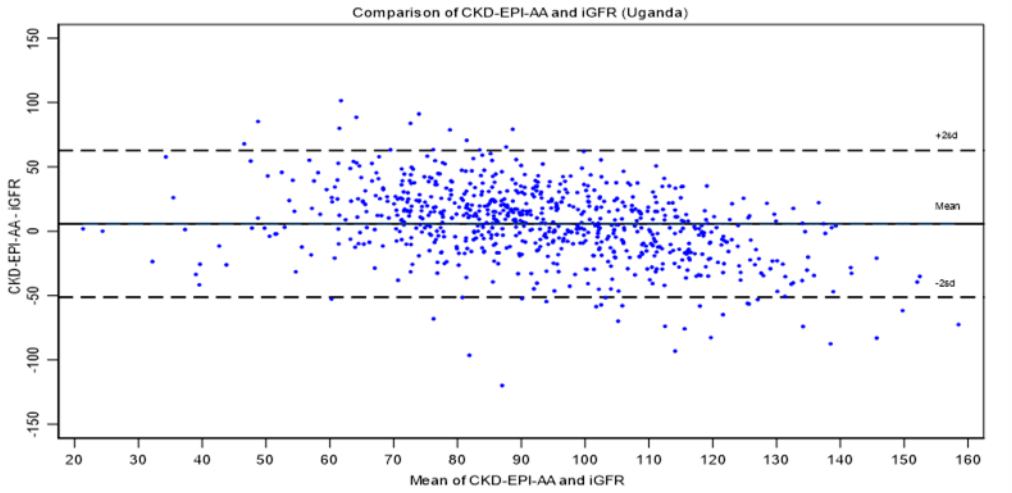
Correlation and regression studies could be used to determine the relationship between eGFR and mGFR. This would only tell us about the relationship between the two methods of

determining kidney function, which falls short of telling us the differences between these two methods. The best way would be to assess the comparability between the two methods. In order to do this, the Bland-Altman analysis which quantifies the agreement between eGFR and mGFR measurements by analyzing the mean difference and constructing limits of agreement around it would be the best option. The Bland-Altman method employs a graphical method of a scatter plot on the X and Y-axis. Where Y-axis shows the difference between eGFR-mGFR and the X-axis represents the average of the two measurements (mGFR+eGFR/2). It is recommended that 95% of the data points should lie within ±2 standard deviations (SD) of the mean difference, often represented by two dotted lines. See as an example of how we generated the Bland-Alman plots for mGFR and eGFR comparisons for Ugandan restricted data using CKD-Epi with and without the ethnicity correlation coefficient in **Figure 8** below. The plot without the ethnicity coefficient (**Panel A**) has minimal bias of 2.8mls/min/1.73m^2 (solid black line) and less scatter beyond 2SD limits of 66 and -50 (beyond dotted lines) while in those with ethnicity factor (**Panel B**) the bias and scatter are much more pronounced at 21.6mls/min/1.73m2 and 76 and -40 respectively.

Figure 8 Bland-Altman plot for CKD-Epi eGFR with and without ethnicity coefficient and mGFR for Uganda

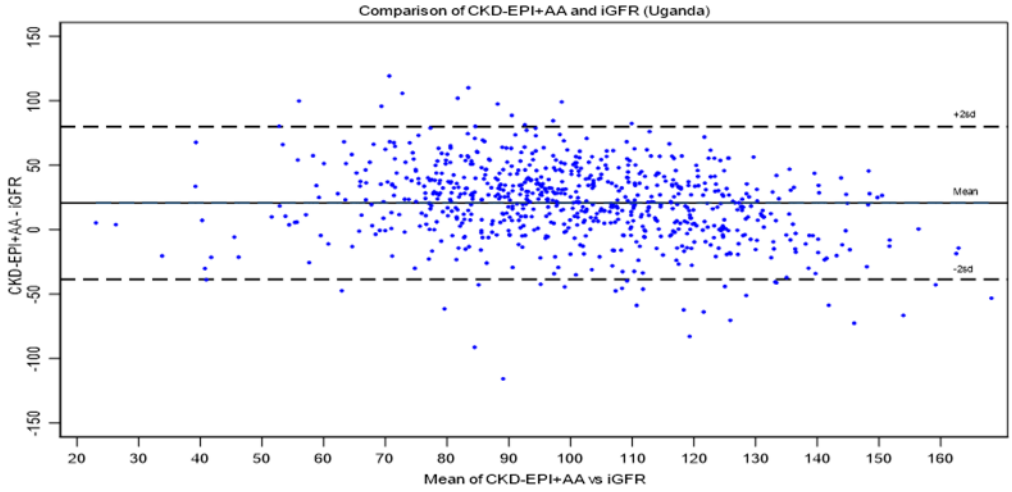
Panel A

Without ethnicity



Panel B

With ethnicity



Performance of eGFR equations compared to iohexol mGFR for Uganda

The final stage of the analysis from individual iohexol measurement to measured GFR was to compare these values to those of the GFR estimating equations. **Table 3** below shows the

performance of GFR estimating equations compared to reference mGFR for 733 participants from Uganda.

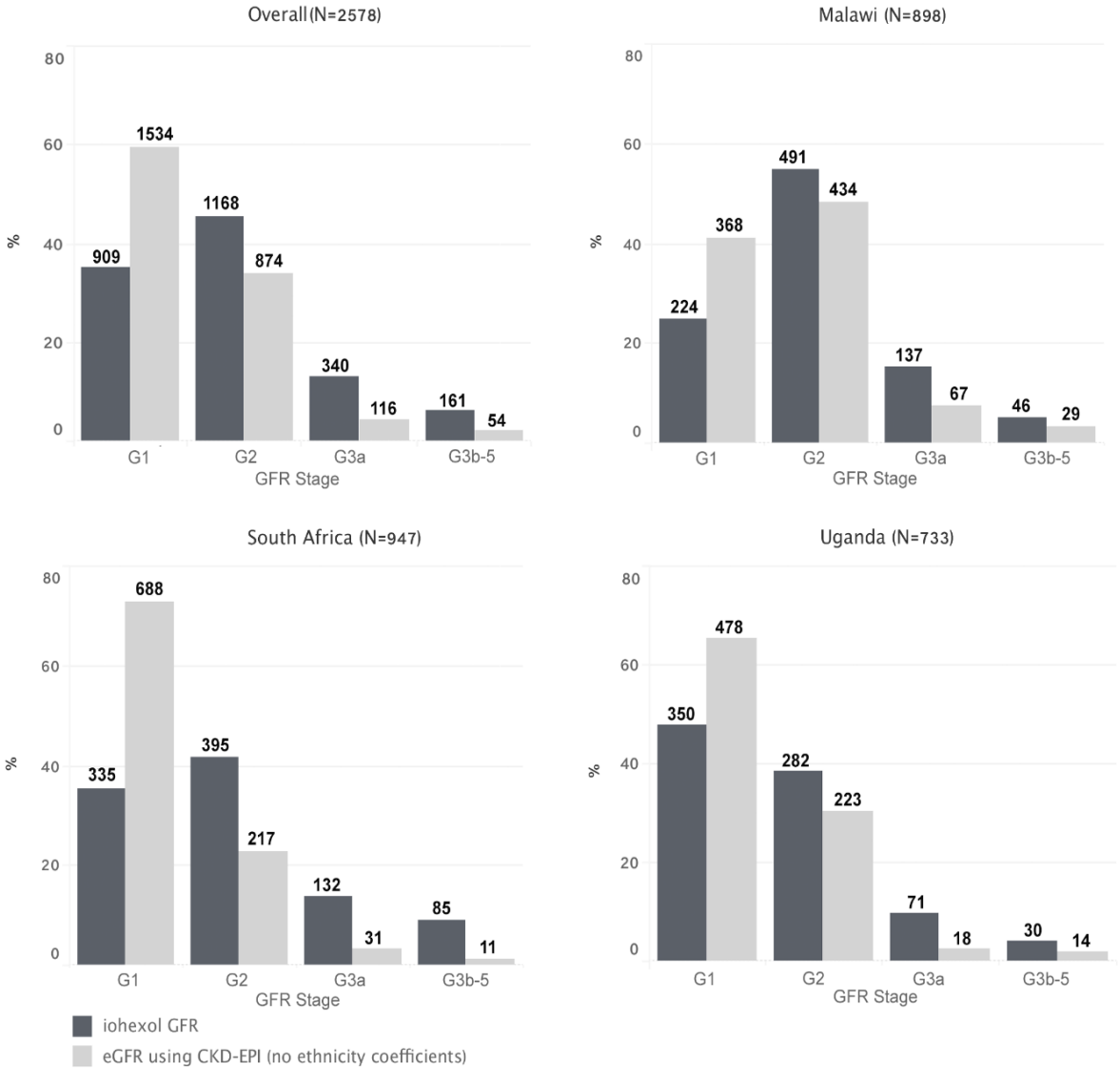
Table 3: Performance of eGFR equations compared to iohexol mGFR for Uganda

GFR estimating equation	[1]Absolute bias	[2]Relative bias	[3]Precision (RMSE)	[4]P_{10}	[5]P_{30}
Cockroft Gault (adjusted for BSA)	0.0	1.00	32.2	0.24	0.65
MDRD	3.0	1.03	31.3	0.25	0.65
MDRD ethnicity coefficient	22.4	1.25	34.4	0.20	0.50
FAS (creatinine)	-0.4	0.99	29.7	0.26	0.68
Lund-Malmö (revised)	-3.0	0.97	27.5	0.27	0.72
CKD-EPI (creatinine)	2.8	1.02	28.0	0.27	0.68
CKD-EPI (creatinine) ethnicity coefficient	21.6	1.24	29.2	0.21	0.52
CKD-EPI (creatinine + cystatin C)	-0.5	0.99	25.8	0.32	0.74
CKD-EPI (cystatin C)	-6.7	0.92	26.9	0.29	0.72

[1]Absolute bias: median of the difference between (estimated GFR - iohexol GFR)
[2]Relative bias: median of the difference between ([estimated GFR - iohexol GFR]/iohexol GFR)
[3]Precision RMSE (Root Mean Square Error): standard deviation of (estimated GFR - iohexol GFR)
[4]Precision IQR (Interquartile Range): IQR for (estimated GFR - iohexol GFR)
[5]Accuracy: proportion of eGFR results within 10% (P_{10}) and 30% (P_{30}) of iohexol GFR
N=733 for creatinine-based equations; N=620 for cystatin C-based equations

In **Figure 9** we compare the iohexol mGFR stage to the GFR stage estimated by CKD-Epi without the ethnicity coefficient showing the mismatch between the two at each stage of kidney function across the different countries of ARK. Though this is not a true population prevalence because we oversampled participants with GFR <60mls/min/1.73m^2 in all the three countries, it gives a good comparison of the performance of CKD-Epi across the different stages. In particular, among patients with normal eGFR, the CKD-Epi over estimates GFR while in the rest of the stages CKD-Epi underestimates the number of participants with abnormal kidney function. These results will be explored in detail in Chapter 7.

Figure 9: Iohexol GFR stage compared to GFR stage estimated by the CKD-EPI (creatinine) equation without ethnicity coefficient, overall and by country


Overall (N=2578)
Malawi (N=898)
South Africa (N=947)
Uganda (N=733)
%
GFR Stage
G1
G2
G3a
G3b-5
909
1534
1168
874
340
116
161
54
224
368
491
434
137
67
46
29
335
688
395
217
132
31
85
11
350
478
282
223
71
18
30
14
iohexol GFR
eGFR using CKD-EPI (no ethnicity coefficients)

3.0 Problem statement, aim, hypothesis, objectives and methods

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3.7 Appendix for methods chapter: Research approvals

UNHRO
Uganda National Health Research Organisation

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REPUBLIC OF UGANDA

Our Ref: GC/127/15/12/543
Your Ref:

17th December 2015

Dr. Anatoli Kamali

RE: UVRI REC review of protocol titled **"Identification and Characterization of Chronic Kidney Disease in Uganda."**

Thank you for submitting the response to the queries addressed to you by the UVRI REC.

This is to inform you that your response dated 15th December 2015 was reviewed and met the requirements of the UVRI REC.

UVRI REC annual approval has been given for you to conduct your research up to 17th December 2016. Annual progress report and request for extension should be submitted to UVRI REC prior to the expiry date, to allow timely review.

The reviewed and approved documents included;

1. UVRI REC Application form
2. Study Protocol: version 1.0 dated 9th November 2015
3. Questionnaire: version 1.0 dated 9th November 2015
4. Information and Consent forms: version 1.0 dated 9th November 2015
5. Applicants' CVs

You can now continue with your study after registration with the Uganda National Council for Science and Technology (UNCST).

Note: UVRI REC requires you to submit a copy of the UNCST approval letter for the above study before commencement.

Yours sincerely,

[redacted]

Mr. Tom Lutalo
Chair, UVRI REC
C.C Secretary, UVRI REC

3.0 Problem statement, aim, hypothesis, objectives and methods

Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS 2231 **25th September 2018**

Dr. Robert Kalyesubula
Principal Investigator
MRC/UVRI and LSHTM Uganda Research Unit
ENTEBBE

RE: APPROVAL OF MATERIAL TRANSFER AGREEMENT (MTA) BETWEEN MRC/UVRI AND LSHTM UGANDA RESEARCH UNIT-UGANDA AND UNIVERSITY OF WITWATERSRAND-SOUTH AFRICA

We refer to the request for approval of MTA in a letter dated 4th September 2018 for the transfer of samples obtained in the research project titled, **'Identification and Characterization of Chronic Kidney Disease in Uganda (CKD Study)'**

The UNCST on **14/09/2018** approved your MTA valid until 2019 of the approved study. The approval is granted to transfer stored plasma and DBS samples in 3 batches of 800, 1600 and 1600 samples through Entebbe International Airport to University of Witwatersrand-South Africa.

The approval is subject to the terms and conditions of the MTA between MRC/UVRI AND LSHTM Uganda Research Unit-Uganda and University of Witwatersrand-South Africa.

The Institutions should observe the conditions set by the Uganda-National Guidelines for Research Involving Humans as Research Participants on the use of the human materials. We also request that you submit to UNCST reports of analysis done on the specimens.

The Commissioner Customs, Uganda Revenue Authority is duly informed by copy of this letter and is kindly requested to give you the necessary assistance to facilitate the transfer of the specimens within the terms of this agreement.

Yours sincerely,

[redacted]

Beth Mutumba
FOR: EXECUTIVE SECRETARY

cc The Secretary, Office of the President

cc The Commissioner Customs, Uganda Revenue Authority

LOCATION/CORRESPONDENCE

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REPUBLIC OF UGANDA

Our Ref: GC/127/16/11/543
Your Ref: MRCU/16/0914

November 29, 2016

Dr. Robert Kalyesubula,

RE: UVRI REC review of progress report titled "**Identification and Characterization of Chronic Kidney Disease (CKD) in Uganda and Malawi.**"

Thank you for submitting your progress report for the above study dated November 8th, 2016 to the UVRI Research Ethics Committee (REC).

This is to inform you that after review of your report, UVRI REC continuation approval has been granted for you to continue with this study up to December 17th, 2017.

At that time, REC would expect you to submit a progress report and request for renewal, prior to the expiry date, to allow timely review.

Yours sincerely,

Dr. Alice Namale
Vice Chair, UVRI REC
C.C Secretary, UVRI REC

3.0 Problem statement, aim, hypothesis, objectives and methods

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Observational / Interventions Research Ethics Committee

Dr Robert Kalyesubula
LSHTM

24 August 2020

Dear Robert,

Study Title: Characterization of Kidney Disease in Sub-Saharan Africa

LSHTM Ethics Ref: 21802

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Local Approval	CKD UVRI REC Approval-1	17/12/2015	1
Local Approval	APROVAL LETTER FROM UNCST - Feb 2016-1	17/02/2016	1
Consent form	CKD ICF_current sample storage_eng	17/12/2017	2
Consent form	CKD CF_ IOHEXOL testing_eng	17/12/2017	2
Consent form	CKD-PIS& ICF-English	17/12/2017	2
Consent form	CKD PIS-ICF Lug_stamped_dec2017	20/12/2017	2
Protocol / Proposal	Protocol for PhD 2019	08/08/2019	1
Investigator CV	LSHTM CV 2019	12/03/2020	1
Investigator CV	CV Prof Liam Smeeth	12/03/2020	1
Investigator CV	Laurie Tomlinson CV for NIHR	12/03/2020	1
Other	GCP_Certificate_Kalyesubula 2020_new (1)	13/03/2020	1
Protocol / Proposal	Protocol for PhD 2019 version 2 dated 28th July 2020	28/07/2020	2
Covering Letter	Cover Letter 2 LSHTM Ethics Kalyesubula	21/08/2020	version 3

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

4.0 Research paper 2: Impaired renal function in a rural population cohort in Uganda

4.1 Introduction

In order to appreciate the current status of kidney disease in Uganda, we needed to conduct a robust baseline study. While several studies existed on the prevalence of kidney disease in Uganda, the majority of these where in high-risk populations such as patients with HIV-AIDS or hospital-based patients or were limited by small sample size [1-3].

In this study we set out to establish the prevalence of impaired renal function using standardized creatinine measurements and calculating the estimated glomerular kidney function according to internationally approved measurement standards. Though we were not able to do a repeat creatinine nor the micro-albumin level, we were able to recruit up to 5,979 participants with Isotope-dilution mass spectrometry (IDMS) standardized measurements. We used the CKD-Epi equation without the ethnicity factor to estimate GFR and noted the factors associated with this abnormal kidney function.

4.0 Research paper 2: Impaired renal function in a rural population cohort in Uganda

4.2 Box 1 Summary of key findings from prevalence study

- The overall prevalence of eGFR <60 ml/min per 1.73 m^2 was 1.6% (95% CI 1.34–1.99) with up to 1,089 (18.2%) having an eGFR <90 ml/min per 1.73 m^2 in a predominantly young population in Uganda.
- Older age, hypertension and anaemia were independently associated with eGFR <60 ml/min per 1.73 m^2
- The traditional risk factors for CKD like diabetes mellitus, hypertension, obesity, alcohol intake and smoking were low in the general population.

4.0 Research paper 2: Impaired renal function in a rural population cohort in Uganda

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4.0 Research paper 2: Impaired renal function in a rural population cohort in Uganda

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1604453/REPH	**Title**	Dr.
First Name(s)	Robert		
Surname/Family Name	Kalyesubula		
Thesis Title	Characterization of kidney disease in sub-Saharan Africa		
Primary Supervisor	Dr Laurie Tomlinson		

If the Research Paper has previously been published, please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Wellcome Open Res.
When was the work published?	May 2019
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A
Have you retained the copyright for the work? *	Published with a Creative Commons Attribution License
Was the work subject to academic peer review?	Yes

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	

4.0 Research paper 2: Impaired renal function in a rural population cohort in Uganda

Please list the paper's authors in the intended authorship order: Stage of publication	

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I am the first author on this paper. I participated by writing the protocol for the study and did data analysis as well as submission of the manuscript. My co-authors supported the work by advising on research design, data analysis and manuscript writing.

SECTION E

Student Signature	[redacted]
Date	28 June 2021

Supervisor Signature	[redacted]
Date	28 June 2021

4.0 Research paper 2: Impaired renal function in a rural population cohort in Uganda

Research Paper 2 https://wellcomeopenresearch.org/articles/4-92

Wellcome Open Research Wellcome Open Research 2019, 3:149 Last updated: 22 APR 2020

Check for updates

RESEARCH ARTICLE

REVISED Impaired renal function in a rural Ugandan population cohort [version 3; peer review: 2 approved]

Robert Kalyesubula [1-3], Jeffrey P. Hau [1], Gershim Asiki [1,4], Billy Ssebunya[1], Sylvia Kusemererwa [1], Janet Seeley [5], Liam Smeeth [3], Laurie A. Tomlinson [3], Robert Newton[1,6]

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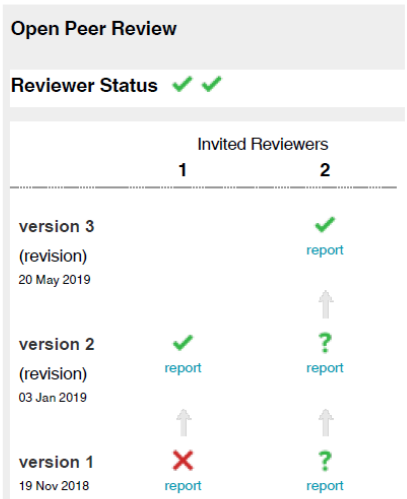
Abstract

Background: Kidney disease is an important cause of morbidity and mortality globally. However, there are limited data on the prevalence of impaired kidney function in sub-Saharan Africa. We aimed to determine the prevalence of reduced kidney function and associated factors in a rural Ugandan population.

Methods: We undertook a study of a representative sample of the General Population Cohort in South-western Uganda. We systematically collected data on cardiovascular disease risk factors, anthropometric measurements and blood tests including haemoglobin, HIV, HbA1c and serum creatinine. The estimated glomerular filtration rate (eGFR) was calculated using the CKD-Epi equation, without the race component of the equation.

Results: A total of 5,979/6,397 (93.5%) participants had valid creatinine results. The mean age was 39 years (Range:16-103 years) and 3,627 (60.7%) were female. HIV prevalence was 9.7% and about 40% of the population were pre-hypertensive or hypertensive. The mean serum creatinine level was 0.75 mg/dl (95% CI 0.74–0.75), and the average eGFR was 109.3 ml/min/1.73 m ^{2} (95% CI 108.8–109.9). The overall prevalence of eGFR <60 ml/min/1.73 m^2 was 1.64% (98/5,979) (95% CI 1.34–1.99). Additionally, 4,792(80.2%) were classified as normal eGFR (≥90 ml/min/1.73 m^2), 1,089(18.2%) as low eGFR (60–89 ml/min/1.73 m^2), 91(1.52%) as moderately reduced eGFR (30–59 ml/min/1.73 m^2), 4(0.07%) as severely reduced eGFR (15-29 ml/min/1.73 m^2), and 3(0.05%) classified as having kidney failure (eGFR<15 ml/min/1.73 m^2). When age-standardised to the WHO Standard Population the prevalence of



4.0 Research paper 2: Impaired renal function in a rural population cohort in Uganda

eGFR<60 ml/min/1.73 m^2 was 1.79%. Age above 35 years and the presence of hypertension (OR 2.86, 95% CI 1.15-7.08) and anaemia (OR 2.14, 95% CI 1.12-4.09) were associated with eGFR<60 ml/min/1.73 m^2.
Conclusion: In a systematic survey of people in rural Uganda, we found a substantial proportion had eGFR<60 ml/min/1.73 m^2. More population based studies are needed to further characterize kidney disease in sub-Saharan Africa.

Keywords
Kidney disease, population cohort, epidemiology, prevalence

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Author roles: Kalyesubula R: Conceptualization, Formal Analysis, Investigation, Methodology, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing; **Hau JP**: Formal Analysis, Writing – Original Draft Preparation, Writing – Review & Editing; **Asiki G**: Data Curation, Investigation, Methodology, Project Administration; **Ssebunya B**: Investigation, Project Administration, Writing – Original Draft Preparation; **Kusemererwa S**: Investigation, Methodology, Project Administration, Writing – Review & Editing; **Seeley J**: Conceptualization, Investigation, Project Administration, Supervision, Writing – Review & Editing; **Smeeth L**: Methodology, Supervision, Writing – Review & Editing; **Tomlinson LA**: Investigation, Methodology, Project Administration, Supervision, Writing – Review & Editing; **Newton R**: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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4.0 Research paper 2: Impaired renal function in a rural population cohort in Uganda

REVISED **Amendments from Version 2**

In this version of the paper we have tried again to highlight the difference between impaired renal function and chronic kidney disease, and why this distinction is not made in the majority of epidemiological studies, and why the utility of the concept of CKD may be different in sub-Saharan Africa. We have also added more details on the selection criteria of the study population.

See referee reports

Introduction

Chronic kidney disease (CKD) is an under-recognized non-communicable disease, associated with a high morbidity and mortality. It is estimated that one in ten people are living with kidney disease worldwide and the prevalence may be higher in low-income countries such as Uganda[1,2]. However, as shown in a recent systematic review the quality of data is often poor, frequently using convenience samples in high-risk populations[3]. Furthermore, only 2% of the studies included in this review used the CKD-EPI equation for calculation of estimated glomerular filtration rate (eGFR) which, based on limited data, has been found to be the best estimate of population CKD prevalence[3,4]. There is little distinction between impaired renal function and chronic kidney diseases in most of the studies from sub-Saharan Africa where these two are used interchangeably[3]. It is thus more appropriate to use impaired renal function rather than CKD when creatinine and albuminuria are measured once with no repeated measure to comfirm chronicity. Community-based studies of the prevalence of impaired renal function have shown marked variation in results. Among people living with HIV/AIDS, estimates range from 0.7% in Rakai, Central Uganda, 14.4% in Gulu, Northern Uganda, 26.5% in Zambia to 41.3% in Tanzania[5–8]. Among HIV-negative populations, estimates range from 2.5% in Wakiso, Central Uganda to 26.5% in Tanzania[8,9]. Hospital-based studies from a National Referral Hospital in Uganda show that most patients with kidney disease are young and have advanced disease by the time of presentation[10]. Thus, in sub-Saharan Africa estimates of kidney disease prevalence vary widely depending on the methods used to determine renal function and the population studied, in particular the age distribution[8,11–19].

Globally, among the known key risk factors for CKD are diabetes mellitus, hypertension and infections such as HIV. Hypertension and HIV are important problems in Uganda with hypertension prevalence estimated to be 26.4%[20] and rising among those with HIV-infection[21]. However, the prevalence of diabetes mellitus is low compared to high-income countries at 2%[22]. Moreover, some studies have also highlighted differences in the prevalence of impaired renal function between urban and rural areas in Africa. A study from Cameroon found the overall prevalence of CKD to be 13.2%: 14.1% and 10.9% among rural and urban dwellers, respectively[23]. Late diagnosis, along with limited health care leading to poor control of hypertension and diabetes may be possible drivers of a higher prevalence in rural populations.

Therefore, we aimed to determine the prevalence and associations of impaired renal function among a representative sample of a rural area of Uganda, within an existing population cohort using high quality sampling methods.

Methods

Study design and setting

The General Population Cohort (GPC) was established in 1989, by the United Kingdom Medical Research Council and the Uganda Virus Research Institute, in Kalungu District, South-western Uganda[24]. The cohort was initially established to examine prevalence, incidence, risk factors and trends of infection with HIV in a rural African population. More recently, research activity has broadened to include the epidemiology and genetics of other communicable and of non-communicable diseases, including cancer, cardiovascular disease and diabetes[24,25]. In brief, the GPC is a community-based open cohort study of residents of 25 neighbouring villages within one-half of a sub-county, lying about 40 km from Lake Victoria. The population is scattered across the countryside in villages defined by administrative boundaries, with a few concentrated in small trading centres. The population under survey includes approximately 22,000 people, less than half of whom are more than 13 years of age. The cohort is dynamic with new births, deaths and migration reported at each round of follow-up. Data are collected through an annual census, an annual questionnaire and serological survey from 1989–2011 and a biennial questionnaire and serological survey thereafter. Details of sexual behaviour, medical, sociodemographic and geographic factors are recorded. Blood specimens are obtained at each biennial survey. Serum is tested for HIV-1 and the remainder is stored at -80°C. Since 1989, the seroprevalence of HIV has remained relatively stable in this population, with about 8% of participants infected; in recent years, prevalence has risen slightly, with the roll out of antiretroviral therapy and consequent improvements in survival.

All eligible participants were evaluated for the study with an acceptance rate of 98%. A total of 6,397 participants were indentified in the two rounds of the GPC (2011–2012 and 2014–2015) with 5,979 (93.5%) individuals having valid creatinine results. The 418 who did not have valid creatinine did not differ significantly from those selected. Variables used for analysis were extracted from two rounds, and participants' information gathered from questionnaire and laboratory data of the survey rounds were linked by unique identifiers. For adults (18+ years for males and 16+ years for females), variables used to develop a socioeconomic score (SES), smoking status, alcohol consumption, fruit and vegetable intake and results of Hepatitis B and C tests were derived from the 2011–2012 survey round. Variables associated with participant's eGFR, age, maximum education level, current marital status, history of stroke, body mass index (BMI) and HIV status were based on the 2014–2015 survey round.

Data collection

Data collected from the GPC questionnaire regarding sexual behaviour and lifestyle factors were self-reported (Supplementary File 1). Anthropometric measurements and blood tests were performed by trained interviewers/nurses using calibrated instruments and following standard operating procedures. We adapted the World Health Organization (WHO) STEP-wise approach to surveillance questionnaire to obtain socio-demographic

characteristics, lifestyle (diet, tobacco, and alcohol consumption), medical history and biophysical measurements. Blood pressure was measured using a digital sphygmomanometer (Omron M4-1). The participant had to be in a sitting position and the mean of the second and third readings taken at 5-minute intervals was used for analysis. Body weight was measured using the Seca 761 mechanical scales and body height was measured using a stadiometer to the nearest 1 kg and 0.1 cm, respectively. Both scales were calibrated according to manufacturer guidelines weekly.

Laboratory tests

Blood tests for haemoglobin, HIV screening, HbA1c, hepatitis B and C viruses, as well as the creatinine level were performed. Venous blood was tested for haemoglobin level using CT -5 Coulter Ac.T 5diff AL (Autoloader) [Beckman Coulter, North America]. HIV testing was performed using an approved national algorithm[26]. Hepatitis B surface antigen, Hepatitis C antibody and creatinine level were tested using a Cobas e 601 Auto Analyzer (Roche Diagnostics, North America). Creatinine was measured using the Jaffe method traceable to an isotope dilution mass spectrometry method[27]. The MRC/UVRI Entebbe laboratories currently have laboratory accreditation through ISO 15189 of the Kenya Accreditation Service, and are enrolled in external quality control programs for South Africa, America, Australia and the United Kingdom.

Definitions and classification

Each participant's SES was derived from conducting principal component analysis (PCA) using variables relating to household infrastructure and property ownership. Urbanicity score used in this study was derived from a previous study using information from the Round 22 survey[28]. BMI was classified according to WHO categories (weight/height^2: kg/m^2): underweight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2) and obese (>30.0 kg/m^2). Blood pressure (BP) classification was derived from the National Institute of Health guidelines: Pre-Hypertension was defined as having a systolic BP greater than 120mmHg but less than 140 mmHg, and a diastolic BP greater than 80 mmHg but less than 90 mmHg. Hypertension was defined as having a diastolic BP greater than or equal to 90 mmHg, systolic BP greater than or equal to 140 mmHg or being on treatment for high BP. Anaemia was defined as having haemoglobin levels less than 130 g/l in men, 120 g/l in non-pregnant women, and 110 g/l in pregnant women. Diabetes mellitus was diagnosed by either having HbA1c >6.5%, through self-reported measures of being previously diagnosed with diabetes, or by current treatment for diabetes.

Classification of renal function

The estimated glomerular filtration rate (eGFR) was calculated using the CKD-Epi equation, without use of the coefficient for African Americans[29]. Impaired renal function was divided into five categories analogous to CKD stages, based on the National Kidney Foundation guidelines (without including proteinuria) as: normal eGFR (≥90 ml/min/1.73 m^2); low eGFR (60–89 ml/min/1.73 m^2); moderately reduced eGFR (30–59 ml/min/1.73 m^2); severely reduced eGFR (15–29 ml/min/1.73 m^2); and kidney failure (eGFR <15 ml/min/1.73 m^2)[30]. We have used impaired renal function for the study because we did not have a second creatinine after 3 or more months or measures of urinary protein excretion to comfirm CKD.

Statistical analysis

Baseline characteristics were tabulated stratified by sex. The prevalence of impaired renal function was also age standardised using the WHO world population as the reference.

We used logistic regression to estimate odd ratios (OR), along with its 95% confidence intervals (95% CIs), to identify potential factors independently associated with CKD. A forward stepwise approach was used in developing our multivariable model adjusting for age, sex, and all independent predictors of CKD.

We also conducted a secondary analysis to compare participants with eGFR<60 ml/min/1.73 m^2 to those with normal renal function excluding individuals in the low eGFR category. The population attributable fraction (PAF) of impaired renal function was estimated for hypertension, and anaemia using the adjusted odds ratios from the final multivariable model.

All statistical analyses were performed using STATA 13 SE (Stata Corp, Texas, USA).

Ethical considerations

All study participants gave written informed consent to participate in the study. The study was approved by Uganda Virus Research Institute Research and Ethics Committee (UVRI-REC) and the Uganda National Council for Science and Technology (UNCST).

Results

Baseline characteristics of study participants

A total of 6,397 individuals participated in the Round 24 GPC survey in 2014–2015 and 5,979 (93.5%) individuals had valid creatinine test results. The average age of study participants was 39 years (range: 16 years to 103 years), consisting of 3,626 (60.7%) females. The majority of patients had primary-level education (60.4%). HIV prevalence was 9.7% (males: 8.4%, females: 10.5%) within this study population, and about 40% of the population was classified as pre-hypertensive. The mean serum creatinine level of the study population was 66.3 mmol/l (95% CI 65.4–66.3), and using the CKD-EPI equation, the average eGFR was 109.3 ml/min/1.73 m^2 (95% CI 108.8–109.9) (Table 1).

Prevalence of impaired renal function

The overall prevalence of eGFR <60 ml/min per 1.73 m^2 was 1.6% (95% CI 1.34–1.99). Of the respondents, 4,792 (80.2%) were classified as normal, 1,089 (18.2%) as low eGFR, 91 (1.5%) as moderately reduced eGFR, 4 (0.1%) as severely reduced eGFR, and 3 (0.1%) classified as having kidney failure (Figure 1, Table 2). The prevalence of impaired renal function among those over the age of 16, age-standardised to the WHO population, was 1.8%.

Factors associated with eGFR <60 ml/min per 1.73 m^2

Age and sex adjusted associations with the presence of eGFR <60 ml/min per 1.73 m^2 are shown in Supplementary Table 1. In multivariable analysis, older age, hypertension (OR 2.86; 95% CI 1.15-7.08) and anaemia (OR 2.14 ; 95% CI 1.12-4.09) were independently associated with eGFR <60 ml/min per 1.73 m^2 (Table 3).

4.0 Research paper 2: Impaired renal function in a rural population cohort in Uganda

Table 1. Characteristics of participants with creatinine results from Survey round 24 among a general population cohort in rural Uganda (N=5,979).

Variable	Male, n (%)	Female, n (%)	Total, n (%)
Age Group			
<35	1,033 (43.90)	1,703 (46.97)	2,736 (45.77)
35–44	460 (19.55)	721 (19.88)	1,181 (19.74)
45–54	378 (16.02)	507 (13.98)	884 (14.79)
55–64	244 (10.37)	336 (9.27)	580 (9.70)
65–74	138 (5.86)	231 (6.37)	369 (6.17)
75+	101 (4.29)	128 (3.53)	229 (3.83)
Max Education			
None	137 (5.82)	394 (10.87)	531 (8.88)
Primary	1,488 (63.24)	2,122 (58.52)	3,610 (60.38)
Secondary	570 (24.21)	946 (26.09)	1,516 (25.35)
Higher Level	158 (6.71)	164 (4.52)	322 (5.38)
Currently Married**			
No	357 (20.62)	1,075 (36.68)	1,432 (30.72)
Yes	1,373 (79.36)	1,856 (63.32)	3,229 (69.28)
Urbanicity*[1]			
Quartile 1	513 (28.71)	756 (26.31)	1,259 (27.24)
Quartile 2	468 (26.19)	733 (25.86)	1,201 (25.98)
Quartile 3	436 (24.40)	697 (24.59)	1,133 (24.51)
Quartile 4	370 (20.71)	659 (23.25)	1,029 (22.26)
SES*[2]			
Lower	565 (35.76)	819 (32.79)	1,384 (33.94)
Middle	521 (33.04)	833 (33.35)	1,354 (33.23)
Upper	493 (31.20)	846 (33.87)	1,339 (32.83)
BMI[3]**			
Normal weight	1,786 (76.47)	2,290 (65.84)	4,076 (70.11)
Underweight	407 (17.42)	302 (8.68)	709 (12.19)
Overweight	122 (5.22)	648 (18.63)	770 (13.24)
Obese	21 (0.90)	238 (6.84)	259 (4.45)
Blood Pressure*[4]			
Normal	668 (40.44)	1,235 (48.82)	1,903 (45.51)
Pre-Hypertension	719 (43.52)	944 (37.28)	1,663 (39.75)
Hypertension	265 (16.04)	352 (13.90)	617 (14.75)
HIV Status**			
Negative	2,150 (91.57)	3,242 (89.51)	5,392 (90.32)
Positive	198 (8.43)	380 (10.49)	578 (9.68)
Hepatitis B*			
Negative	1,588 (96.48)	2,479 (98.10)	4,067 (97.46)
Positive	58 (3.52)	48 (1.90)	106 (2.54)
Hepatitis C*			
Negative	1,582 (96.17)	2,439 (96.52)	4,021 (96.38)
Positive	63 (3.83)	88 (3.48)	151 (3.62)
Anaemia*[5]			
Negative	1,078 (86.87)	1,583 (83.40)	2,661 (84.77)
Positive	163 (13.13)	315 (16.60)	478 (15.23)
Diabetes*[6]			
No	1,603 (97.74)	2,467 (97.94)	4,070 (97.53)
Yes	37 (2.26)	52 (2.06)	89 (2.14)
Current smoking status*			
Not current smoker	1,301 (78.80)	2,478 (97.87)	3,779 (90.34)
Non-daily smoker	83 (5.03)	17 (0.67)	100 (2.39)
Daily smoker	267 (16.17)	37 (1.46)	304 (7.27)
Alcohol consumption*			
Never drinkers	831 (54.64)	1,589 (69.27)	2,420 (63.43)
No alcohol in past 30 days	90 (5.92)	250 (10.90)	340 (8.91)
Alcohol in past 30 days	600 (39.45)	455 (19.83)	1,055 (27.65)

*Variables from a previous round (R22) of the GPC where total number of participants may vary: Urbanicity (n=4,622), SES (n=4,077), Blood Pressure (BP) (n=4,184), Hepatitis B (n=4,173), Hepatitis C (n=4,172), smoking status (n=4,183), alcohol consumption in the last 30 days (n=3,815), and anaemia (n=3,139). [1]Urbanicity score derived from Riha *et al.* (2014). [2]Socio-economic Score (SES) derived from conducting Principle Component Analysis (PCA) on a statistical software using variables relating to household infrastructure and property ownership [3]Body Mass Index (BMI) Classification according to WHO (weight/height^2: kg/m^2): Underweight (<18.5 kg/m^2), Normal weight (18.5–24.99 kg/m^2), Overweight (25.0–29.99 kg/m^2), Obese (>30.0 kg/m^2). [4]BP classification derived from the National Institute of Health guidelines: Pre-Hypertension was defined as having a systolic BP >120 mmHg but <140 mmHg, and a diastolic BP >80 mmHg but <90 mmHg. Hypertension was defined as having a systolic BP ≥90 mmHg, diastolic BP ≥140 mmHg. [5]Anaemia was defined as having haemoglobin levels less than 130 g/L in men, 120 g/L in non-pregnant women, and 110 g/L in pregnant women. Only 2,064 individuals had anaemia results from the R24 of the GPC [6]Diabetes was defined as having HbA1C >6.5%, or being previously diagnosed with diabetes, or are currently on treatment for diabetes. **Variables in R24 with missing individuals: Currently Married (n=4,661), BMI (n=5,814), HIV (n=5,970)

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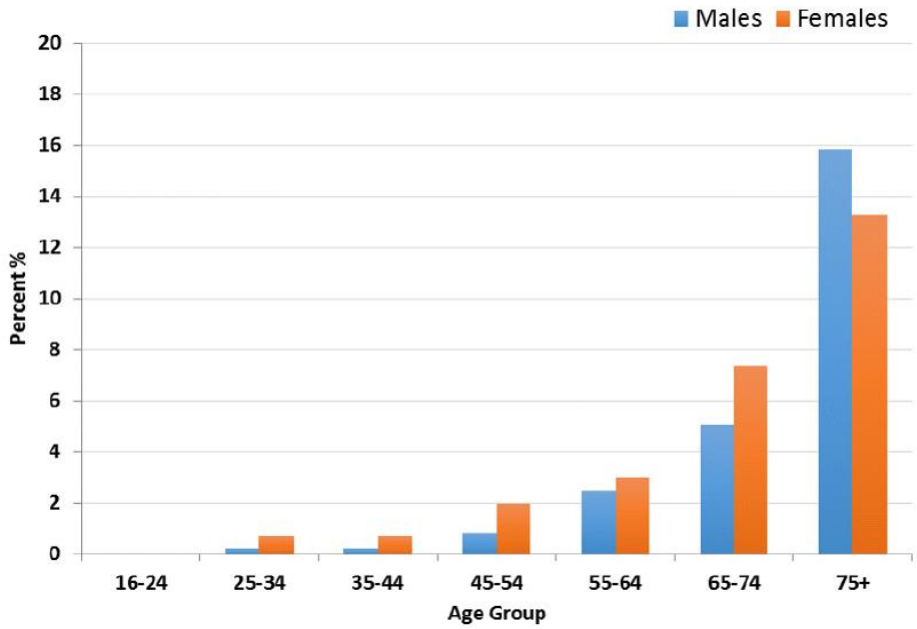

Figure 1. Prevalence of estimated glomerular filtration rate <60 ml/min/1.73 m² by age group among a rural Ugandan cohort.

Table 2. Mean serum creatinine and categories of estimated glomerular filtration rate (eGFR) in the general population cohort.

Variables	Mean (95% CI)
Measurements	
Serum creatinine (mg/dl)	0.75 (0.74–0.75)
eGFR (ml/min/1.73m²)	
CKD-EPI equation*[1]	109.3 (108.8–109.9)
MDRD equation*	106.2 (105.4–107.1)
	Individuals n(%)
Category of level of eGFR	
Normal eGFR (≥90 ml/min per 1.73 m²)	4,792 (80.15)
Low eGFR (60–89 ml/min per 1.73 m²)	1,089 (18.21)
Moderately reduced eGFR (30–59 ml/min per 1.73 m²)	91 (1.52)
Severely reduced eGFR (15–29 ml/min per 1.73 m²)	4 (0.07)
Kidney Failure (eGFR <15 ml/min per 1.73 m²)	3 (0.05)

*The CKD-EPI eGFR calculations were used as the primary outcomes in this study; the MDRD equation was used to contrast the difference between the two equations. [1]The coefficient for black race was omitted while using this equation.

Age and sex adjusted associations of variables with the presence of eGFR of <90 mls/min/1.73m² are shown in Supplementary Table 2. In multivariable analysis, female sex (OR 1.56, 95% CI 1.27-1.93); older age, higher urbanicity score, being overweight or obese; having hypertension (OR 1.60, 95 % CI 1.22-2.11) and HIV-positive status (OR 1.55, 95% CI 1.13-2.04) were associated with impaired kidney function (Supplementary Table 3).

Comparison of participants with eGFR <60 ml/min/1.73m² to those with eGFR >90 ml/min/1.73m² revealed that older age,

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Table 3. Final multivariable model of factors independently associated with estimated glomerular filtration rate <60 ml/min per 1.73m².

Variable	Adjusted OR (95% CI)[1]
Sex	**P=0.56**
Male	*Reference*
Female	1.19 (0.64–2.24)
Age Group	**P<0.001**
<35	*Reference*
35–44	0.53 (0.05–5.14)
45–54	3.49 (0.86–14.09)
55–64	5.73 (1.47–22.25)
65–74	12.24 (3.27–45.82)
75 +	29.68 (7.99–110.19)
Blood Pressure[*2]	**P=0.05**
Normal	*Reference*
Pre-Hypertension	1.92 (0.81–4.57)
Hypertension	2.86 (1.15–7.08)
Anaemia[3]	**P=0.02**
Negative	*Reference*
Positive	2.14 (1.12–4.09)

*Variables from a previous round (R22) of the GPC where total number of participants may vary: Blood Pressure (n=3,039). [1]Multivariable model adjusted for age, sex and all independent predictors of eGFR <60mls/min per 1.73 m². OR, odds ratio; 95% CI, 95% confidence interval. [2]Blood pressure classification derived from the National Institute of Health guidelines: Pre-Hypertension was defined as having a systolic blood pressure greater than 120 mmHg but less than 140 mmHg, and a diastolic blood pressure greater than 80 mmHg but less than 90 mmHg. Hypertension was defined as having a systolic blood pressure (BP) greater than or equal to 90mmHg, diastolic BP greater than or equal to 140mmHg. [3]Anaemia was defined as having haemoglobin levels less than 130 g/l in men, 120 g/L in non-pregnant women, and 110 g/l in pregnant women. Only 2,064 individuals had anaemia results from the R24 of the GPC

hypertension and anaemia were independently associated with impaired renal function (Supplementary Table 4).

The adjusted population attributable fraction of decreased renal function attributable to hypertension and anaemia was 26.4% and 12.8%, respectively.

Discussion

We found a prevalence of eGFR <60 mL/min per 1.73 m² of 1.64% in this predominantly young rural community of Uganda with more than one-fifth of the study participants having eGFR <90 mL/min per 1.73 m². Impaired renal function was strongly associated with age, high blood pressure and anaemia.

Comparing different prevalence estimates of impaired renal function from studies across sub-Saharan Africa is challenging for many reasons. In a meta-analysis of CKD in sub-Saharan Africa by Stanifer *et al.*[3] the overall prevalence was 13.9% but the majority of the studies were conducted among patients with known risk factors for renal disease such as diabetes mellitus, HIV infection and hypertension. Furthermore, only 2% of the included studies used the CKD-EPI equation for calculation of eGFR although, based on limited data, it has been found to be the best estimate of population CKD prevalence[3,4]. The age structure of population varies widely between countries in sub-Saharan Africa making standardisation to a reference population crucial for comparisons between regions or countries. In addition, the prevalence of risk factors such as HIV infection vary substantially across and within countries. We found a lower prevalence of of eGFR <60 mL/min per 1.73 m² in this rural setting than would be expected according to previous studies[3,23]. This unexpected finding could be explained by the characteristics of the population under study. Over 66% of our study participants were less than 45 years of age yet CKD prevalence increases with age. The traditional risk factors for CKD like diabetes mellitus, hypertension, obesity, alcohol intake and smoking were low in our population (see Table 1).

We found that older age, hypertension and anaemia were associated with impaired renal function. Age is known to be strongly associated with eGFR[3,23,29]. Hypertension is both a cause and a consequence of kidney disease, and in this cross-sectional survey it was not possible to tell whether the participants had hypertension as a cause or consequence of the kidney disease. However there has been a rise in reported levels of hypertension in Uganda, from 13.7%[31] in 1969 to 26.4% in 2015[20]. Anaemia is also often a consequence of kidney disease, or may be due to shared risk-factors such as other chronic diseases. We found that anaemia was associated with kidney disease, even for patients with eGFR <90 ml/min/1.73m², a level of kidney function at which a direct causal effect would not be anticipated.

We only measured creatinine on one occasion while two results of eGFR <60 mL/min per 1.73 m² more than 3 months apart are required for the formal definition of CKD. This may have led to an overestimate of the prevalence of impaired renal function. However, most large scale epidemiological surveys have also used one measurement of creatinine[32]. In addition, our study was prospectively sampled from well people and are thus likely to be affected by a transient fall in eGFR associated with acute illness. This is in contrast to many studies using routinely collected data to define renal function where misclassification is likely if blood tests are measured during when patients are unwell[33]. Even if we had two measures of creatinine we would not have been able to confidently assert that patients with eGFR <60 mL/min per 1.73 m² had 'chronic kidney disease' as the estimating equations are not validated in sub-Saharan Africa and the long-term outcome implications, on which the CKD categorisation was defined, are not yet understood in this setting. From the multivariable analysis hypertension and anaemia are likely manifestations of chronic kidney disease, so it is not unreasonable to presume that impaired kidney function is an estimate of CKD. It is also very difficult to have a repeat creatinine and urinalysis in community studies. More studies are needed to establish the utility of the second creatinine/urinary protein in establishing chronicity of kidney disease. There is

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also need to put this in the context of poorly resourced countries where patients are likely to be lost to follow up. The initial contact may be the only opportunity to diagnose them and put them into formal care with the aim of reducing progression to serious complications[10].

Factors which have been traditionally associated with kidney disease in high-income countries such as smoking, alcohol intake and obesity were not associated with the presence of eGFR <60 mL/min per 1.73 m^2 in this population. This may be because of the low prevalence of these factors in the community, or may suggest that the risk factors for CKD are different in this region. Indeed, other researchers have found that the majority of kidney disease in sub-Saharan Africa is not explained by traditional risk factors[34].

Study strengths and limitations

This was a large community-based study conducted within a well-characterized population cohort. We used the CKD-Epi equation to determine eGFR, which is thought to be the best estimate of true GFR in sub-Saharan Africa. We measured a wide range of social and anthropometric factors, chronic diseases and biochemical measurements in a structured and validated manner. In addition, our prevalence estimates have been standardized to the WHO population to enable comparability with other studies across the world.

However, there were limitations, including lack of screening for urine abnormalities (proteinuria and hematuria) which could have led us to underestimate the prevalence of kidney disease. Newer classifications of CKD require measurement of proteinuria to define kidney disease[4]. We only measured creatinine on one occasion while two results of eGFR <60 mL/min per 1.73 m^2 more than 3 months apart are required for the formal definition of CKD. This may have led to an overestimate of the prevalence of impaired renal function.

Implications of the study

Interventions for end-stage renal disease are currently limited for most countries in sub-Saharan Africa with very poor access to dialysis and kidney transplantation[9,35]. This study has established a significant prevalence of impaired renal function, highlighting the need to focus efforts on preventive strategies to delay onset and slow progression of renal disease. However, marked uncertainty remains about how best to estimate GFR in black Africans. This highlights the importance of our ongoing prospective study to determine the best way to measure renal function in sub-Saharan Africa: http://blogs.lshtm.ac.uk/ark/.

Conclusions

We found that approximately one in five adults in rural Uganda had abnormal function despite a low prevalence of diabetes and obesity. More population based studies are needed to further characterize kidney disease in sub-Saharan Africa.

Data availability

Owing to data protection concerns, there are restrictions on access to the underlying data. The GPC database contains 25 years of longitudinal data sets on demographics and disease surveillance. All data (census, survey and laboratory) generated through the cohort are stored and curated at the MRC/UVRI and the LSHTM Research Unit. Data access for specific research purposes is possible and has been granted previously. For any data access inquiries, you may contact the director, MRC/UVRI and the LSHTM Research Unit or by email to mrc@mrcuganda.org or the corresponding author.

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Supplementary material

Supplementary Table 1. Factors associated with estimated glomerular filtration rate (eGFR) <60 ml/min per 1.73 m^2 among a general population cohort from rural Uganda.

Click here to access the data.

Supplementary Table 2. Factors associated with estimated glomerular filtration rate (eGFR) <90 ml/min per 1.73 m^2 among a general population cohort from rural Uganda.

Click here to access the data.

Supplementary Table 3. Final multivariable model of factors independently associated with estimated glomerular filtration rate (eGFR) <90 ml/min per 1.73 m^2 among a general population cohort from rural Uganda.

Click here to access the data.

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Supplementary material research paper 2

Supplementary Table 1: Factors associated with eGFR <60 mL/min per 1.73 m^2 among a general population cohort from rural Uganda (N=5,979)

	Total Individuals n (%)	Individuals with eGFR <60 mL/min/1.73 m^2 n (%)	Unadjusted OR (95% CI)[1]	Age and sex adjusted OR (95% CI)[1]
Sex			P=0.37	P=0.16
Male	2,353 (39.35)	34 (1.45)	*Reference*	*Reference*
Female	3,626 (60.65)	64 (1.76)	1.20 (0.79-1.83)	1.35 (0.87-2.08)
Age Group			P<0.001	P<0.001
<35	2,736 (45.77)	6 (0.22)	*Reference*	*Reference*
35-44	1,181 (19.74)	6 (0.51)	2.32 (0.74-7.22)	2.33 (0.75-7.25)
45-54	884 (14.79)	13 (1.47)	6.79 (2.57-17.92)	6.89 (2.61-18.19)
55-64	580 (9.70)	16 (2.76)	12.90 (5.02-33.13)	13.07 (5.09-33.58)
65-74	369 (6.17)	24 (6.50)	31.65 (12.84-77.97)	31.66 (12.85-78.01)
75+	229 (3.83)	33 (14.41)	76.60 (31.71-185.03)	78.28 (32.38-189.26)
Max Education[**]			P<0.001	P=0.007
None	531 (8.88)	15 (2.82)	*Reference*	*Reference*
Primary	3,610 (60.38)	76 (2.10)	0.73 (0.42-1.29)	2.00 (1.10-3.62)
Secondary	1,516 (25.35)	3 (0.20)	0.04 (0.01-0.19)	0.56 (0.15-2.10)
Higher Level	322 (5.38)	4 (1.24)	0.43 (0.14-1.31)	2.66 (0.82-8.65)
Currently Married[**]			P<0.001	P=0.49
No	1,432 (30.72)	55 (3.84)	*Reference*	*Reference*
Yes	3,229 (69.28)	40 (1.24)	0.32 (0.21-0.48)	0.84 (0.51-1.37)
*Urbanicity**[2]			P=0.35	P=0.59
Quartile 1	1,259 (27.24)	26 (2.07)	*Reference*	*Reference*
Quartile 2	1,201 (25.98)	17 (1.42)	0.68 (0.36-1.26)	0.69 (0.37-1.31)
Quartile 3	1,133 (24.51)	25 (2.21)	1.07 (0.61-1.86)	1.04 (0.59-1.86)
Quartile 4	1,029 (22.26)	15 (1.46)	0.70 (0.26-1.33)	0.96 (0.49-1.87)
*SES**[3]			P=0.64	P=0.29
Lower	1,384 (33.94)	24 (1.73)	*Reference*	*Reference*
Middle	1,354 (33.23)	30 (2.21)	1.28 (0.74-2.20)	1.54 (0.88-2.71)
Upper	1,339 (32.83)	25 (1.87)	1.07 (0.61-1.89)	1.33 (0.74-2.39)
BMI[4**]			P=0.05	P=0.33
Normal weight	4,076 (70.11)	56 (1.37)	*Reference*	*Reference*
Underweight	709 (12.19)	19 (2.68)	2.01 (1.18-3.41)	0.93 (0.53-1.62)
Overweight	770 (13.24)	17 (2.21)	1.65 (0.93-2.85)	1.72 (0.95-3.10)
Obese	259 (4.45)	4 (1.54)	1.14 (0.41-3.19)	1.10 (0.38-3.18)
*Blood Pressure**[5]			P<0.001	P=0.005
Normal	1,903 (45.51)	12 (0.63)	*Reference*	*Reference*
Pre-Hypertension	1,663 (39.75)	33 (1.98)	3.19 (1.64-6.20)	2.10 (1.06-4.16)
Hypertension	617 (14.75)	39 (6.32)	10.63 (5.53-20.45)	2.98 (1.47-6.02)
HIV Status[**]			P=0.83	P=0.12
Negative	5,392 (90.32)	88 (1.63)	*Reference*	*Reference*
Positive	578 (9.68)	10 (1.73)	1.07 (0.55-2.07)	1.78 (0.88-3.58)
*Hepatitis B**			P=0.92	P=0.60
Negative	4,067 (97.46)	82 (2.02)	*Reference*	*Reference*
Positive	106 (2.54)	2 (1.89)	0.93 (0.22-3.85)	1.49 (0.34-6.48)
*Hepatitis C**			P=0.51	P=0.12
Negative	4,021 (96.38)	82 (2.04)	*Reference*	*Reference*
Positive	151 (3.62)	2 (1.32)	0.64 (0.15-2.64)	0.37 (0.08-1.59)
Anaemia[6]			P<0.001	P=0.003
Negative	2,661 (84.77)	33 (1.24)	*Reference*	*Reference*
Positive	478 (15.23)	21 (4.39)	3.65 (2.09-6.38)	2.47 (1.37-4.42)
Diabetes[7]			P=0.14	P=0.42
No	4,070 (97.53)	80 (1.97)	*Reference*	*Reference*
Yes	89 (2.14)	4 (4.49)	2.34 (0.84-6.55)	1.59 (0.54-4.65)
*Current Smoking Status**			P=0.16	P=0.51
Not current smoker	3,779 (90.34)	71 (1.88)	*Reference*	*Reference*
Non-daily smoker	100 (2.39)	2 (2.00)	1.06 (0.25-4.40)	0.62 (0.14-2.72)
Daily smoker	304 (7.27)	11 (3.62)	1.96 (1.02-3.74)	1.37 (0.66-2.81)
*Alcohol Consumption**			P=0.12	P=0.74
Never drinkers	2,420 (63.43)	34 (1.40)	*Reference*	*Reference*
No alcohol in past 30 days	340 (8.91)	9 (2.65)	1.90 (0.90-4.01)	1.11 (0.51-2.42)
Alcohol in past 30 days	1,055 (27.65)	23(2.18)	1.56 (0.91-2.66)	0.83 (0.46-1.50)

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[*]Variables from a previous round (R22) of the GPC where total number of participants may vary: Urbanicity (n=4,622), SES (n=4,077), Blood Pressure (BP) (n=4,184), Hepatitis B (n=4,173), Hepatitis C (n=4,172), smoking status (n=4,183), alcohol consumption in the last 30 days (n=3,815), and anaemia (n=3,139). [1]Urbanicity score derived from Riha et al (2014). [2]Socio-economic Score (SES) derived from conducting Principle Component Analysis (PCA) on a statistical software using variables relating to household infrastructure and property ownership

[3]Body Mass Index (BMI) Classification according to WHO (weight/height^2: kg/m2): Underweight (<18.5 kg/m^2), Normal weight (18.5 – 24.99 kg/m^2), Overweight (25.0 – 29.99 kg/m^2), Obese (>30.0 kg/m^2). [4]BP classification derived from the National Institute of Health guidelines: Pre-Hypertension was defined as having a systolic BP >120mmHg but <140 mmHg, and a diastolic BP >80 mmHg but <90 mmHg. Hypertension was defined as having a systolic BP ≥90mmHg, diastolic BP ≥140mmHg. [5]Anaemia was defined as having haemoglobin levels less than 130 g/L in men, 120 g/L in non-pregnant women, and 110 g/L in pregnant women. Only 2,064 individuals had anaemia results from the R24 of the GPC

[6]Diabetes was defined as having HbA1C >6.5%, or being previously diagnosed with diabetes, or are currently on treatment for diabetes.

[**]Variables in R24 with missing individuals: Currently Married (n=4,661), BMI (n=5,814), HIV (n=5,970)

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Supplementary Table 2: Factors associated with eGFR <90 mL/min per 1.73 m^2 among a general population cohort from rural Uganda

	Total Individuals N(%)	Individuals with eGFR <90 mL/min/1.73 m^2 N(%)	Unadjusted OR (95% CI)[1]	Age and sex adjusted OR (95% CI)[1]
Sex			P<0.001	P<0.001
Male	2,352 (39.34)	400 (17.01)	*Reference*	*Reference*
Female	3,627 (60.66)	787 (21.70)	1.35 (1.18,1.54)	1.71 (1.46,2.01)
Age Group			P<0.001	P<0.001
<35	2,736 (45.77)	94 (3.44)	*Reference*	*Reference*
35-44	1,181 (19.74)	186 (15.75)	5.25 (4.05,6.80)	5.32 (4.11,6.90)
45-54	884 (14.79)	231 (26.13)	9.94 (7.07,12.82)	10.34 (8.01,13.36)
55-64	580 (9.70)	248 (42.76)	20.99 (16.13,27.32)	22.03 (16.90,28.73)
65-74	369 (6.17)	255 (60.98)	43.91 (32.75,58.88)	45.51 (33.86,61.16)
75+	229 (3.83)	203 (88.65)	219.44 (138.92,346.63)	240.87 (151,381.85)
*Max Education***			P<0.001	P=0.002
None	531 (8.88)	205 (38.61)	*Reference*	*Reference*
Primary	3,610 (60.38)	778 (21.56)	0.43 (0.36,0.52)	1.21 (0.95,1.55)
Secondary	1,516 (25.35)	147 (9.69)	0.17 (0.13,0.21)	1.29 (0.95,1.75)
Higher Level	322 (5.38)	57 (17.70)	0.34 (0.24,0.47)	2.21 (1.47,3.33)
*Currently Married***			P<0.001	P=0.61
No	1,432 (30.72)	529 (36.94)	*Reference*	*Reference*
Yes	3,229 (69.28)	612 (18.95)	0.39 (0.34,0.45)	1.04 (0.87,1.24)
Urbanicity[2]			P<0.001	P=0.018
Quartile 1	1,259 (27.24)	264 (20.97)	*Reference*	*Reference*
Quartile 2	1,201 (25.98)	241 (20.05)	0.94 (0.77,1.14)	0.96 (0.76,1.22)
Quartile 3	1,133 (24.51)	294 (25.95)	1.32 (1.09,1.59)	1.34 (1.06,1.68)
Quartile 4	1,029 (22.26)	182 (17.67)	0.80 (0.65,0.99)	1.18 (0.91,1.52)
SES[3]			P=0.69	P=0.20
Lower	1,384 (33.94)	314 (22.69)	*Reference*	*Reference*
Middle	1,354 (33.23)	289 (21.34)	0.92 (0.77,1.10)	1.08 (0.87,1.35)
Upper	1,339 (32.83)	297 (22.20)	0.97 (0.81,1.16)	1.22 (0.98,1.51)
BMI[4]**			P<0.001	P<0.001
Normal weight	4,076 (70.11)	714 (17.52)	*Reference*	*Reference*
Underweight	709 (12.19)	176 (24.82)	1.55 (1.28,1.87)	0.68 (0.53,0.87)
Overweight	770 (13.24)	193 (25.06)	1.57 (1.31,1.88)	1.45 (1.17,1.80)
Obese	259 (4.45)	86 (33.20)	2.34 (1.78,3.06)	1.97 (1.44,2.70)
Blood Pressure[5]			P<0.001	P<0.001
Normal	1,903 (45.51)	290 (15.24)	*Reference*	*Reference*
Pre-Hypertension	1,663 (39.75)	402 (24.17)	1.77 (1.49,2.09)	1.31 (1.08,1.59)
Hypertension	617 (14.75)	281 (45.47)	4.63 (3.79,5.67)	1.56 (1.22,2.00)
*HIV Status***			P=0.046	P=0.001
Negative	5,392 (90.32)	1,050 (19.47)	*Reference*	*Reference*
Positive	578 (9.68)	133 (23.01)	1.23 (1.00,1.51)	1.47 (1.17,1.86)
*Hepatitis B**			P=0.11	P=0.63
Negative	4,067 (97.46)	949 (23.33)	*Reference*	*Reference*
Positive	106 (2.54)	18 (16.98)	0.67 (0.40,1.12)	0.87 (0.49,1.53)
*Hepatitis C**			P=0.084	P=0.70
Negative	4,021 (96.38)	923 (22.95)	*Reference*	*Reference*
Positive	151 (3.62)	44 (29.14)	1.38 (0.96,1.97)	0.91 (0.58,1.43)
Anaemia[6]			P<0.001	P=0.83
Negative	2,661 (84.77)	504 (18.94)	*Reference*	*Reference*
Positive	478 (15.23)	123 (25.73)	1.48 (1.18,1.86)	0.97 (0.73,1.27)
Diabetes[7]			P=0.40	P=0.17
No	4,070 (97.53)	940 (21.10)	*Reference*	*Reference*
Yes	89 (2.14)	24 (26.97)	1.22 (0.76,1.97)	0.69 (0.39,1.19)
*Current Smoking Status**			P=0.024	P=0.23
Not current smoker	3,779 (90.34)	859 (22.73)	*Reference*	*Reference*
Non-daily smoker	100 (2.39)	33 (33.00)	1.67 (1.09,2.55)	0.95 (0.57,1.58)
Daily smoker	304 (7.27)	81 (26.64)	1.23 (0.94,1.61)	0.75 (0.54,1.04)
*Alcohol Consumption**			P<0.001	P=0.082
Never drinkers	2,420 (63.43)	441 (18.22)	*Reference*	*Reference*
No alcohol in past 30 days	340 (8.91)	107 (31.47)	2.06 (1.60,2.65)	1.39 (1.02,1.88)
Alcohol in past 30 days	1,055 (27.65)	302 (28.65)	1.80 (1.52,2.13)	0.97 (0.79,1.20)

*Variables from a previous round (R22) of the GPC where total number of participants may vary: Urbanicity (n=4,622), SES (n=4,077), Blood Pressure (BP) (n=4,184), Hepatitis B (n=4,173), Hepatitis C (n=4,172), smoking status (n=4,183), alcohol consumption in the last 30 days (n=3,815), and anaemia (n=3,139). [1]Urbanicity score derived from Riha et al (2014). [2]Socio-economic Score (SES) derived from

conducting Principle Component Analysis (PCA) on a statistical software using variables relating to household infrastructure and property ownership

[3]Body Mass Index (BMI) Classification according to WHO (weight/height^2: kg/m2): Underweight (<18.5 kg/m^2), Normal weight (18.5 – 24.99 kg/m^2), Overweight (25.0 – 29.99 kg/m^2), Obese (>30.0 kg/m^2). [4]BP classification derived from the National Institute of Health guidelines: Pre-Hypertension was defined as having a systolic BP >120mmHg but <140 mmHg, and a diastolic BP >80 mmHg but <90 mmHg. Hypertension was defined as having a systolic BP ≥90mmHg, diastolic BP ≥140mmHg. [5]Anaemia was defined as having haemogloblin levels less than 130 g/L in men, 120 g/L in non-pregnant women, and 110 g/L in pregnant women. Only 2,064 individuals had anaemia results from the R24 of the GPC

[6]Diabetes was defined as having HbA1C >6.5%, or being previously diagnosed with diabetes, or are currently on treatment for diabetes.

[**]Variables in R24 with missing individuals: Currently Married (n=4,661), BMI (n=5,814), HIV (n=5,970)

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Supplementary Table 3: Final multivariable model of factors independently associated with eGFR<90 mL/min per 1.73 m^2 among a general population cohort from rural Uganda

Variable	Adjusted OR (95% CI)[1]
Sex	P<0.001
Male	*Reference*
Female	1.56 (1.27-1.93)
Age Group	P<0.001
<35	*Reference*
35-44	4.35 (3.00-6.31)
45-54	8.77 (6.14-12.54)
55-64	16.95 (11.70-24.57)
65-74	36.63 (24.45-54.89)
75 +	278.15 (148.28-521.79)
Urbanicity[2]*	P=0.013
Quartile 1	*Reference*
Quartile 2	0.96 (0.74-1.25)
Quartile 3	1.41 (1.09-1.82)
Quartile 4	1.18 (0.88-1.57)
*BMI[3]***	P<0.001
Normal weight	*Reference*
Underweight	0.74 (0.55-0.99)
Overweight	1.47 (1.13-1.91)
Obese	1.78 (1.21-2.63)
*Blood Pressure**	P=0.002
Normal	*Reference*
Pre-Hypertension	1.19 (0.96-1.48)
Hypertension	1.60 (1.22-2.11)
*HIV Status***	P=0.006
Negative	*Reference*
Positive	1.55 (1.13-2.04)

**Variables in R24 with missing individuals: Currently Married (n=4-661)- BMI (n=5-814)- HIV (n=5-970) * Variables from a previous round (R22) of the GPC where total number of participants may vary: Urbanicity (n=4-622)-SES (n=4-077)- Blood Pressure (n=4-184)
[1]OR denotes odds ratio; 95% CI denotes 95% confidence interval. [2]Urbanicity score derived from Riha et al (2014). [3]Body Mass Index (BMI) Classification according to WHO (weight/height^2: kg/m^2): Underweight (<18.5 kg/m^2)- Normal weight (18.5 – 24.99 kg/m^2)- Overweight (25.0 – 29.99 kg/m^2)- Obese (>30.0 kg/m^2). [4]Blood pressure classification derived from the National Institute of Health guidelines: Pre-Hypertension was defined as having a systolic blood pressure greater than 120mmHg but less than 140 mmHg and a diastolic blood pressure greater than 80 mmHg but less than 90 mmHg. Hypertension was defined as having a systolic blood pressure (BP) greater than or equal to 90mmHg, diastolic BP greater than or equal to 140mmHg.

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Questionnaire appendix

PERSONAL IDENTIFIER INFORMATION

1. Consent obtained? |__| CONS

1 = yes, 2 = no
If no, do not continue

DDEXAM MDEXAM YDEXAM
2. Interviewer name & code no.……..…|__|__| INTCODE 3. Date of interview: |__|_| |__|__| |__|__|__|__|
dd mm yyyy

STICKER with participant's personal identifier information
Residence code: |__|__| VNO |__|__| BNO |__|__|__| HNO |__|__| STM

4. PARTICIPANT NAME: .....NAMEC |__|__|__|__|__|__|__|__|__|__| IDNO

5. SEX |__| 6. DOB |__|__| |__|__| |__|__|__|__|→ If year of birth unknown, ask or estimate age (years) |__|__| AGE
1 = M, 2 = F dd mm yy

6. What is your ethnicity?
a. Muganda |__| ETN1
b. Rwandese/ Barundi |__| ETN2
c. Other |__| ETN3

7. What is your tribe? (use code list) |__| TRB

Information for survey clerks and data manager:
If person listed on Enumeration List, indicate any differences in age, name etc.
8. a. Revised name:……. RVNAME
b. Revised date of birth: |__|__| DDOB |__|__| MDOB |__|__|__| YDOB or |__|__| AGER

Remarks: .....……...

EDUCATION, OCCUPATION AND LIVELIHOOD

9. Are you in full-time education? |__| PSCH
1 = yes, 2 = no, 3 = don't know

If yes,
10. What level of education? |__| FTED
1 = pre-primary school; 2 = primary school; 3 = secondary school; 4 = higher education (e.g. college, university)
5 = vocational college

If no,
11. What is your source of livelihood? (use code list L) |__|__| OCCUP1
|__|__| OCCUP2
|__|__| OCCUP3
|__|__| OCCUP4
|__|__| OCCUP5

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12. What level of education are you at (if still in education) or did you reach (if finished education)? |__|__| LEV
99 = nil; 18 = preprimary; 1-7 = P1-P7; 8-10 = J1-J3; 11-16 = S1- S6; 17 = college/university; 19 = vocational college

MARITAL STATUS
I'm going to ask you about your marital status. This means if you have ever regarded someone as your spouse.

13. Have you ever been married, that is, have you ever had someone you called your wife/husband? |__|EVM

1 =yes, 2 =no, 3 =don't know
If no, go to question 17

If yes,

14. How old were you when you first got married? State age (years) |__|__| AGEMG

PREGNANCY - for all female participants aged 13-49 (for male and 50+ female participants, go to question 20) FEMALE PARTICIPANTS

15. In the past 12 months, have you become pregnant? |__| PREGYR
1 = yes, 2 = no, 3 = don't know, 4 = not applicable
If no, go to question 20

If yes,
16. Did you attend antenatal clinic? 1 = yes, 2 = no, 3 = don't know, 4 = not applicable |__| ANCP

17. Have you ever had high blood pressure in pregnancy? |__| BPP
1 = yes, 2 = no, 3 = don't know

18. Have you ever had diabetes in pregnancy? |__| DMP
1 = yes, 2 = no, 3 = don't know

19. Have you ever had a miscarriage and or still birth? |__| MSB
1 = yes, 2 = no, 3 = don't know

HEALTH – for all participants

Interviewer: Please read this to the participant.
MRC has mainly been finding out about HIV. However it's also important to know about some other conditions in this community. So I'm now going to ask about some other conditions.

I am now going to ask you some questions about your health and lifestyle behaviours. This includes things like smoking, drinking alcohol, eating fruit and vegetables and physical activity. Let's start with tobacco.

TOBACCO USE

20. Do you currently smoke any tobacco products, such as cigarettes, cigars or pipes? |__|TOBAC1
1 = yes, 2 = no,

If no, go to question 22

21. Do you currently smoke tobacco products daily? |__|TOBAC2

1 = yes, 2 = no,
If yes go to question 23

22. In the past, did you ever smoke daily? |__|TOBAC3

1 = yes, 2 = no
If no, go to question 25

23. How old were you when you first started smoking daily? (Age in years) |__|__|TOBAC4
888 = don't know
If question 21 = 1 (you currently smoke), go to question 25)
If question 22 = 1 (you have smoked in the past, but do not currently smoke) go to question 24

24. How long ago did you stop smoking daily? |__|TOBAC5
1 = less than 4 weeks ago; 2 = more than 1 month but less than 12 months ago; 3 = more than one year, but less than 5 years ago; 4 = more than 5 years ago, 8 = don't know

25. Do you commonly chew any tobacco products?
1 = yes, 2 = no, 3 = don't know **If no go to Qn 27** |__|TOBAC6

26. If yes, how frequently?
1=Daily, 2 = 2-3x per week 3=once a week 4= once a month |__|TOBAC7

ALCOHOL CONSUMPTION

The next questions ask about the consumption of alcohol.

27. Have you ever consumed an alcoholic drink such as beer, wine, spirits, fermented cider or local products?
|__|ALC1

1 = yes, 2 = no,
If no, go to question 33

28. Have you consumed an alcoholic drink within the past 12 months?
|__|ALC2

1 = yes, 2 = no,
If no, go to question 32

29. During the past 12 months, how frequently have you had at least one alcoholic drink? |__|ALC3
1 = daily, 2 = 5-6 days per week, 3 = 1-4 days per week, 4 = 1-3 days per month, 5 = less than once a month

30. Have you consumed an alcoholic drink within the past 30 days? |__|ALC4

1 = yes, 2 = no
If no, go to question 32

31. During the past 30 days, on how many occasions did you have at least one alcoholic drink? Number |__|__|ALC5
88 = don't know

32. When was the last time you had an alcoholic drink? |__|ALC6
1 = today, 2 = yesterday, 3 = between 3 and 7 days ago, 4 = between 8 and 30 days ago

PHYSICAL ACTIVITY - WORK

33. Does your work involve activity that causes large increases in breathing or heart rate like *carrying or lifting heavy loads, very brisk walking, digging or construction work* for at least 10 minutes continuously? |__| PHYS1
1 = yes, 2 = no
If no, go to question 36

34. In a typical week, on how many days do you do these activities as part of your work?
Number of days |__|__| PHYS2

35. How much time do you spend doing these activities at work on a typical day?
Hours : minutes |__|__|:|__|__| PHYS3
Hrs mins

FRUITS, VEGETABLES, SALT AND WATER INTAKE

36. How many times each week do you eat fresh fruit or uncooked vegetable? Number |__|__| FV1
88= don't know

37. How often do you add salt to your food?
Rarely (<1x/wk) |__| SI1
Sometime (1-3x/wk) |__| SI2
Often (almost daily) |__| SI3
Frequently (multiple per day) |__| SI4

38. How much water do you drink in a day?
1 ☐ ≤ 1 L 2☐ 1-2 L 3☐ 2-3 L 4☐ 3-4 L 5☐ 4-5 L 6☐ ≥ 5 L |__|WI
88= don't know

FAMILY MEDICAL HISTORY

39. Does any family member (parents, siblings, or children) ever had or currently have any of the following diseases?
1 = yes, 2 = no 88= don't know
Diabetes |__| FDM
Hypertension |__| FHT
Heart disease |__| FHD

Dyslipidaemia |__| FDL
Chronic Kidney disease |__| FCKD

HISTORY OF RAISED BLOOD PRESSURE

40. Have you ever had your blood pressure measured by a doctor or other health worker? |__| HBP1
1 = yes, 2 = no

41. Have you ever been told by a doctor or other health worker that you have raised blood pressure or hypertension?
1 = yes, 2 = no |__| HBP2

If no, go to question 45

42. How long have you had raised blood pressure? Number |__|__| HBP3
88= don't know

43. Have you been told in the past 12 months that you have raised blood pressure or hypertension? |__| HBP4
1 = yes, 2 = no

44. During the past two weeks, have you been treated for raised blood pressure with drugs (medication) prescribed by a doctor or other health worker? |__| HBP5
1 = yes, 2 = no

HISTORY OF DIABETES

45. Have you ever had your blood sugar measured by a doctor or other health worker? |__| HD1
1 = yes, 2 = no

46. Have you ever been told by a doctor or other health worker that you have raised blood sugar or diabetes?
1 = yes, 2 = no |__| HD2
If no, go to question 50

47. How long have you had raised blood sugar? Number |__|__| HD3
88= don't know

48. Have you been told in the past 12 months that you have raised blood sugar or diabetes? |__| HD4
1 = yes, 2 = no **If no go to question 50**

49. Today, have you taken insulin or other drugs (medication) that have been prescribed by a doctor or other health worker for raised blood sugar? |__| HD5
1 = yes, 2 = no

HISTORY OF HIGH CHOLESTEROL

50. Have you ever had your cholesterol measured (by blood test) by a doctor or other health worker? |__| CHOLM
1 = yes, 2 = no

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51. Have you ever been told by a doctor or other health worker that you have high cholesterol? |__| CHOLD
    1 = yes, 2 = no
    If no, go to question 54
52. Have you been told in the past 12 months that you have high cholesterol? |__| HCHOL

    1 = yes, 2 = no

53. During the past two weeks, have you been treated for high cholesterol with drugs (medication) prescribed by a doctor or other health worker? |__| CHOLTR
    1 = yes, 2 = no

HISTORY OF CHRONIC KIDNEY DISEASE

54. Have you ever suffered from Loin pain? |__|H LP
    1 = yes, 2 = no

55. Have you ever had repeated episodes of urinary tract infection? |__| HUTI
    1 = yes, 2 = no

56. Have you ever been told by a doctor or other health worker that you have Kidney disease?
    1 = yes, 2 = no |__| HCKD1
    If no, go to question 60

57. How long have you had kidney disease? Number |__|__| HCKD2
88= don't know

58. Have you been told in the past 12 months that you have Kidney Disease? |__| HCKD3
    1 = yes, 2 = no

59. Today, have you taken drugs (medication) that have been prescribed by a doctor or other health worker for Kidney disease? |__| CKDT
    1 = yes, 2 = no

HISTORY OF TAKING TRADITIONAL MEDICINE

60. Have you ever taken traditional medicine for any medical condition or for any other reasons including cultural rituals?
    1 = yes, 2 = no **If no go to question 63** |__| TRDE

61. Have you taken in the last 12 months traditional medicine for any medical condition or for any other reasons including cultural rituals?
1 = yes, 2 = no **If no go to question 63** |__| TRDY

62. When did you last take traditional medicine for any medical condition or for any other reasons including cultural rituals? |__| TRD
1= within last one month; 2= within last one week; 3= within less than one week
88= don't know

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HISTORY OF TAKING TREATMENT FOR ANY CHRONIC DISEASE

63. Have you ever taken treatment for any chronic disease including HIV/AIDS? |__| TRT1
1 = yes, 2 = no **If no go to question 70**

If yes,

64. For what disease was it? (tick all that apply)
1= Diabetes, 2= High Blood pressure, 3= HIV/AIDS, 4= Chronic Kidney disease, 5=TB |__| TRT2
6= Heart disease, 7= Cancer 8= Back pain

65. For how long did you take the treatment? |__| TRTD
1= one month, 2= 2-6 months, 3= 7-12 months, 4= more than 12 months

66. Are you still taking the treatment? |__| TRTC
1 = yes, 2 = no
67. Are you currently taking any pain killers?
1 = yes, 2 = no **If no go to question 69** |__| PRT1

If yes,

68. How long have you been taking the pain killers?
1= one month, 2= 2-6 months, 3= 7-12 months, 4= more than 12 months |__| PRT2

69. Please list all the current medicines participant is taking. (Tick all that apply)

a. ACE/ARB Enalpril, Captopril, Lisonopril or Lorsartan/ Ibersatan etc |__| CRT1
b. Beta blockers eg propranolol; atenolol, carvedilol, bisoprolol etc |__| CRT2
c. Calcium channel blocker eg Nifedipine/ Amilodipine/Adalat XL |__| CRT3
d. Diuretics; Bendrofluazide/ Hydrochlorothalidone Lasix/ other diuretic |__| CRT4
e. NSAIDS, asprin, diclofenac, ibubrofen, aceclofenac etc |__| CRT5
f. HAART; TDF/ AZT, 3TC. ABC. NVP. EFV, PIs, |__| CRT6
p. Others specify…….. |__| CRT7

PHYSICAL MEASUREMENTS (if not done, enter code 888)

70. Consent obtained for physical measurements?
1 = yes, 2 = no |__| CONSPHYS

Blood pressure (mm Hg) and Pulse

71. Time blood pressure taken: (HH:MM) |__|__|:|__|__|BPT

72. Blood pressure measured on right arm |__|BPARM
1 = yes, 2 = no
If it is not possible to use the right arm and the left arm is used, state reason
|_____|REASNARM

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73. Arm circumference (cm) |__|__| AC

If arm circumference is under 24 cm use paediatric cuff size; if 24 – 32 cm use regular arm cuff size; if 33 – 41 cm use large arm cuff size; or if over 41 cm use thigh cuff size

74. systolic/diastolic blood pressure (mm Hg) and pulse (number / minute) - *take 3 readings*

1st systolic |__|__|__| SYST1 1st diastolic |__|__|__|DIAST1 1st pulse |__|__|__|PLS1

2nd systolic |__|__|__| SYST2 2nd diastolic |__|__|__|DIAST2 2nd pulse |__|__|__|PLS2

3rd systolic |__|__|__| SYST3 3rd diastolic |__|__|__|DIAST3 3rd pulse |__|__|__|PLS3

Average of 2nd & 3rd|__|__|__| SYSTAVG |__|__|__|DIASTAVG |__|__|__| PLSAVG

Blood pressure comment |_____| BPCOM

Anthropometry

75. Height (cm) |__|__|__|•|__| HT

State if hairdo prevents sliding part of measuring rod from pressing flat against head:

Height comment |_____| HTCOM

76. Weight (kg) |__|__|__|•|__| WT

77. Waist circumference (cm) |__|__|__|•|__| WC1

|__|__|__|•|__| WC2

If there is a difference greater than 3cm between WC1 and WC2, measure a third time: |__|__|__|•|__| WC3

78. Hips circumference (cm) |__|__|__|•|__| HC1

|__|__|__|•|__| HC2

If there is a difference greater than 3cm between HC1 and HC2, measure a third time: |__|__|__|•|__| HC3

BLOOD AND URINE SAMPLES

79. Consent obtained for taking blood for screening for HIV, Hepatitis B, Hepatitis C, diabetes, cholesterol, biochemistry, full blood count, creatinine and for gene sequencing as well as urinalysis?

1 = yes, 2 = no |__| CONSBLD

80. Interviewer code of the person taking the blood sample if different from the interviewer |__|__|DINTCODE

|__|__|__|__|__|__|__|__|LABNO

4.0 Research paper 2: Impaired renal function in a rural population cohort in Uganda

81. 8.5ml with plain serum
1 = specimen obtained, 2 = specimen to be obtained later, 7 = refused, 9 = failed |__| VAC

82. 6ml with EDTA
1 = specimen obtained, 2 = specimen to be obtained later, 7 = refused, 9 = failed |__| EDTA

83. Would you like to know the result of this HIV test? |__|KVCT
1 = yes, 2 = no, 8 = don't know/not sure

84. Would you like to know your results for possible diabetes, high cholesterol and kidney function?
|__| DCLRES
1 = yes, 2 = no, 8 = don't know/not sure

85. Consent obtained for taking blood and urine for future use and storage of blood and urine samples?
|__| CONBLDG
1 = yes, 2 = no

TREATMENT
Instruction to interviewer: please record here if any treatment provided to participant on the spot

Diagnosis:

Treatment:

5.0 Research paper 3: Association of impaired kidney function with mortality in rural Uganda: results of a general population cohort study.

5.0 Research paper 3: Association of impaired kidney function with mortality in rural Uganda: results of a general population cohort study.

5.1 Introduction

As outlined in chapter 4, kidney disease presents a big problem across the world while disproportionately affecting resource limited settings. We are increasingly becoming aware that the prototype of kidney dysfunction seen in high-income countries may not align with what we see in sub-Saharan Africa. For example, people presenting to clinical services in Uganda with overt kidney dysfunction are young with low prevalence of common kidney drivers like diabetes mellitus, smoking, alcohol and obesity[1]. Given this, and the low estimated prevalence of CKD from conventional eGFR equations, we might anticipate that an association of kidney function with mortality would not be observed in this population. Studies that have examined associations of GFR with mortality in sub-Saharan Africa have been predominantly hospital based; disease specific with limited follow-up periods [2-4]. Studies looking at largely infection-related acute kidney injury in children and ESKD in adults have shown that kidney disease is associated with increased mortality compared to those with normal kidney function [2, 5, 6].

The General Population Cohort with the longitudinal follow-up of patients and robust records of several non-communicable disease related covariates, provided us with an opportunity to look at the consequences of having an abnormal kidney function at baseline. In this study we sought to determine the association between baseline kidney function and subsequent all-cause mortality.

5.0 Research paper 3: Association of impaired kidney function with mortality in rural Uganda: results of a general population cohort study.

Box 2 Summary of key findings for mortality paper

- The baseline population was young with a median age of 36 years (IQR 24-50) with a relatively low prevalence of hypertension (14·6%) and diabetes mellitus (1·8%) compared to developed countries.
- We registered 140 deaths with a median follow-up of 5·0 years with an incidence rate of death for the participants enrolled in the study of 4 deaths per 1000 person-years at risk.
- Adjusting for age and sex, HIV, hypertension, diabetes, BMI, marital status, and alcohol and tobacco use; participants with eGFR ≤45 mls/min/1·73m^2 had six-fold higher mortality compared to those with eGFR ≥90mls/min/1·73m^2 (HR 6·12, 95% CI 2·27-16·45) with strong evidence of a linear trend for risk of mortality as renal function declined (P<0·001).
- This research suggests that kidney function plays a key role in overall health status in sub-Saharan Africa.

5.0 Research paper 3: Association of impaired kidney function with mortality in rural Uganda: results of a general population cohort study.

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5.0 Research paper 3: Association of impaired kidney function with mortality in rural Uganda: results of a general population cohort study.

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed <u>for each</u> research paper included within a thesis.

<u>SECTION A – Student Details</u>

Student ID Number	1604453/REPH	**Title**	Dr.
First Name(s)	Robert		
Surname/Family Name	Kalyesubula		
Thesis Title	Characterization of kidney disease in sub-Saharan Africa		
Primary Supervisor	Dr Laurie Tomlinson		

If the Research Paper has previously been published, please complete Section B, if not please move to Section C.

<u>SECTION B – Paper already published</u>

Where was the work published? When was the work published?		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion		
Have you retained the copyright for the work? *	Was the work subject to academic peer review?	

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

<u>SECTION C – Prepared for publication, but not yet published</u>

Where is the work intended to be published?	BMJ Open
Please list the paper's authors in the intended authorship order:	Robert Kalyesubula, Isaac Sekitoleko, Keith Tomlin, Christian Hansen, Billy Ssebunya, Ronald Makanga, Moses Kwizera Mbonye, Janet Seeley, Liam Smeeth, Robert Newton, Laurie A. Tomlinson

5.0 Research paper 3: Association of impaired kidney function with mortality in rural Uganda: results of a general population cohort study.

Stage of publication	Under peer review

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I collected the data, participated in data analysis, drafted the first manuscript, submitted the manuscript as first author and did all correspondence with the reviewers and the editors.

SECTION E

Student Signature	[redacted]
Date	28 June 2021

Supervisor Signature	[redacted]
Date	28 June 2021

5.0 Research paper 3: Association of impaired kidney function with mortality in rural Uganda: results of a general population cohort study.

Research paper 3

BMJ Open

Association of impaired kidney function with mortality in rural Uganda: results of a general population cohort study

Journal:	*BMJ Open*
Manuscript ID	Draft
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5.0 Research paper 3: Association of impaired kidney function with mortality in rural Uganda: results of a general population cohort study.

Association of impaired kidney function with mortality in rural Uganda: results of a general population cohort study

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Abstract

Objectives To determine the association between baseline kidney function and subsequent all-cause mortality.

Design and settings A general population-based cohort (GPC) study from rural Uganda.

Participants 5,678 participants aged 18 years and above with baseline estimated glomerular filtration rate (eGFR) recruited between 2011-2014 and followed up to March 2019.

Primary and secondary outcome measures The associations between baseline eGFR and all-cause mortality was determined using multivariable cox regression.

Results The median age of the participants at baseline was 36 years (IQR 24-50), 60.7% were female, 14.6% hypertensive, 9.7% HIV-positive and 1.8% diabetic. We registered 140 deaths with a median follow-up of 5.0 years. Adjusting for age and sex, HIV, hypertension, diabetes, BMI, marital status, and alcohol and tobacco use participants with eGFR ≤45 mls/min/1.73m^2 had six-fold higher mortality compared to those with eGFR ≥90mls/min/1·73m^2 (HR 6·12 (95% CI 2.27-16.45)) with strong evidence of a linear trend for risk of mortality as renal function declined (P<0.001).

Conclusion In a prospective cohort with high rates of follow-up we found that baseline kidney function was associated with subsequently increased mortality in a graded manner. Improved understanding of the determinants of kidney disease and its progression are needed in order to inform interventions for prevention and treatment.

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Strengths and limitations of this study

- This is a large well-established population cohort with robust standardised procedures for detailed measurements of covariates such as kidney function and blood pressure, and creatinine was measured according to recommended international standards.
- We had a high participation and retention in the study within the local community and regular reporting of mortality and migration leading to limited loss to follow-up.
- To our knowledge this is the first time that a long-term prospective association between baseline impaired kidney function and mortality in an adult general population of this size has been demonstrated in sub-Saharan Africa.
- Outward migration by predominantly younger participants and use of a single measurement of creatinine without microalbuminuria may have led to a degree of bias.
- Information such as smoking, diabetes and blood pressure were not measured in both the 2011 and 2014 surveys of the GPC so our complete case analysis led to reduction in power for the fully-adjusted model.

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Introduction

Chronic kidney disease (CKD) affects approximately one in every ten adults in high-income countries and is strongly associated with morbidity and mortality [1 2]. The leading causes of death among people with CKD include cardiovascular disease and infections while a proportion progress to end-stage kidney disease (ESKD) [3 4]. In high-income countries, ESKD is a chronic disease that can be managed with dialysis or kidney transplantation [5]. For low-income countries such as Uganda there is very limited access to kidney replacement therapies and most patients with ESKD die prematurely [6 7]. Compounding the challenge of managing kidney disease in sub-Saharan Africa (SSA) is that population prevalence estimates are limited [1], biochemical methods of measuring kidney function have been suboptimal [8], and the appropriate way to estimate kidney function is uncertain, since equations developed in high-income countries have not been validated for SSA [8-11]. In order to determine the value of developing health services to measure and manage kidney disease, we need to understand the relative importance of kidney function in determining prognosis compared to other chronic diseases in this setting. At present no population-based studies have examined the association between kidney function and mortality in sub-Saharan Africa. Therefore, we sought to determine the association between baseline kidney function and all-cause mortality in a large, well-described general population cohort of people in rural Uganda from 2011-2019.

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Methods

Study design and setting

This was a prospective general population cohort (GPC) of people in Kyamulibwa, a rural community 132 kilometres from Kampala, the capital of Uganda. The GPC was established in 1989 by the Medical Research Council (MRC) UK and the Uganda Virus Research Institute (UVRI) to study the epidemiology of HIV infection. Subsequently, other diseases including non-communicable diseases have been examined in the same cohort. The population consists of rural subsistence farmers with a

few peri-urban dwellers and is similar to the broader rural populations of Uganda which constitute about 76% of the country[12]. This is an open cohort with new births, migrations (inward and outward) and deaths recorded regularly by community volunteers through the annual census. The participant response rate is high, at 95% [13]. Once every two years a survey is conducted to determine diseases and outcomes of interest. In 2011-12 and 2014-15, the major foci were diabetes, hypertension, obesity and chronic kidney disease. We collected data on subsequent mortality for participants who had a baseline creatinine measured in either 2011-2012 or 2014-2015.

Patient and Public Involvement in Research

This research was carried out with participation from members of the public of Kyamulibwa. As a research centre we have a community advisory board (CAB) which gets involved from inception of the studies to their implementation. The community was consulted on the priorities of research for the area in 2012 and non-communicable disease were identified as one of the key areas of research. Through seminars and face to face meetings community members were involved in the development of the key questions and outcome measures of the study on kidney disease. We developed simple tools on how the kidneys work and conducted several awareness campaigns in the villages to explain and get more input from the participants and other members of the community. We also undertook a qualitative study to understand the way community members appreciated kidney disease and this work has been published elsewhere[14] . Plans to disseminate our work involve sharing results with the community of Kyamulibwa as well as presentation to both national and international forum.

Participants

We identified potential participants above 18 years who had been recruited from household visits to a homestead in the community for creatinine analysis [15]. All participants had given informed consent for specimen storage and future use of their stored samples.

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Variables

We extracted baseline demographic characteristics including age, sex, tribe, home address, socioeconomic status and maximum level of education. We also collected information on smoking, alcohol use, exercise patterns and diet in the census rounds of 2011-12 *or* 2014-15 (thus this information was not available for all participants for both rounds). We used participants' height and weight to determine their body mass index (BMI) using weight (kg) /height^2 (m^2). Participants with systolic blood pressure ≥140mmHg and/or diastolic blood pressures ≥90mmHg and those who were on treatment for hypertension were classified as hypertensive.

We also analysed blood for creatinine levels, hepatitis B, hepatitis C, HIV-infection and cholesterol levels. Diabetes mellitus was defined as having HbA1C >6.5%, being previously diagnosed with diabetes, or being on current treatment for diabetes. Detailed descriptions of these measurements have been published elsewhere [15]. Creatinine was measured using the enzymatic method traceable to an isotope dilution mass spectrometry method and 93.5% of all participants had a valid creatinine result (6.5% had no blood samples taken, usually because consent for this was declined) [15 16]. We calculated eGFR based on the CKD-Epi-creatinine equation without adjustment for ethnicity [5] and classified impaired kidney function in categories analogous to those used to define CKD stages [17]. We collected data on mortality through registers updated monthly through reports from community health workers (verified by verbal autopsy conducted in accordance with standard guidelines by WHO [18]) and cross-checked via annual census.

Statistical Analysis

We conducted a complete-case analysis with regard to baseline creatinine measurements and also excluded participants with missing data regarding outcome or with probable linkage errors. We described the population characteristics according to pre-defined categories of eGFR. We used multivariable Cox modelling to determine the Hazard Ratio (HR) for mortality for each category of eGFR. We tested the proportional hazards assumption using log-minus-log survival plots and visual

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inspection of Kaplan-Meier curves. Due to missing data for some covariates (such as smoking and alcohol use) which were not collected in *both* the 2011 and 2014 surveys, we present models adjusted for age and sex, and then additionally for baseline HIV status, hypertension, diabetes mellitus, BMI, smoking, alcohol and marital status. Confounders were decided *a priori* based on research regarding associations of chronic conditions with eGFR in this population [15] and after investigation of co-linearity and data sparsity. We present the rate of mortality per 1,000 person-years, and report HR and 95% confidence intervals (CIs).

Because HIV is common in this population and has been associated with increased risk of both renal impairment and mortality, we examined potential interaction between eGFR and HIV status in the association with mortality by fitting an interaction term in the final adjusted model. In additional analysis, to examine for a 'J-shaped curve' in the association between eGFR and mortality we repeated the main analysis after re-categorisation of eGFR with those ≥120mls/min/1.73m^2 as a separate group. We analysed all data using STATA version 15.0 (StataCorp, College Station, TX, USA) statistical software.

Ethics

All study participants gave written informed consent to participate in the study. The study was approved by Uganda Virus Research Institute Research and Ethics Committee (UVRI-REC-#HS 1978), the Uganda National Council for Science and Technology (UNCST-#SS 4283) and the London School of Hygiene and Tropical Medicine Observational / Interventions Research Ethics Committee (LSHTM Ethics #21802)

Role of the funding source

The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

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Results

We included 5,678 participants with kidney function and mortality data available for the analysis (**Figure 1**). The median age of the participants was 36 years (interquartile range (IQR) 24-50 years) with most being female (60.7%) and of normal weight (70.2%) while 9.7% were HIV-positive. The majority (90.5%) of the participants were non-smokers and 58.5% did not consume alcohol. 14.6% were hypertensive and 1.8% of the participants were diabetic (**Table 1**). Comparison of characteristics with included participants showed that those with missing data were younger but otherwise there were no important differences (**Supplementary Table 1**). We also included the baseline characteristics of participants by five-level stages of kidney function including eGFR > 120mls/min/1.73m^2 (**Supplementary Table 2**).

During follow-up there were 140 deaths with a median follow-up of 5.0 years (IQR 3.7-6.0). The incidence rate of death for the participants enrolled in the study was 4 deaths per 1000 person-years at risk (PYAR). For participants with eGFR >90mls/min/1.73m^2 the incident rate was about 3 deaths per 1000 (PYAR) person-years at risk and for those <60mls/min/1.73m^2 it was 27 deaths per 1000 PYAR. During follow-up, 26.4% (1,500/5,678) of participants migrated and 3.0% (169/5,678) were lost to follow-up.

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Table 1. Baseline characteristics of participants categorised by level of kidney function in the general population cohort (N=5,678)

	Estimated glomerular filtration rate (mls/min/1.73m^2)				
Variable	≥90	60-89	45-59	<45	Total
Sex					
Male	1853 (40.6)	343 (33.6)	26 (35.6)	7 (35.0)	2229 (39.3)
Female	2710 (59.4)	679 (66.4)	47 (64.4)	13 (65.0)	3449 (60.7)
Age (years), median (IQR)	32 (22-44)	56 (45-70)	66 (58-76)	68 (42-76)	36 (24-50)
Age groups, N (%)					
<35	2531 (55.5)	76 (7.4)	3 (4.1)	3 (15.0)	2613 (46.0)
35-44	946 (20.7)	176 (17.2)	4 (5.5)	2 (10.0)	1128 (19.9)
45-54	619 (13.6)	210 (20.6)	10 (13.7)	3 (15.0)	842 (14.8)
55-64	311 (6.8)	214 (20.9)	14 (19.2)	1 (5.0)	540 (9.5)
65-75	133 (2.9)	186 (18.2)	19 (26.0)	5 (25.0)	343 (6.0)
>75	23 (0.5)	160 (15.7)	23 (31.5)	6 (30.0)	212 (3.7)
Marital status, N (%)					
Single/widowed	853 (18.7)	444 (43.4)	37 (50.7)	13 (65.0)	1347 (23.7)
Married	2496 (54.7)	536 (52.5)	33 (45.2)	7 (35.0)	3072 (54.1)
Missing	1214 (26.6)	42 (4.1)	3 (4.1)	0 (0.0)	1259 (22.2)
HIV status, N (%)					
Negative	4134 (90.6)	901 (88.2)	70 (95.9)	13 (65.0)	5118 (90.1)
Positive	424 (9.3)	117 (11.5)	3 (4.1)	7 (35.0)	551 (9.7)
Missing	5 (0.1)	4 (0.4)	0 (0.0)	0 (0.0)	9 (0.2)
Alcohol Consumption, N (%)					
Infrequent/no alcohol	1911 (41.9)	393 (38.5)	29 (39.7)	4 (20.0)	2337 (41.2)
Regular alcohol	1164 (25.5)	449 (43.9)	36 (49.3)	12 (60.0)	1661 (29.3)
Missing	1488 (32.6)	180 (17.6)	8 (11.0)	4 (20.0)	1680 (29.6)
Smoking Status, N (%)					
Not current smokers	2801 (61.4)	748 (73.2)	55 (75.3)	13 (65.0)	3617 (63.7)
Non-daily smokers	64 (1.4)	30 (2.9)	1 (1.4)	1 (5.0)	96 (1.7)
Daily smokers	209 (4.6)	64 (6.3)	9 (12.3)	2 (10.0)	284 (5.0)
Missing	1489 (32.6)	180 (17.6)	8 (11.0)	4 (20.0)	1681 (29.6)
Socioeconomic status					
Lower	1064 (23.3)	289 (28.3)	21 (28.8)	3 (15.0)	1377 (24.3)
Middle	1059 (23.5)	257 (25.2)	23 (31.5)	7 (35.0)	1346 (23.7)
Upper	1029 (22.6)	272 (26.6)	21 (28.8)	4 (20.0)	1326 (23.4)
Missing	1411 (30.9)	204 (20.0)	8 (11.0)	6 (30.0)	1629 (28.7)
Hypertension, N (%)					
Normal	2756 (60.4)	614 (60.1)	35 (48.0)	9 (45.0)	3414 (60.1)
Hypertensive	319 (7.0)	228 (22.3)	30 (41.1)	7 (35.0)	584 (10.3)
Missing	1488 (32.6)	180 (17.6)	8 (11.0)	4 (20.0)	1680 (29.6)
Diabetes mellitus, N (%)					
No	2998 (65.7)	815 (79.8)	62 (84.9)	16 (100.0)	3891 (68.5)
Yes	62 (1.4)	20 (2.0)	3 (4.1)	0 (0.0)	85 (1.5)
Missing	1503 (32.9)	187 (18.3)	8 (11.0)	4 (20.0)	1702 (30.0)
BMI classification, (kg/m^2) N (%)					
Underweight (<18.5)	504 (11.1)	143 (14.0)	14 (19.2)	4 (10.0)	665 (11.7)
Normal weight (18.5–24.99)	3201 (70.2)	623 (61.0)	41 (56.2)	14 (70.0)	3879 (68.3)
Overweight (25.0–29.99)	552 (12.1)	166 (16.2)	14 (19.2)	2 (10.0)	734 (12.9)
Obese (>30.0)	167 (3.7)	77 (7.5)	3 (4.1)	0 (0.0)	247 (4.4)
Missing	139 (3.1)	13 (1.3)	1 (1.4)	0 (0.0)	153 (2.7)

Missing data mainly arises due to different variables being collected in the 2011 and 2014 surveys of the GPC.

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Age-sex and fully adjusted associations with mortality are shown in **Table 2** and **Figure 2**. In age and sex adjusted analyses, mortality was associated with poorer kidney function, being HIV positive, being unmarried or widowed, low BMI and regular smoking. In the fully-adjusted model, low kidney function remained associated with increased risk of mortality, (HR 6.12, 95% CI 2.27-16.45) for those with eGFR <45mls/min/1.73m^2 compared to those with eGFR ≥90mls/min/1.73m^2. The test for trend showed strong evidence (P<0.001) that the mortality rate increased progressively as category of baseline kidney function decreased. There was no evidence of an interaction between HIV and kidney function with risk of mortality in the fully adjusted model (P=0.672). In an additional analysis, when high eGFR was included as a separate category there was weak evidence of a 'J-shaped curve' (**Supplementary Table 3**). In age-sex adjusted analysis, baseline eGFR ≥120mls/min/1.73m^2 was associated with increased risk of mortality (HR 2.68, 95% CI 1.47-4.87) compared to the reference category of 90-119mls/min/1.73m^2 while in fully-adjusted analyses confidence intervals crossed the null (HR 1.65, 95% CI 0.61-4.44).

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Table 2. Results of age-sex and fully-adjusted regression models for the association between kidney function and mortality in the general population cohort

	Age and sex adjusted		Fully adjusted#	
	HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
eGFR (mls/min/1.73m^2)				
≥90	**Reference**		**Reference**	
60-89	1.11 (0.73-1.71)		1.21 (0.73-2.03)	
45-59	1.72 (0.81-3.69)		1.91 (0.80-4.54)	
<45	5.97 (2.55-13.98)	<0.001	6.12 (2.27-16.45)	0.003
Age (years)*	1.06 (1.05-1.07)	<0.001	1.05 (1.03-1.07)	<0.001
Sex*				
Male	**Reference**		**Reference**	
Female	0.50 (0.36-0.70)	<0.001	0.70 (0.42-1.07)	0.188
HIV status				
Positive	2.29 (1.43-3.65)	<0.001	1.67 (0.87-3.18)	0.120
Negative	**Reference**		**Reference**	
Hypertension				
Normotensive	**Reference**		**Reference**	
Hypertensive	0.93 (0.60-1.43)	0.725	1.08 (0.58-2.01)	0.433
Diabetes mellitus				
No	**Reference**		**Reference**	
Yes	0.85 (0.27-2.67)	0.777	1.11 (0.35-3.57)	0.861
BMI classification				
Under weight	2.14 (1.49-3.09)		1.71 (1.10-2.67)	
Normal weight	**Reference**		**Reference**	
Over weight	0.21 (0.07-0.66)		0.20 (0.05-0.84)	
Obese	0.21 (0.03-1.50)	<0.001	0.35 (0.48-2.55)	0.006
Marital status				
Currently married	0.55 (0.37-0.81)	0.003	0.77 (0.48-1.23)	0.273
Single/widowed	**Reference**		**Reference**	
Alcohol consumption				
Regular alcohol	1.49 (0.99-2.26)	0.057	1.50 (0.92-2.46)	0.104
No/infrequent alcohol	**Reference**		**Reference**	
Smoking status				
Not current smokers	**Reference**		**Reference**	
Non-daily smokers	1.69 (0.79 – 3.58)		1.22 (0.50-2.97)	
Daily smokers	2.13 (1.32 – 3.43)	0.007	1.63 (0.93-2.87)	0.236

*Age adjusted for sex and sex for age: #Fully Adjusted Hazard Ratios adjusted for age, sex, HIV status, hypertension, diabetes mellitus, BMI, marital status, alcohol use and smoking.

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Discussion

In a rural Ugandan population cohort, we found a graded association between low baseline kidney function and subsequent mortality. Participants with severe kidney impairment (eGFR <45mls/min/1.73m^2) had a more than six-fold risk of dying compared to those with eGFR >90mls/min/1.73m^2. This was despite a low prevalence of obesity, diabetes and regular smoking, all key risk factors for kidney disease in high-income countries.

Our study has a number of strengths. It is large and conducted within a well-established population cohort with robust standardised procedures for detailed measurements of covariates such as kidney function and blood pressure, and creatinine was measured according to recommended standards [13]. There is high participation and retention in the study within the local community and regular reporting of mortality and migration by local study workers leading to limited loss to follow-up [13]. Outward migration by predominantly younger participants leads to a degree of selection bias. However, people with chronic health problems often return home so we anticipate capturing the majority of deaths among the baseline cohort, even among those who previously migrated.

However, there were also limitations. This cohort covers a region that is mostly rural, although this is in keeping with the vast majority of people living in Uganda and in many regions of sub-Saharan Africa. Information such as smoking, diabetes and blood pressure were not measured in *both* the 2011 and 2014 surveys of the GPC so our complete case analysis led to reduction in power for the fully-adjusted model. However, there are no substantial differences in characteristics between those with complete and incomplete covariate information, and we see little difference in the effect estimates between age-sex adjusted and fully-adjusted models. We used baseline measures of covariates and were not able to update health related confounders over time. There is likely to be inaccuracy in measurement of kidney function which we categorised using estimated GFR calculated from serum creatinine, a breakdown product of muscle and related to muscle mass and dietary meat intake [16]. Therefore, the prevalence of low BMI in this population where food scarcity is common [19] means that we may have underestimated the prevalence of impaired GFR in this population. In

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addition, validation of GFR estimating equations is currently limited in sub-Saharan Africa [8]. We measured kidney function only once while two measures 3 months apart are required to confirm a diagnosis of chronic kidney disease, and we do not have measures of proteinuria, an important early marker of kidney damage (and predictor of mortality), which may have led to misclassification of some participants [17]. Migration and subsequent loss to follow-up among younger, healthier participants may have led to selection bias with overrepresentation of older participants with health problems including impaired kidney function remaining in the cohort. Finally, we do not know the causes of death, and small numbers of people in lower kidney function subgroups led to limited power and imprecise estimates with wide confidence intervals.

Recent community-based studies of associations of kidney disease suggest important differences in prevalence and associations of impaired kidney function between countries in sub-Saharan Africa. Those including participants from urban areas in South Africa and Nairobi found similar risk factors for those well-established in high-income countries: age, hypertension, diabetes, HIV and female sex [20]. However, cohorts in Malawi and Uganda where the populations are younger, often living in rural areas and with low levels of smoking, obesity and diabetes showed low prevalence of impaired renal function and found a protective association with diabetes, possibly due to hyperfiltration [15 21].

The prospective studies that have looked at the association between mortality and kidney disease in sub-Saharan Africa are limited to cohorts of people which are hospital based [22-25], include patients with only ESKD [26] or HIV [27], or study albuminuria alone [28], and are also limited to short-term follow-up. Our study is the first to demonstrate this association in a general population cohort in sub-Saharan Africa and the results are consistent with studies from high-income countries which have shown a consistent, strong and graded association between impaired (as well as very high) kidney function and risk of subsequent mortality [29]. However, the key difference for our study is that the importance of baseline kidney function can be demonstrated despite the co-existent burden of infectious disease mortality and relatively limited health care system.

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While HIV can cause kidney disease [30] we did not find an interaction between HIV and kidney disease, suggesting that low kidney function has the same pattern of association with mortality among people who are HIV negative. Unexpectedly, we did not find an association between hypertension and mortality in our study despite evidence that it is a key driver of disease and outcomes in sub-Saharan Africa and globally [31]. It is possible that repeated screening within this cohort population has led to higher rates of treatment and control than in other populations, and prevalence of hypertension in this region (14.6%) was lower than the estimated national prevalence of 26.4% [32]. However, it is also possible that the causal relationship between hypertension and kidney damage in sub-Saharan Africa has been oversimplified due to lack of kidney function measurement and longitudinal follow-up. Recent discovery of strong genetic risks for kidney disease in African-Americans has suggested that hypertension and kidney dysfunction may develop in part from shared risk factors [33]. Similarly, diabetes is an independent predictor of mortality among patients with kidney disease and is the leading cause of CKD globally [34]. However, in this population with a low prevalence of diabetes it was not associated with the presence of impaired kidney function or mortality.

In conclusion, in this prospective cohort study based in a rural Ugandan community we found that baseline impaired kidney function was associated with mortality, which remained after adjusting for known shared risk factors such as diabetes, hypertension and tobacco. This suggests that kidney function plays a key role in overall health status in sub-Saharan Africa and, since options for treatment of kidney failure are very restricted, should be included within public health targets. Improved understanding of the determinants of kidney disease and its progression are needed in order to inform interventions for prevention and treatment.

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Author Contributions Statement

RK conceived the idea. RK, KT, JS and RN and CHH advised on methodology. JS, RN, LS and LAT obtained the funding. RK, KT, BS, RM, PH, MKM, JS and RN collected and curated the data and administered the project. RK and IS analysed the data. RK and LAT wrote the original draft and all authors contributed to and approved the final manuscript.

Additional Information

Collaborators Statement

IS and KT have verified the underlying data. All authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication.

Declaration of interests

The authors declare that there are no conflicts of interest

Data sharing

Owing to data protection concerns, there are restrictions on access to the underlying data. The GPC database contains 25 years of longitudinal data sets on demographics and disease surveillance. All data (census, survey and laboratory) generated through the cohort are stored and curated at the MRC/UVRI and the LSHTM Research Unit. Data access for specific research purposes is possible and has been granted previously. For any data access inquiries, you may contact the director, MRC/UVRI and the LSHTM Research Unit or by email to mrc@mrcuganda.org or the corresponding author.

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Figure Legends

Figure 1 Title: Study Flow Chart

Figure 2 Title: Hazard ratios for the fully adjusted associations of baseline eGFR and mortality in Uganda

Legend: eGFR- estimated glomerular filtration rate

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5.0 Research paper 3: Association of impaired kidney function with mortality in rural Uganda: results of a general population cohort study.

Figure 1 Study flow chart

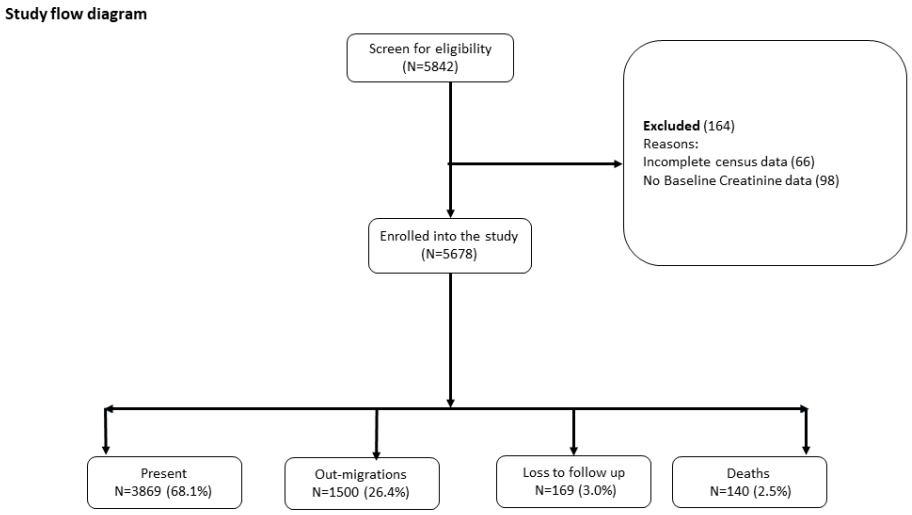
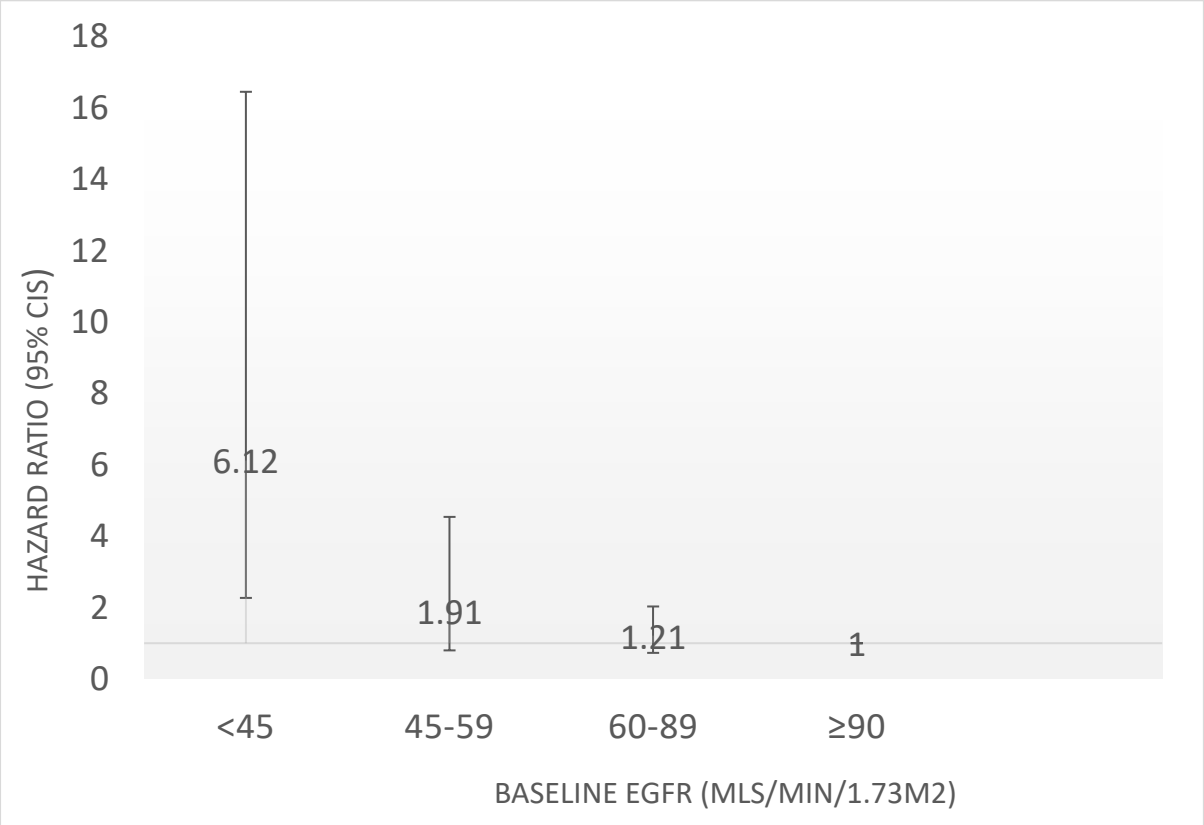


Figure 2 Hazard ratios for fully adjusted associations of baseline e GFR and mortality in Uganda



5.0 Research paper 3: Association of impaired kidney function with mortality in rural Uganda: results of a general population cohort study.

Supplementary appendix for research paper 3

Supplementary Table 1. Comparison between participants in the main analysis and all enrolled participants.

Variable	Participants with complete data included in main model N=3102	All enrolled participants N=5,678
eGFR (mls/min/1.73m^2)		
≥90	2231 (71·9)	4563 (80·3)
60-89	792 (25·6)	1022 (18·0)
45-59	63 (2·0)	73 (1·3)
<45	16 (0·5)	20 (0·4)
Gender		
Male	1150 (37·1)	2229 (39·3)
Female	1952 (62·9)	3449 (60·7)
Age (years), median (IQR)	45 (35-57)	36 (24-50)
Age groups, N (%)		
<35	733 (23·6)	2613 (46·0)
35-44	772 (24·9)	1128 (19·9)
45-54	682 (22·0)	842 (14·9)
55-64	451 (14·5)	540 (9·5)
65-75	291 (9·4)	343 (6·0)
>75	173 (5·6)	212 (3·7)
Currently married, *N(%)		
Married	978 (31·5)	1347 (23·7)
Single/widowed	2124 (68·5)	3072 (54·1)
Missing	..	1259 (22·2)
HIV status, **N (%)		
Negative	2756 (88·8)	5118 (90·3)
Positive	346 (11·2)	551 (9·7)
Missing	..	9 (0·2)
Alcohol Consumption, * N (%)**		
Regular alcohol	1601 (51·6)	2337 (41.1·)
Infrequent/no alcohol	1501 (48·4)	1661 (29.3·)
Missing	..	1680 (29·6)
Smoking Status, ** N (%)**		
Not current smokers	2757 (88·9)	3617 (63·8)
Non-daily smokers	79 (2·6)	96 (1·6)
Daily smokers	266 (8·5)	284 (5·0)
Missing	..	1681 (29·6)
Socioeconomic status[#]		
Lower	855 (27·5)	1377 (24·2)
Middle	815 (26·3)	1346 (23·7)
Upper	766 (26·7)	1326 (23.4·)
Missing	666 (21·5)	1629 (28·7)
Hypertension,[##] N (%)		
Normal	2583 (83·3)	3414 (85·4)
Hypertensive	519 (16·7)	584 (14·6)
Missing	..	1680 (29·6)
Diabetes mellitus, ### N (%)		
No	3025 (97·5)	3896 (68.·6)
Yes	77 (2·5)	85 (1·.4)
Missing	..	1702 (30·0)
BMI classification,[#####] (kg/m^2) N (%)		
Underweight (<18·5)	415 (13·4)	665 (11·7)
Normal weight (18·5–24·99)	2029 (65·4)	3879 (68.3·2)
Overweight (25·0–29·99)	484 (15·6)	734 (13·0)
Obese (>30·0)	174 (5·6)	247 (4·3)
Missing	..	153 (2·7)

5.0 Research paper 3: Association of impaired kidney function with mortality in rural Uganda: results of a general population cohort study.

Supplementary Table 2. Baseline characteristics of participants by five-level categories of kidney function in the general population cohort (n=5,678)

	Estimated glomerular filtration rate (mls/min/1.73m^2)					
Variable	≥120	90-119	60-89	45-59	<45	Total
Sex						
Male	709 (36·1)	1144 (44·1)	343 (33·6)	26 (35·6)	7 (35·0)	2229 (39·3)
Female	1257 (63·9)	1453 (56·0)	679 (66·4)	47 (64·4)	13 (65·0)	3449 (60·7)
Age (years), median (IQR)	22 (19-28)	41 (33-51)	56 (45-70)	66 (58-76)	68 (42-76)	36 (24-50)
Age groups, N (%)						
<35	1784 (90·7)	747 (28·8)	76 (7·4)	3 (4·1)	3 (15·0)	2613 (46·0)
35-44	158 (8·0)	788 (30·3)	176 (17·2)	4 (5·5)	2 (10·0)	1128 (19·9)
45-54	17 (0·9)	602 (23·2)	210 (20·6)	10 (13·7)	3 (15·0)	842 (14·8)
55-64	5 (0·3)	306 (11·8)	214 (20·9)	14 (19·2)	1 (5·0)	540 (9·5)
65-75	2 (0·1)	131 (5·0)	186 (18·2)	19 (26·0)	5 (25·0)	343 (6·0)
>75	0 (0·0)	23 (0·9)	160 (15·7)	23 (31·5)	6 (30·0)	212 (3·7)
Marital status, N (%)						
Married	162 (8·2)	691 (26·6)	444 (43·4)	37 (50·7)	13 (65·0)	1347 (23·7)
Single/widowed	888 (45·2)	1608 (61·9)	536 (52·5)	33 (45·2)	7 (35·0)	3072 (54·1)
Missing	916 (46·6)	298 (11·5)	42 (4·1)	3 (4·1)	0 (0·0)	1259 (22·2)
HIV status, N (%)						
Negative	1836 (93·4)	2298 (88·5)	901 (88·2)	70 (96·0)	13 (65·0)	5118 (90·1)
Positive	127 (6·5)	297 (11·4)	117 (11·5)	3 (4·1)	7 (35·0)	551 (9·7)
Missing	3 (0·2)	2 (0·1)	4 (0·4)	0 (0·0)	0 (0·0)	9 (0·2)
Alcohol Consumption, N (%)						
No	854 (43·4)	1057 (40·7)	393 (38·5)	29 (39·7)	4 (20·0)	2337 (41·2)
Yes	258 (13·1)	906 (34·9)	449 (43·9)	36 (49·3)	12 (60·0)	1661 (29·3)
Missing	854 (43·4)	634 (24·4)	180 (17·6)	8 (11·0)	4 (20·0)	1680 (29·6)
Smoking Status, N (%)						
Not current smokers	1061 (54·0)	1740 (67·0)	748 (73·2)	55 (75·3)	13 (65·0)	3617 (63·7)
Non-daily smokers	13 (0·7)	51 (2·0)	30 (2·9)	1 (1·4)	1 (5·0)	96 (1·7)
Daily smokers	38 (1·9)	171 (6·6)	64 (6·3)	9 (12·3)	2 (10·0)	284 (5·0)
Missing	854 (43·4)	635 (24·5)	180 (17·6)	8 (11·0)	4 (20·0)	1681 (29·6)
Socioeconomic status[#]						
Lower	417 (21·2)	647 (24·9)	289 (28·3)	21 (28·8)	3 (15·0)	1377 (24·3)
Middle	413 (21·0)	646 (24·9)	257 (25·2)	23 (31·5)	7 (35·0)	1346 (23·7)
Upper	431 (21·9)	598 (23·0)	272 (26·6)	21 (28·8)	4 (20·0)	1326 (23·4)
Missing	705 (35·9)	706 (27·2)	204 (20·0)	8 (11·0)	6 (30·0)	1629 (28·7)
Hypertension, [##] N (%)						
Normal	1064 (54·1)	1692 (65·2)	614 (60·1)	35 (47·9)	9 (45·0)	3414 (60·1)
Hypertensive	48 (2·4)	271 (10·4)	228 (22·3)	30 (41·1)	7 (35·0)	584 (10·3)
Missing	854 (43·4)	634 (24·4)	180 (17·6)	8 (11·0)	4 (20·0)	1680 (29·6)
Diabetes mellitus, [###] N (%)						
No	1096 (55·8)	1902 (73·2)	815 (79·8)	62 (84·9)	16 (80·0)	3926 (68·5)
Yes	13 (0·7)	49 (1·9)	20 (2·0)	3 (4·1)	0 (0·0)	85 (1·5)
Missing	857 (43·6)	646 (24·9)	187(18·3)	8 (11·0)	4 (20·0)	1702 (30·0)
BMI classification, [####] (kg/m^2) N (%)						
Underweight (<18·5)	185 (9·4)	319 (12·3)	143 (14·0)	14 (19·2)	4 (20·0)	665 (11·7)
Normal weight (18·5–24·99)	1429 (72·7)	1772 (68·2)	623 (61·0)	41 (56·2)	14 (70·0)	3879 (68·3)
Overweight (25·0–29·99)	195 (9·9)	357 (13·8)	166 (16·2)	14 (19·2)	2 (10·0)	734 (12·9)
Obese (≥30·0)	46 (2·3)	121 (4·7)	77 (7·6)	3 (4·1)	0 (0·0)	247 (4·4)
Missing	111 (5·7)	28 (1·1)	13 (1·3)	1 (1·4)	0 (0)	153 (2·7)

**Missing data largely due to different variables being collected in the 2011 and 2014 surveys of the GPC: Socio-economic status (SES) derived from conducting Principle Component Analysis (PCA) using variables relating to household infrastructure and property ownership [##]Hypertension was defined as having a systolic BP ≥90 mmHg, diastolic BP ≥140 mmHg. [###]Diabetes mellitus defined as HBA1C >6.5%, previously diagnosed with diabetes mellitus or being on treatment for diabetes. [####]Body Mass Index (BMI) Classification according to WHO (weight/height^2: kg/m^2).*

5.0 Research paper 3: Association of impaired kidney function with mortality in rural Uganda: results of a general population cohort study.

Supplementary Table 3. Results of age-sex and fully-adjusted regression models for the association between five category kidney function and mortality in the general population cohort

	Age and sex adjusted		Fully adjusted[#]	
	HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
eGFR (mls/min/1.73m^2)				
≥120	2·68 (1·47-4·87)		1·65 (0·61-4·44)	
90-119	**reference**		**reference**	
60-89	1·14 (0·73-1·77)		1·23 (0·73-2·06)	
45-59	1·67 (0·77-3·60)		1·90 (0·79-4·52)	
<45	5·58 (2·36-13·24)	<0·001	6·06 (2·25-16·37)	0·005
Age (years)*	1·06 (1·05-1·07)	<0·001	1·06 (1·04-1·08)	<0·001
Sex*				
Male	**reference**		**reference**	
Female	0·50 (0·36-0·70)	<0·001	0·71 (0·42-1·19)	0·19
HIV status				
Positive	2·29 (1·44-3·65)	<0·001	1·69 (0·89-3·23)	0·11
Negative				
Hypertension				
Normotensive	**reference**		**reference**	
Hypertensive	0·93 (0·60-1·43)	0·73	0·82 (0·51-1·33)	0·42
Diabetes mellitus				
No	**reference**		**reference**	
Yes	0·85 (0·27-2·67)	0·78	1·11 (0·34-3·56)	0·87
BMI classification				
Under weight	2·14 (1·49-3·09)		1·70 (1·09-2·66)	
Normal weight	**reference**		**reference**	
Over weight	0·21 (0·07-0·66)		0·20 (0·05-0·84)	
Obese	0·21 (0·03-1·50)	<0·001	0·35 (0·05-2·58)	0·006
Marital status				
Married	0·55 (0·37-0·81)	0·003	0·77 (0·48-1·22)	0·27
Single/widowed	**reference**		**reference**	
Alcohol consumption				
Regular alcohol	1·49 (0·99-2·26)	0·057	1·50 (0·92-2·45)	0·11
Infrequent/no alcohol	**reference**		**reference**	
Tobacco use				
Not current smokers	**reference**		**reference**	
Non-daily smokers	1·69 (0·79-3·58)		1·23 (0·51 - 3·00)	
Daily smokers	2·13 (1·32-3·43)	0·007	1·63 (0·92 - 2·86)	0·24

*Age adjusted for sex and sex for age: [#] Fully Adjusted Hazard Ratios adjusted for age, sex, HIV status, hypertension, diabetes mellitus, BMI, marital status, alcohol use and smoking. Number of people in the fully adjusted model is 3,102.

6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

6.1 Introduction

The best way to estimate kidney function in sub-Saharan Africa (sSA) is not well established. The current equations for estimating kidney function were developed in high income countries and may not directly be transferable to the individuals from sSA. Studies done so far in sSA show that the estimating equations may be inaccurate in estimating the kidney function in this population but have not had adequate sample size particularly for patients with lower levels of kidney function (eGFR <60mls/min/1.73m^2)[1-4]. All the afore mentioned studies provide growing evidence that the use of the ethnicity coefficient in estimating GFR among people of black colour may lead to greater overestimation and misclassification of kidney disease stage. In this chapter I share the findings from one of the largest studies globally, conducted in Malawi, South Africa and Uganda, where we measured GFR using iohexol and determined the accuracy of the most frequently used eGFR equations. The paper also explores attempts to develop a more accurate equation for sSA and use of other cohorts from Africa to estimate (impute) the prevalence of kidney disease in seven countries in Africa.

6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

6.2 Box summary of key findings for the ARK study

- Creatinine-based equations overestimate kidney function compared to measurement with plasma iohexol clearance or cystatin C among 2,578 participants.
- The adjustments for ethnicity in the existing equations lead to greater overestimation of GFR.
- None of the existing eGFR equations achieved substantial accuracy to guide clinical decision making: no equation estimated GFR within ±30% of measured GFR for 75% or more participants.
- We were not able to develop a more accurate sSA-specific eGFR formula largely because of the inaccuracies arising out of creatinine as a marker of kidney function measurement.
- Using a model to impute kidney function based on measured GFR, we estimated CKD prevalence to be two to three-fold higher compared to creatinine-based estimates in populations across six countries in sSA.

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6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1604453/REPH	**Title**	Dr.
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Thesis Title	Characterization of kidney disease in sub-Saharan Africa		
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If the Research Paper has previously been published, please complete Section B, if not please move to Section C.

SECTION B – Paper already published

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	The New England Journal of Medicine
Please list the paper's authors in the intended authorship order:	June Fabian, Robert Kalyesubula (co-first author), Joseph Mkandawire, Christian Holm Hansen, Dorothea Nitsch, Eustasius Musenge, Wisdom P Nakanga, Josephine E Prynn, Gavin Dreyer, Tracy Snyman, Billy Ssebunnya,

6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

	Michele Ramsay, Liam Smeeth, Stephen Tollman, Saraladevi Naicker, Amelia Crampin, Robert Newton, Jaya A George, Laurie Tomlinson on behalf of the African Research on Kidney Disease (ARK) Consortium
Stage of publication	Resubmission after appeal

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I collected the data, participated in data analysis, write up as first co-author and participated in all correspondence with the reviewers and the editors.

SECTION E

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Date	28 June 2021

Supervisor Signature	[redacted]
Date	28 June 2021

6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

Research paper 4

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Measurement of kidney function in Sub-Saharan Africa

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6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

	Tomlinson, Laurie; London School of Hygiene and Tropical Medicine Department of Non-communicable Disease Epidemiology
Abstract:	Background The burden of kidney disease in Sub-Saharan Africa (SSA) is unknown. Equations used to estimate kidney function from serum creatinine have limited regional validation. We sought to determine the most accurate way to measure kidney function and thus estimate the prevalence of impaired kidney function in SSA. Methods We measured serum creatinine, cystatin C, and glomerular filtration rate using the slope-intercept method for iohexol plasma clearance (mGFR). We compared performance of creatinine and cystatin C-based estimating equations to mGFR, modelled and validated a new equation, and used multiple imputation based on mGFR to predict the population prevalence of impaired kidney function. Results Among 2578 included participants, creatinine-based equations overestimated kidney function compared to mGFR. The greatest bias occurred at low kidney function so that the proportion with impaired kidney function (GFR <60ml/min/1.73m2) by mGFR or estimated by cystatin C was more than double that estimated from creatinine. A new creatinine-based equation did not outperform existing equations, and no equation estimated GFR within ±30% of mGFR for 75% or more participants. Using a model to impute kidney function based on mGFR, the estimated prevalence of impaired kidney function was two- to three-fold higher than creatinine-based estimates in populations across six SSA countries. Conclusion Estimating GFR using serum creatinine substantially underestimates the individual and population-level burden of impaired kidney function in SSA with implications for understanding disease progression and complications, clinical care and service provision. Scalable and affordable ways to accurately identify impaired kidney function in SSA are urgently needed.

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6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

Measurement of kidney function in Sub-Saharan Africa

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6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

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6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

Abstract

Background

The burden of kidney disease in Sub-Saharan Africa (SSA) is unknown. Equations used to estimate kidney function from serum creatinine have limited regional validation. We sought to determine the most accurate way to measure kidney function and thus estimate the prevalence of impaired kidney function in SSA.

Methods

We measured serum creatinine, cystatin C, and glomerular filtration rate using the slope-intercept method for iohexol plasma clearance (mGFR). We compared performance of creatinine and cystatin C-based estimating equations to mGFR, modelled and validated a new equation, and used multiple imputation based on mGFR to predict the population prevalence of impaired kidney function.

Results

Among 2578 included participants, creatinine-based equations overestimated kidney function compared to mGFR. The greatest bias occurred at low kidney function so that the proportion with impaired kidney function (GFR <60ml/min/1.73m^2) by mGFR or estimated by cystatin C was more than double that estimated from creatinine. A new creatinine-based equation did not outperform existing equations, and no equation estimated GFR within ±30% of mGFR for 75% or more participants. Using a model to impute kidney function based on mGFR, the estimated prevalence of impaired kidney function was two- to three-fold higher than creatinine-based estimates in populations across six SSA countries.

Conclusion

Estimating GFR using serum creatinine substantially underestimates the individual and population-level burden of impaired kidney function in SSA with implications for understanding disease progression and complications, clinical care and service provision. Scalable and affordable ways to accurately identify impaired kidney function in SSA are urgently needed.

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6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

Introduction

The prevalence of chronic kidney disease (CKD) in Sub-Saharan Africa (SSA) is unknown. Current estimates are 11-16% in those at high risk and 3-6% in population-representative studies. There is concern that these figures are not capturing the true burden of kidney disease for several reasons.[1] Creatinine-based equations to estimate glomerular filtration rate (eGFR) were developed in high-income countries with limited validation in African populations. Direct measurement of glomerular filtration rate (mGFR) using exogenous biomarkers such as iohexol or iothalamate needed to assess the accuracy of these equations is expensive and inaccessible in many SSA countries. The most commonly used Modification of Diet in Renal Disease (MDRD) Study and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations include coefficients adjusting eGFR for individuals with African ancestry (henceforward 'ethnicity coefficient'). These adjustments are based on studies showing participants with African ancestry had relatively higher mGFR for a given creatinine than other US population groups.[2] Studies in Africa suggest that ethnicity coefficients overestimate mGFR in continental Africans, possibly from smaller body surface area (BSA) and lower muscle mass compared to the African diaspora.[3-6] Additional sources of inaccuracy in determining CKD prevalence are wide biologic and analytic variation in creatinine measurement, leading to difficulties comparing results within and between countries and over time.[7,8]

Limited data on the accuracy of kidney function measurement in SSA impacts individuals with CKD, and more broadly, African populations. For an individual, missed opportunities to implement targeted interventions may result in progression to severe kidney disease with consequent premature disability or death.[9] The absence of accurate population data for prevalent CKD limits implementation of public health measures and integration of CKD into chronic disease management platforms.[10]

To address these knowledge gaps, we formed the African Research on Kidney Disease (ARK) Consortium, comprising centers of excellence from three longitudinal population studies in Malawi (urban and rural)[11] and Uganda (rural),[12] and a Health and Demographic Surveillance System site in South Africa (rural).[13] Our primary aim was to measure GFR using plasma clearance of iohexol in large, population-based samples from each country, and to compare the performance of available eGFR equations to iohexol mGFR. Our secondary aim was to model and externally validate an eGFR equation that better predicted kidney function in SSA, enabling accurate determination of the regional burden of impaired kidney function.

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6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

Methods

Study setting and sampling strategy

Our study methods are published.[14] In summary, all partners obtained ethics approval for their respective studies and all participants provided written informed consent. First, each partner measured kidney function in a large, population-representative sample..[16,17] From this, subsamples of potential adult participants for the iohexol GFR (mGFR) study were identified, with the estimated sample size (N=730) calculated for 90% power to detect whether eGFR was within 5% of mGFR at an eGFR of 60ml/min/1.73m^2, assuming a standard deviation of 25ml/min and two-sided α=0.05.[14] Samples were increased to 1000 per country to accommodate non-participation, technical and screening failures, and stratified by sex and eGFR stage to overrepresent lower levels of kidney function (**Section S1**).

Study procedures

All study protocols were harmonised prior to starting the study. We administered 5milliliters of Omnipaque™ (350mg iodine/ml, GE Healthcare, USA) as an intravenous bolus and calculated the dose of iohexol from pre- and post-administration syringe weights, measured in milligrams to two decimal points. We drew venous samples from the contralateral arm at 5,120, 180, and 240 minutes after iohexol administration, recording exact times for iohexol administration and sampling (**Section S2**).[18] We used the slope-intercept method to calculate mGFR for three time points in the slow exponential phase of iohexol elimination.

Laboratory methods and testing

Iohexol plasma samples were processed at each partner laboratory, stored at -112F, and shipped to a national reference laboratory in Johannesburg. Iohexol plasma concentrations were assayed using high-performance liquid chromatography-tandem mass spectrometry.[19] Coefficients of variation for internal quality control with the certified reference material for iohexol at 100 and 1000mg/L were 4.1% and 4.2% respectively. The laboratory complied with Equalis external quality assurance requirements for iohexol (Uppsala, Sweden) (**Section S3.1; Table S3.1**).[20]

In each country, laboratories performed serum creatinine measurements using an IDMS-traceable assay calibrated to a standard reference material for creatinine. The modified Jaffe method was used in Malawi and South Africa, and the enzymatic method in Uganda. For cystatin C, Malawian and Ugandan samples were analyzed in Uganda, while South African samples were measured in South Africa (**Section S3.2; Table S3.2**). Intersite analytic bias was assessed with a split sample recalibration study for creatinine and cystatin C (**Section S3.3; Table S3.3**).[21,22] Creatinine values for Malawi and South Africa

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were recalibrated by a constant factor of +0.11mg/dL to adjust for method-related bias and align with the Uganda enzymatic method (**Figures S3.1-2**). For cystatin C we used Passing-Bablok regression analysis to determine constants for recalibration and Uganda and Malawi cystatin C measurements were adjusted with South Africa as the reference (**Figures S3.3-4**). Recalibrated values for both analytes were used for all analyses.

GFR estimating equations

We evaluated the following eGFR equations: Cockroft Gault (adjusted for BSA);[23] Four variable MDRD re-expressed for IDMS-traceable assays;[24] CKD-EPI creatinine,[25] cystatin C and creatinine-cystatin C;[26] Revised Lund-Malmö Study;[27] and Full Age Spectrum (FAS) creatinine (**Section S4**).[28] For the FAS equation we derived country-specific healthy population creatinine values (Q) (**Table S4.1**).

Data management and statistical analysis

We included participants who met all the following criteria: complete recordings of age, sex, height, weight; exact times for administering intravenous iohexol (T0) and subsequent sampling; iohexol plasma concentrations at each timepoint and demonstrating a monotonic decline; pre- and post-administration syringe weights; serum creatinine concentration ≥0.34mg/dL. We examined distribution of data overall and by country, and evaluated agreement between mGFR and creatinine and cystatin C-based eGFR equations using Bland Altman plots. For MDRD and CKD-EPI equations, we evaluated performance with and without ethnicity coefficients. Using mGFR as the reference, for each eGFR equation, we compared performance using bias, precision, and accuracy and compared the proportion of participants correctly classified by mGFR stage. The parameters for performance expressed as GFR (mL/min/1.73m^2) were absolute bias: median difference of each individual's eGFR and their respective mGFR (eGFR-mGFR); relative bias: median percentage difference of (eGFR-mGFR), expressed as a percent; precision: interquartile range (IQR) of (eGFR-mGFR); precision: root mean square error (RMSE), representing standard deviation of (eGFR-mGFR); accuracy: median of the absolute difference of (eGFR-mGFR) expressed as a percent of mGFR for estimates within 10% (P_{10}) and 30% (P_{30}) of mGFR.Based on the British Nuclear Medicine Society Guidelines,[18] to evaluate whether our findings were influenced by systematic error we performed sensitivity analyses with three restricted datasets (i) r>0.985 for the slope-intercept measured GFR derivation; (ii) normal sex-specific calculated volumes of distribution (11-17 liters for women; 13-20 liters for men) (iii) a double-restricted dataset combining (i) and (ii).

Development of a new creatinine-based GFR estimating equation

We chose creatinine over cystatin C to ensure clinical utility in SSA. Using the whole mGFR sample we sought to develop a new eGFR model by regressing log mGFR on log creatinine as well as age and sex, with and without body mass index (BMI),

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mirroring the functional form of the CKD-EPI and Lund-Malmö eGFR equations. Regression coefficients were allowed to vary by sex, and the model included a spline knot with the position chosen using lowess plots. We used likelihood ratio tests and adjusted R^2 to decide between candidate models and assessed model performance as previously defined, compared to existing creatinine-based eGFR equations. For external validation, we compared the performance of eGFR from our model and existing equations to mGFR from radionuclide studies of 651 individuals (along with their creatinine measurements, age, and sex) obtained during cohort and clinical studies in South Africa.

Estimating true population prevalence of impaired kidney function in SSA

Based on population-based studies in SSA we sought to estimate the prevalence of impaired kidney function defined as eGFR<60mls/min/1.73m² (analogous to CKD stages G3a-5), using the most accurate eGFR equation. However, since the predictive power of all estimating equations was limited, we used a multiple imputation model trained on the measured mGFR sample, to predict individual GFR based on creatinine, age, and sex with predictions were based on 100 imputed datasets. The imputation model builds a predictive distribution around each unobserved GFR with variance and mean determined through a linear regression on the chosen predictor variables. We tested the model on the datasets from which it was trained and then used this approach within six distinct, large population-representative datasets: (i) the baseline prevalence studies in the ARK Consortium of adults 20-80 years;[16,17] (ii) the Africa Wits-International Network for the Demographic Evaluation of Populations and their Health Partnership for Genomic Studies (AWI-Gen) in Ghana, Kenya, and Burkina Faso and South Africa, which recruited adults 40-60 years.[29,30]

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Results

Characteristics of ARK participants in Malawi, South Africa, and Uganda

Across four study sites in three countries, we measured GFR using iohexol plasma excretion in 3,025 adults and included 2,578 participants in the final analysis (**Table 1**). The predominant reason for exclusion (n=266) was missing syringe weight during the pilot phase: a flowchart of derivation of the final study sample is shown in **Figure S4** and comparison of characteristics with the whole sample in **Table S5**. Mean age was 50±15 years, mean height and weight were 162±9 cm and 67±17 kg, respectively, and mean mGFR was 82±26 ml/min/1.73m². South African participants were taller and heavier with disproportionate obesity in women, contrasting with Ugandan participants who had the smallest body mass indices in the

study. The distribution of mGFR, volume of distribution (Vd) and correlation coefficient (r) was similar across countries (**Figures S5-7**).

Performance of eGFR equations against mGFR

Across all three countries, creatinine-based equations overestimated GFR compared to mGFR (**Figure 1; Figures S8-9**). The greatest bias, least precision, and least accuracy were observed with mGFR <45ml/min/1.73m^2 (**Figure 2; Table 2; Figures S10-11; Table S6**). When classifying by mGFR within categories analogous to CKD stages, eGFR equations overestimated G1 and underestimated lower stages of kidney function. All creatinine-based equations underestimated GFR stages 3a-5 by at least 50% (**Table S7**). Overestimation of individual GFR and misclassification by GFR stage was exacerbated when using ethnicity coefficients for the MDRD and CKD-EPI equations (**Figure 1; Table 2; Figure S12; Table S7**). The Revised Lund-Malmö Study equation correctly classified the highest proportion (54%) of participants by mGFR stage (**Table S7**).

Performance of estimating equations using cystatin C alone, or in combination with creatinine, was higher than with creatinine alone (**Figure 1; Table 2; Tables S6-7**). Although cystatin C underestimated stage G1, the estimates of prevalence of impaired kidney function were almost two-fold higher than creatinine-based estimates, mostly in stage G3a (**Table S7**). This translated to 76% of individuals being correctly classified as having a kidney function stage as or more severe than their corresponding mGFR stage, compared to 72% with the Lund-Malmö (revised) equation and 59% with the CKD-EPI equation (**Table S7**). The performance of equations at estimating GFR compared to mGFR was similar between countries (**Tables S8-10**).

Sensitivity analyses

We compared mGFR distribution (**Figure S13a-c**), agreement between eGFR and mGFR for each equation both overall (**Figure S14a-c**) and by GFR stage (**Table S11a-c**) for single and double-restricted datasets. Accuracy, as P_{30}, for CKD-EPI compared to mGFR was 65% in the unrestricted dataset, 69% and 67% in the single restricted datasets, and 71% in the dataset restricted for both Vd and r (**Table 2; Table S12a-c**). The best performing equation, Lund-Malmö, had a P_{30} of 87% in the double-restricted dataset. The P_{30} for CKD-EPI cystatin increased from 70% in the main dataset to 78% in the double-restricted dataset. However, there was also a disproportionate loss of people with low mGFR, from 6% to 2% in the double-restricted dataset (**Figure S15, Table S13a-c**). Measurements of low GFR have lower r without necessarily indicating substantial error in GFR measurement.[31] In addition, people with impaired kidney function may have abnormal fluid balance, while normal volumes of distribution have not been validated in SSA. Given the risk of creating systematic bias through data restriction and

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the only modest increase in performance for the equations in sensitivity analyses we used the complete dataset for further analyses.

Development of a new creatinine-based GFR estimating equation

We plotted the relationship between creatinine and mGFR and inspection of lowess curves (**Figures S16**) supported a piecewise linear model with one knot at 0.82mg/dL for men with no evidence to support fitting separate coefficients for creatinine among men and women before the knot, nor to support separate age coefficients for men and women. Including BMI in the model increased the adjusted R^2 but only from 0.225 to 0.234. We examined agreement between mGFR and the ARK models (**Figure S17**) and all equations had limited predictive power with chosen predictors accounting for 20-25% of the total variation in the data irrespective of the model form (**Table S14**). The final model was:

If male: $eGFR = 124 \times \min(1, SCr/0.82)^{-0.339} \times \max(1, SCr/0.82)^{-0.574} \times 0.993^{age}$

If female: $eGFR = 103 \times (SCr/0.82)^{-0.339} \times 0.993^{age}$

We used this equation to estimate GFR measurements among the 2,578 people in our primary dataset. Although model predictions were less biased than those of the CKD-EPI equation, our model showed limited discrimination and was not able to better predict kidney function than existing equations, categorizing 54% of people correctly for all GFR stages (**Tables S15-17**). In the external validation dataset (**Table S18**) our model did not have better performance than the CKD-EPI or Revised Lund-Malmö equations (**Tables S19-20**).

Estimating true population prevalence of impaired kidney function in SSA

The multiple imputation model predicted the proportion of people with impaired kidney function in the primary ARK datasets in which it was developed to be between 3.0% higher and 4.2% lower than mGFR (Uganda: observed 13.8% versus predicted 10.8%; Malawi: observed 20.4% versus predicted 24.6%; South Africa: observed 22.9% versus predicted 21.0%). In the external validation dataset from South Africa the same model predicted the proportion of people with impaired kidney function to be 20.9% versus observed 17.2%. With this method, we estimated the prevalence of impaired kidney function to be between 4.6% and 15.1% higher than when estimated using CKD-EPI creatinine in population-based countries in West, East, and southern Africa (**Figure 3**, **Tables S21-22**).

Discussion

Our results show that across three Sub-Saharan African countries, in diverse urban and rural populations, creatinine-based eGFR equations substantially overestimate kidney function compared to GFR measured with iohexol (mGFR). The

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overestimation worsened at lower levels of mGFR and was further exacerbated by inclusion of ethnicity coefficients. Cystatin C-based equations performed better than creatinine-based equations but none of the eGFR equations achieved an accuracy (P_{30}) considered appropriate for individual clinical decision-making.[32] At population level, using creatinine-based eGFR equations substantially underestimated the prevalence of impaired kidney function. A new creatinine-based equation to better estimate GFR in SSA was not possible due to wide age- and sex-independent variability in the relationship between creatinine and mGFR, even with weight or BMI adjustment. Therefore, we used a multiple imputation method to estimate population prevalence of impaired kidney function in countries across SSA and found the prevalence was substantially higher than that measured using creatinine-based estimates of GFR.

Our large, prospective study used population-representative samples with oversampling of those with poor kidney function, and used both creatinine and cystatin to estimate GFR. Aside from US-derived eGFR equations, we included the FAS equation[28] and Revised Lund-Malmö Study equation[27] not previously evaluated in SSA. We used a gold-standard multi-sample method for iohexol plasma clearance with centralized measurements in a reference laboratory with extensive experience using mass-spectrometry assays, compliant with the Equalis quality assurance program for iohexol. We addressed intersite analytic bias for creatinine and cystatin C and recalibrated measurements accordingly. However, there are also limitations to this work. Because we measured creatinine and mGFR at a single time point, we did not fulfil the temporal requirements for the definition of CKD[32] although our approach is in keeping with other large cohort studies of GFR.[34] In addition, participants were well, and therefore (aside from seasonal changes in eGFR),[33] we would not anticipate substantial variation in kidney function. Our sampling frame was population-based with high mean kidney function compared to CKD cohorts used to develop GFR equations.[2,26,35] Measurement of low creatinine values is more prone to biological and analytic variation than high level.s[36] It is possible that variability in direct GFR measurement inherent to a study largely conducted in rural SSA, despite rigorous quality control procedures, may have contributed to the reduced performance of GFR estimating equations. We have quantified this with transparently reported sensitivity analyses and in these estimating equations (compared to mGFR) did not attain the level of performance reported in studies in different settings.[6,26,37] These restricted analyses resulted in disproportionate exclusion of people with low GFR, where methods of determining quality of GFR measurement may not be appropriate,[31] and so we undertook further analysis in the whole dataset. In addition, prevalence of impaired kidney function measured with iohexol GFR was very similar to that estimated from cystatin, reinforcing that it is variability in the association between creatinine and GFR that leads to poor performance of eGFR in this population rather than measurement error of iohexol GFR. Finally, while our study is the largest of its kind from SSA and includes measured

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GFR from three countries, the diversity of African populations means that there is likely to be greater variability in the relationship between creatinine and GFR than we have measured. This could affect the accuracy of the imputed GFR estimates in the AWI-GEN datasets but in internal validation the model estimates prevalence of impaired kidney function close to that measured in the ARK datasets, and with differences substantially less than the variation we see between creatinine-based and imputed estimates.

Our finding that creatinine-based eGFR equations overestimate well-preserved kidney function (stages G1-2) but underestimate declining kidney function (stages G3-5) has been replicated in studies from Scandinavia and West Africa, [37,38] but differs from those in the Japan and the USA.[24,26,39] Greater inaccuracy in GFR estimation from creatinine-based equations has been described in Asia,[40] and there are reasons why the relationship between creatinine and GFR may be more variable in SSA compared to US or European populations. Our study and others report lower BMI, with likely lower muscle mass and therefore lower baseline creatinine, in continental Africans when compared to the African diaspora.[4,5,16,17] Perinatal and early childhood factors resulting in undernutrition and growth stunting, which predisposes to low lean muscle mass and short stature in adulthood even in the presence of adult obesity, are common in SSA.[13,41-43] Wasting from chronic infection or inflammation, such as tuberculosis and HIV, low dietary protein ingestion, and undiagnosed liver disease also impact muscle mass and creatinine generation.[44-47] Finally, variants in genes affecting pathways of creatinine production and tubular secretion may impact the association between serum creatinine and true GFR in SSA.[48,49]

The first implication of our results is to confirm conclusively that ethnicity coefficients exaggerate the overestimation of GFR by creatinine and should not be used in SSA. Secondly, overestimation of kidney function at lower levels of GFR impacts clinical care and public health. For an individual, inaccurate estimation of GFR risks a missed diagnosis of CKD and the opportunity to address modifiable risk factors to slow progression of kidney disease, and possible harm from unadjusted doses of renally cleared drugs. At population level underestimation of the burden of CKD in SSA limits prioritization of country-specific and regional strategies to minimize CKD progression and manage end-stage kidney disease. Limited access to specialist renal care in SSA compounds the individual risk for progression, premature disability, and death. While cystatin C identified a greater proportion of people with CKD and might be a valuable confirmatory test, assays are more expensive than creatinine and not widely available in SSA. There is therefore urgent need for further research to develop accurate and low-cost ways to measure kidney function in SSA.

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Conclusion

In this large collaborative study from three Sub-Saharan African countries, we prospectively measured kidney function using consistent and robust techniques. We showed that creatinine-based GFR estimating equations overestimate kidney function compared to iohexol and cystatin C measures. Our results suggest the burden of kidney disease is markedly underestimated in SSA, with substantial implications for individual health care and public health interventions to address the challenge of kidney disease in resource-limited settings.

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6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

Table 1: Characteristics of ARK participants by sex, for each country, and overall (N=2,578)

	MALAWI		SOUTH AFRICA		UGANDA		OVERALL
Characteristic[1]	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	
	N=474	**N=424**	**N=636**	**N=311**	**N=413**	**N=320**	**N=2,578[2]**
Age - year	53.1 (13.5)	52.8 (16.3)	45.6 (14.4)	44.5 (16.3)	50.6(14.2)	51.5 (15.1)	49.5 (15.2)
Age category							
<40 year	70 (14.8%)	87 (20.5%)	244 (38.4%)	138 (44.4%)	92 (22.3%)	74 (23.1%)	705 (27.4%)
40-60 year	269 (56.8%)	209 (49.3%)	280 (44.0%)	109 (35.1%)	232 (56.2%)	158 (49.4%)	1257 (48.8%)
>60 year	135 (28.5%)	128 (30.2%)	112 (17.6%)	64 (20.6%)	89 (21.6%)	88 (27.5%)	616 (23.9%)
BMI[3]	26.76 (5.86)	23.11 (3.86)	30.14 (6.41)	25.02 (5.08)	23.92 (4.47)	21.03 (3.12)	25.61 (5.98)
BMI category[4]							
<18.5 (underweight)	14 (3.0%)	25 (5.9%)	9 (1.4%)	14 (4.5%)	21 (5.1%)	63 (19.7%)	146 (5.7%)
18.5-24.9 (normal)	194 (40.9%)	293 (69.1%)	140 (22.0%)	162 (52.1%)	259 (62.7%)	222 (69.4%)	1270 (49.3%)
25.0-29.9 (overweight)	148 (31.2%)	86 (20.3%)	182 (28.6%)	84 (27.0%)	91 (22.0%)	32 (10.0%)	623 (24.2%)
≥30.0 (obese)	118 (24.9%)	20 (4.7%)	305 (48.0%)	51 (16.4%)	42 (10.2%)	3 (0.9%)	539 (20.9%)
Weight (kg)	65.00 (15.48)	63.24 (11.82)	78.70 (17.37)	74.64 (16.71)	57.56 (11.89)	56.95 (9.64)	67.06 (16.72)
Height (cm)	155.67 (5.99)	165.28 (6.83)	161.56 (5.91)	172.60 (7.14)	154.98 (6.83)	164.42 (6.36)	161.72 (8.53)
BSA(m^2)[5]	1.68 (0.22)	1.70 (0.18)	1.90 (0.23)	1.89 (0.24)	1.58 (0.19)	1.61 (0.16)	1.74 (0.24)
Serum creatinine[6]	0.73 (0.20)	0.92 (0.30)	0.61 (0.16)	0.83 (0.23)	0.74 (0.20)	0.89 (0.31)	0.77 (0.25)
Serum cystatin C[7]	0.97 (0.34)	1.01 (0.30)	0.99 (0.27)	1.01 (0.28)	0.88 (0.23)	0.91 (0.31)	0.97 (0.29)
Iohexol GFR[8]	74.8 (20.1)	79.1 (20.4)	78.6 (25.0)	82.0 (26.4)	86.1 (26.3)	97.1 (31.2)	81.9 (25.6)
Iohexol GFR category[9]							
G1: ≥90	100 (21.1%)	124 (29.3%)	205 (32.2%)	130 (41.8%)	160 (38.7%)	190 (59.4%)	909 (35.3%)

G2: 60-89	271 (57.2%)	220 (51.9%)	282 (44.3%)	113 (36.3%)	195 (47.2%)	87 (27.2%)	1168 (45.3%)
G3a: 45-59	76 (16.0%)	61 (14.4%)	92 (14.5%)	40 (12.9%)	42 (10.2%)	29 (9.1%)	340 (13.2%)
G3b: 30-44	21 (4.4%)	18 (4.3%)	49 (7.7%)	21 (6.8%)	10 (2.4%)	10 (3.1%)	129 (5.0%)
G4-5: <30	6 (1.3%)	1 (0.2%)	8 (1.3%)	7 (2.3%)	6 (1.45%)	4 (1.3%)	32 (1.2%)
Estimated GFR[9]	82.6 (19.4)	86.2 (20.1)	99.7 (19.1)	98.5 (21.3)	93.7 (19.9)	97.8 (20.5)	93.2 (21.0)
Estimated GFR category[10]							
G1: ≥90	174 (36.7%)	194 (45.8%)	468 (73.6%)	220 (70.7%)	241 (58.4%)	237 (74.1%)	1534 (59.5%)
G2: 60-89	245 (51.7%)	189 (44.6%)	146 (23.0%)	71 (22.8%)	154 (37.3%)	69 (21.6%)	874 (33.9%)
G3a: 45-59	41 (8.7%)	26 (6.1%)	17 (2.7%)	14 (4.5%)	13 (3.2%)	5 (1.6%)	116 (4.5%)
G3b: 30-44	12 (2.5%)	10 (2.4%)	4 (0.6%)	5 (1.6%)	3 (0.7%)	4 (1.3%)	38 (1.5%)
G4-5: <30	2 (0.4%)	5 (1.2%)	1 (0.2%)	1 (0.3%)	2 (0.5%)	5 (1.6%)	16 (0.6%)

[1]All data reported as mean (standard deviation); categories reported as number (%); percentages may sum to +/-100 due to rounding; [2]N=2,578 for creatinine samples; N=2433 for cystatin-C samples; [3]Body mass index (BMI) calculated by dividing weight (kilograms) by height squared (meters); [4]BMI category: WHO classification for obesity[46]; [5]Body surface area calculated using the Haycock formula[20]; [6]Serum creatinine in mg/dL (conventional units): to convert to µmol/L, multiply by 88.4; creatinine data unadjusted for intersite calibration study; creatinine data unadjusted for intersite calibration study; [7]Serum cystatin C in mg/dL: cystatin C data unadjusted for intersite calibration study; [8]Iohexol GFR: ml/min/1.73m^2; [9]Iohexol GFR category: GFR stage defined by KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease[32]; [10]Estimated GFR: ml/min/1.73m^2 using CKD-EPI creatinine equation (no ethnicity co-efficient)

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Table 2: Overall performance of GFR estimating equations compared to iohexol GFR (N=2,578)

Variable	Estimated GFR (ml/min/1.73m² of body surface area)				
Iohexol GFR (ml/min/1.73m² of BSA) GFR Stage	>=90 G1	60 – 89 G2	45 – 59 G3a	<45 G3b-5	overall
[1]Absolute bias (95% CI)					
Cockroft Gault	-1.6 (-3.9 - 0.3)	13.5 (11.8 - 15.1)	21.9 (18.0 - 25.7)	36.5 (31.3 - 44.5)	11.3 (10.1 - 12.7)
MDRD	-8.1 (-9.8 - -6.5)	8.6 (7.6 - 9.8)	20.3 (17.8 - 23.6)	34.5 (28.2 - 40.9)	6.6 (5.6 - 7.7)
MDRD + ethnicity coefficient	12.2 (10.9 - 14.5)	26.2 (25.0 - 27.7)	35.8 (33.2 - 40.2)	48.8 (42.0 - 57.0)	24.7 (23.4 - 25.7)
Full Age Spectrum$_{(creatinine)}$	-0.9 (-3.6 - 0.7)	14.1 (12.4 - 15.9)	23.2 (19.1 - 26.7)	36.0 (30.0 - 41.1)	11.2 (10.1 - 12.3)
Revised Lund-Malmö Study	-13.0 (-14.5- -11.6)	6.5 (5.7 - 7.4)	19.3 (16.7 - 22.3)	33.8 (27.5 - 38.8)	2.3 (1.3- 3.3)
CKD-EPI$_{(creatinine)}$	-1.1 (-3.4 - 0.35)	16.1 (14.9 - 17.2)	26.1 (23.5 - 29.9)	40.7 (32.7- 47.2)	11.5 (10.6 - 12.7)
CKD-EPI$_{(creatinine)}$ + ethnicity coefficient	15.2 (13.0 - 17.4)	30.9 (29.3 - 32.0)	39.0 (35.6 - 43.4)	53.9 (44.2 - 59.7)	26.7 (25.4 - 27.6)
CKD-EPI$_{(creatinine + cystatin C)}$	-7.6 (-9.5 - -6.2)	6.9 (5.7 - 8.5)	16.5 (13.4 - 21.0)	27.2 (22.9 - 36.4)	4.1 (3.1 - 5.1)
CKD-EPI$_{(cystatin C)}$	-15.0 (-17.2- -13.0)	0.5 (-1.4 - 2.1)	9.6 (6.8 - 12.4)	20.6 (13.9 - 27.7)	-1.7 (-2.9 - -0.8)
[2]Relative bias (95% CI)					
Cockroft Gault	0.99 (0.96 - 1.00)	1.18 (1.16 - 1.20)	1.40 (1.35 - 1.47)	1.97 (1.83 - 2.27)	1.15 (1.13 - 1.17)
MDRD	0.92 (0.91 - 0.94)	1.12 (1.10 - 1.13)	1.39 (1.34 - 1.45)	1.99 (1.75 - 2.11)	1.08 (1.07 - 1.10)
MDRD + ethnicity coefficient	1.12 (1.10 - 1.14)	1.35 (1.34 - 1.37)	1.68 (1.62 - 1.76)	2.41 (2.12 - 2.56)	1.31 (1.30 - 1.33)
Full Age Spectrum$_{(creatinine)}$	0.99 (0.97 - 1.01)	1.19 (1.17 - 1.21)	1.43 (1.37 - 1.49)	2.00 (1.81 - 2.15)	1.14 (1.13 - 1.16)
Revised Lund-Malmö Study	0.88 (0.87 – 0.89)	1.08 (1.07 - 1.10)	1.36 (1.30 - 1.42)	1.94 (1.68 - 2.06)	1.03 (1.02 - 1.04)
CKD-EPI$_{(creatinine)}$	0.99 (0.97- 1.00)	1.21 (1.19 - 1.23)	1.50 (1.43-1.56) l	2.12 (1.93 - 2.26)	1.15 (1.13 - 1.16)
CKD-EPI$_{(creatinine)}$ + ethnicity coefficient	1.15 (1.12 - 1.16)	1.41 (1.38 - 1.43)	1.74 (1.66 - 1.81)	2.46 (2.23 - 2.62)	1.33 (1.31 - 1.35)
CKD-EPI$_{(creatinine + cystatin C)}$	0.93 (0.91 - 0.94)	1.09 (1.08 - 1.11)	1.31 (1.24 - 1.39)	1.72 (1.58 - 1.96)	1.05 (1.04 - 1.07)
CKD-EPI$_{(cystatin C)}$	0.86 (0.84 - 0.88)	1.01 (0.98 - 1.03)	1.17 (1.13 - 1.24)	1.55 (1.37 - 1.73)	0.98 (0.96 - 0.99)

[3]Precision (RMSE) (95% CI)					
Cockroft Gault	31.5 (30.1 - 33.0)	26.7 (25.7 - 27.9)	29.0 (27.0 - 31.4)	34.9 (31.4 - 39.2)	31.7 (30.8 - 32.6)
MDRD	26.5 (25.4 - 27.8)	20.9 (20.1 - 21.8)	21.4 (19.9 - 23.2)	28.3 (25.5 - 31.8)	27.1 (26.4 - 27.9)
MDRD + ethnicity coefficient	30.5 (29.2 - 32.0)	25.0 (24.0 - 26.1)	25.9 (24.1 - 28.0)	33.8 (30.5 - 37.9)	30.1 (29.3 - 30.9)
Full Age Spectrum$_{(creatinine)}$	28.0 (26.8 - 29.3)	21.4 (20.6 - 22.3)	23.5 (21.9 - 25.5)	29.9 (27.0 - 33.6)	27.8 (27.0 - 28.6)
Revised Lund-Malmö Study	21.2 (20.3 - 22.3)	15.3 (14.7 - 16.0)	18.1 (16.9 - 19.6)	25.0 (22.6 - 28.1)	24.0 (23.3 - 24.6)
CKD-EPI$_{(creatinine)}$	22.9 (21.9 - 24.0)	17.6 (16.9 - 18.4)	21.2 (19.7 - 22.9)	28.5 (25.7 - 32.0)	24.9 (24.3 - 25.6)
CKD-EPI$_{(creatinine)}$ + ethnicity coefficient	24.9 (23.8 - 26.1)	20.2 (19.4 - 21.0)	24.4 (22.7 - 26.4)	32.6 (29.4 - 36.7)	26.4 (25.7 - 27.2)
CKD-EPI$_{(creatinine + cystatin C)}$	21.9 (20.9 - 23.0)	17.5 (16.8 - 18.2)	20.3 (18.9 - 22.0)	27.4 (24.6 - 30.8)	23.8 (23.1 - 24.5)
CKD-EPI$_{(cystatin C)}$	24.1 (23.0 - 25.3)	20.6 (19.8 - 21.5)	21.9 (20.3 - 23.8)	27.3 (24.6 - 30.8)	25.9 (25.2 - 26.6)
[4]Precision (IQR) (95% CI)					
Cockroft Gault	40.4 (37.6 - 43.2)	34.2 (31.6 - 36.7)	42.0 (35.6 - 48.5)	51.0 (42.6 - 59.5)	37.6 (35.7 - 39.5)
MDRD	32.2 (29.3 - 35.1)	26.0 (24.1 - 27.8)	28.2 (23.7 - 32.7)	35.9 (30.5 - 41.4)	31.1 (29.6 - 32.6)
MDRD + ethnicity coefficient	37.8 (34.9 - 40.7)	30.5 (28.5 - 32.6)	33.7 (28.0 - 39.3)	44.2 (37.3 - 51.2)	35.7 (34.0 - 37.3)
Full Age Spectrum$_{(creatinine)}$	35.6 (32.5 - 38.6)	26.1 (24.3 - 30.0)	34.2 (30.5 - 37.8)	44.7 (35.5 - 54.0)	31.4 (29.8 - 33.0)
Revised Lund-Malmö Study	24.4 (22.6 - 26.1)	19.9 (18.5 - 21.2)	25.6 (22.3 - 28.9)	35.7 (29.2 - 42.3)	26.9 (25.6 - 28.2)
CKD-EPI$_{(creatinine)}$	27.3 (25.2 - 29.4)	24.1 (22.3 - 25.9)	31.3 (27.8 - 34.8)	40.5 (33.8 - 47.2)	28.5 (27.1 - 30.0)
CKD-EPI$_{(creatinine)}$ + ethnicity coefficient	30.5 (27.8 - 33.2)	27.2 (25.3 - 29.2)	36.5 (32.3 - 40.7)	46.9 (39.0 - 54.8)	31.3 (29.7 - 32.7)
CKD-EPI$_{(creatinine + cystatin C)}$	27.1 (24.8 - 29.4)	24.4 (22.9 - 25.9)	28.8 (26.0 - 31.7)	39.7 (32.6 - 46.8)	27.4 (26.0 - 28.8)
CKD-EPI$_{(cystatin C)}$	31.2 (28.7 - 33.6)	27.9 (26.0 - 29.9)	28.9 (23.1 - 34.7)	35.2 (27.0 - 43.3)	30.7 (28.9 - 32.5)
[5]Accuracy % P_{10} (95% CI)					
Cockroft Gault	0.27 (0.24 - 0.30)	0.24 (0.22 - 0.27)	0.17 (0.13 - 0.21)	0.08 (0.04 - 0.13)	0.23 (0.22 - 0.25)
MDRD	0.32 (0.29 - 0.35)	0.29 (0.27 - 0.32)	0.14 (0.10 - 0.18)	0.05 (0.02 - 0.10)	0.27 (0.25 - 0.28)
MDRD + ethnicity coefficient	0.27 (0.25 - 0.30)	0.15 (0.13 - 0.17)	0.04 (0.02 - 0.07)	0.04 (0.02 - 0.09)	0.17 (0.16 - 0.19)

Full Age Spectrum$_{(creatinine)}$	0.32 (0.29 - 0.35)	0.25 (0.22 - 0.28)	0.14 (0.10 - 0.18)	0.06 (0.03 - 0.11)	0.25 (0.23 - 0.26)
Revised Lund-Malmö Study	0.36 (0.33 - 0.40)	0.36 (0.34 - 0.39)	0.16 (0.12 - 0.20)	0.08 (0.04 - 0.13)	0.32 (0.30 - 0.34)
CKD-EPI$_{(creatinine)}$	0.42 (0.39 - 0.45)	0.23 (0.21 - 0.26)	0.12 (0.09 - 0.16)	0.06 (0.03 - 0.11)	0.27 (0.26 - 0.29)
CKD-EPI$_{(creatinine)}$ + ethnicity coefficient	0.26 (0.23 - 0.29)	0.11 (0.09 - 0.13)	0.04 (0.02 - 0.07)	0.04 (0.01 - 0.08)	0.15 (0.14 - 0.16)
CKD-EPI$_{(creatinine + cystatin C)}$	0.36 (0.33 - 0.40)	0.32 (0.29 - 0.34)	0.18 (0.14 - 0.22)	0.07 (0.04 - 0.12)	0.30 (0.28 - 0.32)
CKD-EPI$_{(cystatin C)}$	0.29 (0.26 - 0.32)	0.27 (0.25 - 0.30)	0.25 (0.20 - 0.30)	0.08 (0.04 - 0.13)	0.26 (0.25 - 0.28)
[6]Accuracy % (1 - P_{10}) (95% CI)					
Cockroft Gault	0.73 (0.70 - 0.76)	0.76 (0.73 - 0.78)	0.83 (0.79 - 0.87)	0.92 (0.87 - 0.96)	0.77 (0.75 - 0.78)
MDRD	0.68 (0.65 - 0.71)	0.71 (0.68 - 0.74)	0.86 (0.82 - 0.90)	0.95 (0.90 - 0.98)	0.73 (0.72 - 0.75)
MDRD + ethnicity coefficient	0.73 (0.70 - 0.76)	0.85 (0.83 - 0.88)	0.96 (0.94 - 0.98)	0.96 (0.91 - 0.98)	0.83 (0.82 - 0.84)
Full Age Spectrum$_{(creatinine)}$	0.68 (0.65 - 0.71)	0.75 (0.73 - 0.78)	0.86 (0.82 - 0.90)	0.94 (0.89 - 0.97)	0.75 (0.74 - 0.77)
Revised Lund-Malmö Study	0.64 (0.60 - 0.67)	0.64 (0.61 - 0.67)	0.84 (0.80 - 0.88)	0.92 (0.87 - 0.96)	0.68 (0.66 - 0.70)
CKD-EPI$_{(creatinine)}$	0.58 (0.55 - 0.61)	0.77 (0.74 - 0.79)	0.88 (0.84 - 0.92)	0.94 (0.89 - 0.97)	0.73 (0.71 - 0.75)
CKD-EPI$_{(creatinine)}$ + ethnicity coefficient	0.74 (0.71 - 0.77)	0.89 (0.87 - 0.91)	0.96 (0.93 - 0.98)	0.96 (0.92 - 0.99)	0.85 (0.84 - 0.86)
CKD-EPI$_{(creatinine + cystatin C)}$	0.64 (0.60 - 0.67)	0.68 (0.66 - 0.71)	0.82 (0.78 - 0.86)	0.93 (0.88 - 0.97)	0.70 (0.68 - 0.72)
CKD-EPI$_{(cystatin C)}$	0.71 (0.68 - 0.74)	0.73 (0.70 - 0.75)	0.75 (0.70 - 0.80)	0.92 (0.87 - 0.96)	0.74 (0.72 - 0.76)
[5]Accuracy % P_{30} (95% CI)					
Cockroft Gault	0.73 (0.70 - 0.76)	0.61 (0.58 - 0.64)	0.39 (0.34 - 0.44)	0.18 (0.12 - 0.25)	0.60 (0.58 - 0.62)
MDRD	0.78 (0.75 - 0.81)	0.72 (0.69 - 0.75)	0.40 (0.35 - 0.45)	0.16 (0.10 - 0.22)	0.66 (0.65 - 0.68)
MDRD + ethnicity coefficient	0.68 (0.65 - 0.71)	0.42 (0.39 - 0.45)	0.17 (0.13 - 0.22)	0.10 (0.06 - 0.16)	0.46 (0.44 - 0.48)
Full Age Spectrum$_{(creatinine)}$	0.78 (0.75 - 0.81)	0.64 (0.61 - 0.66)	0.38 (0.33 - 0.44)	0.19 (0.14 - 0.26)	0.63 (0.61 - 0.64)
Revised Lund-Malmö Study	0.83 (0.81 - 0.86)	0.83 (0.81 - 0.85)	0.43 (0.38 - 0.48)	0.20 (0.14 - 0.27)	0.74 (0.72 - 0.76)
CKD-EPI$_{(creatinine)}$	0.88 (0.85 - 0.90)	0.63 (0.61 - 0.66)	0.32 (0.27 - 0.37)	0.15 (0.10 - 0.21)	0.65 (0.63 - 0.67)
CKD-EPI$_{(creatinine)}$ + ethnicity coefficient	0.75 (0.72 - 0.78)	0.35 (0.32 - 0.38)	0.16 (0.13 - 0.21)	0.11 (0.07 - 0.17)	0.45 (0.43 - 0.47)

CKD-EPI$_{(creatinine + cystatin C)}$	0.80 (0.77 - 0.82)	0.73 (0.71 - 0.76)	0.45 (0.39 - 0.50)	0.20 (0.14 - 0.27)	0.69 (0.67 - 0.7
CKD-EPI$_{(cystatin C)}$	0.71 (0.68 - 0.74)	0.71 (0.69 - 0.74)	0.54 (0.49 - 0.60)	0.32 (0.25 - 0.40)	0.67 (0.65 - 0.6
[6]Accuracy % (1 - P_{30}) (95% CI)					
Cockroft Gault	0.27 (0.24 - 0.30)	0.39 (0.36 - 0.42)	0.61 (0.55 - 0.66)	0.82 (0.75 - 0.88)	0.40 (0.38 - 0.4
MDRD	0.22 (0.19 - 0.25)	0.28 (0.25 - 0.31)	0.60 (0.55 - 0.66)	0.84 (0.78 - 0.90)	0.34 (0.32 - 0.3
MDRD + ethnicity coefficient	0.32 (0.29 - 0.35)	0.58 (0.55 - 0.61)	0.83 (0.78 - 0.87)	0.90 (0.84 - 0.94)	0.54 (0.52 - 0.5
Full Age Spectrum$_{(creatinine)}$	0.22 (0.20 - 0.25)	0.36 (0.34 - 0.39)	0.62 (0.56 - 0.67)	0.81 (0.74 - 0.87)	0.37 (0.36 - 0.3
Revised Lund-Malmö Study	0.17 (0.14 - 0.19)	0.17 (0.15 - 0.19)	0.57 (0.52 - 0.62)	0.80 (0.73 - 0.86)	0.26 (0.25 - 0.2
CKD-EPI$_{(creatinine)}$	0.12 (0.10 - 0.15)	0.37 (0.34 - 0.39)	0.68 (0.63 - 0.73)	0.85 (0.79 - 0.90)	0.35 (0.33 - 0.3
CKD-EPI$_{(creatinine)}$ + ethnicity coefficient	0.25 (0.22 - 0.28)	0.65 (0.62 - 0.68)	0.84 (0.79 - 0.87)	0.89 (0.83 - 0.93)	0.55 (0.53 - 0.5
CKD-EPI$_{(creatinine + cystatin C)}$	0.20 (0.18 - 0.23)	0.27 (0.24 - 0.29)	0.55 (0.50 - 0.61)	0.80 (0.73 - 0.86)	0.31 (0.30 - 0.3
CKD-EPI$_{(cystatin C)}$	0.29 (0.26 - 0.32)	0.29 (0.26 - 0.31)	0.46 (0.41 - 0.51)	0.68 (0.60 - 0.75)	0.33 (0.31 - 0.3

N=2,578 for creatinine-based equations; N=2433 for cystatin C-based equations; all values reported with 95% confidence intervals in parentheses; [1]Absolute bias: median (estimated GFR - iohexol GFR); [2]Relative bias: median ([estimated GFR - iohexol GFR]/iohexol GFR); [3] Precision RMSE (Root Mean Square Error): standard deviation (estimated GFR - iohexol GFR);[4]Precision IQR (Interquartile Range): IQR (estimated GFR - iohexol GFR); [5]Accuracy: median (estimated GFR - iohexol GFR) expressed as a percent of iohexol GFR for estimates within 10% (P10) and within 30% of iohexol GFR; [6] Accuracy: median (estimated GFR - iohexol GFR) expressed as a percent of iohexol GFR for estimates that differed by more than 10% (1-P10) and more than 30% (1-P30) of iohexol GFR.

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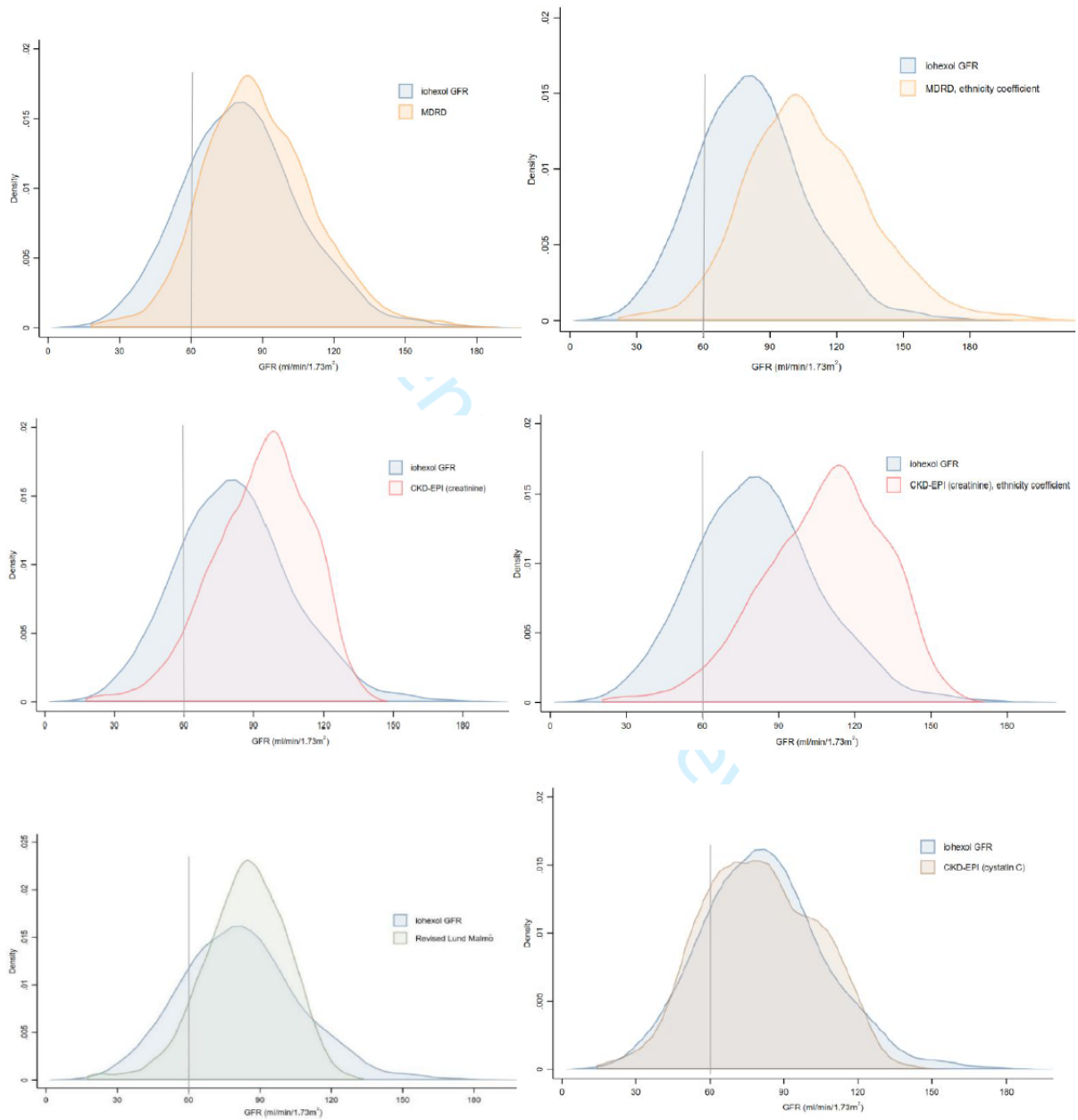
Figure 1: Comparison of GFR distributions between GFR estimating equations and iohexol GFR for the ARK Study



Data shown are from the pooled ARK Iohexol Study including Malawi, South Africa, and Uganda; N=2578 for creatinine-based equations; N=2433 for cystatin C-based equations; vertical line represents GFR cut-off of 60ml/min/1.73m²

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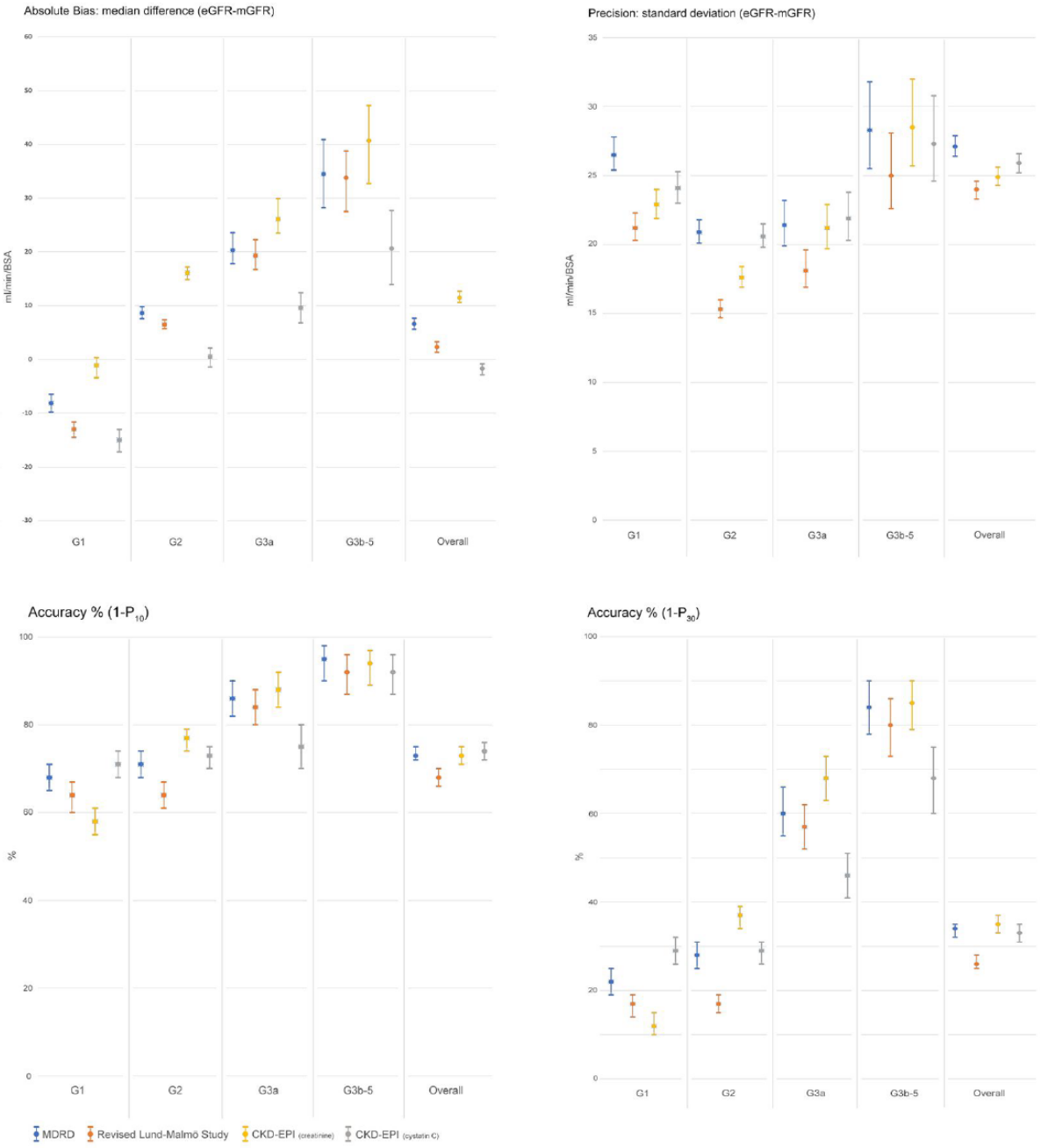
Figure 2: Bias, precision, accuracy of GFR estimating equations compared to iohexol GFR for the ARK Study



Accuracy depicted by the percentage of estimates that were greater than 10% (1-P_{10}), and 30% (1-P_{30}) of iohexol GFR. Vertical bars for data points represent the 95% confidence intervals

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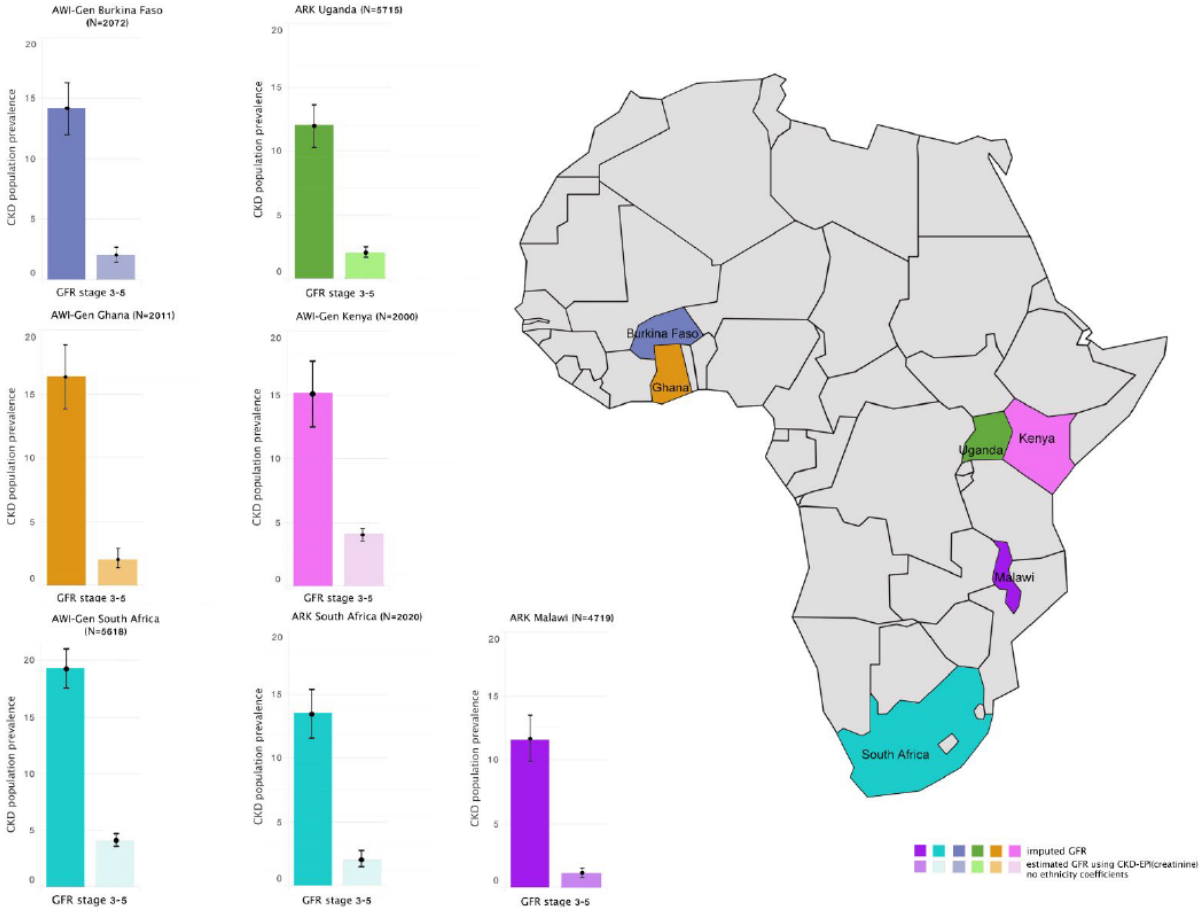
Figure 3: Predicted prevalence of impaired kidney function in Sub-Saharan Africa using imputed GFR compared to estimated GFR (CKD-EPI creatinine) across West, East, and Southern Africa*



Population-based datasets were derived from ARK Malawi, South Africa and Uganda for ages 20 - 80 years; and AWI-GEN countries including Burkina Faso, Ghana, Kenya, and South Africa for ages 40 - 60 years; datasets for ARK and AWI-Gen South Africa did not overlap; prevalence is not adjusted for age distributions

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For the South African study site, study data were collected and managed using REDCap electronic data capture tools hosted at the University of the Witwatersrand.[44,45] We would like to acknowledge Professor Mboyo-Di-Tamba Vangu, Head of the Department of Nuclear Medicine, Charlotte Maxeke Johannesburg Academic Hospital, Emeritus Professor Geoffrey Candy, University of the Witwatersrand, and Professor Jean F. Botha, Head of the Wits Transplant Unit, Wits Donald Gordon Medical Centre, University of the Witwatersrand for granting the ARK Consortium access to clinical records and radionuclide GFR test results that comprised the external validation dataset. We would like to acknowledge Marike Mapham for the design and layout of the graphics.

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6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

Appendix for Research Paper 4 Measurement of kidney function in Sub-Saharan Africa

Table of Contents

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6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

Because of the density of the data in these tables, the only way we could ensure readability, was to insert them in PDF format:

6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

SECTION S1 – STUDY SETTING AND SAMPLING STRATEGY

The African Research on Kidney Disease (ARK) Study is a multicentre study nested in three longitudinal population studies in Malawi (urban and rural) and Uganda (rural), and a Health and Demographic Surveillance System (HDSS) site in South Africa (rural). The Malawi Epidemiology and Intervention Research Unit (MEIRU) has an urban site in Lilongwe and a rural site in the northern Karonga district; in Uganda the General Population Cohort is located in the Kalunga district, south western Uganda; and in South Africa, the Medical Research Council/Wits University Rural Public Health and Health Transitions Research Unit is located in Bushbuckridge, a rural subdistrict of the Mpumalanga province, in the north eastern part of South Africa.[1] Each partner country obtained prior ethics approval for their respective studies and all participants signed written informed consent. For Uganda, ethics approvals were obtained from the Uganda Virus Research Institute (UVRI-REC-#HS 1978) and the Uganda National Council for Science and Technology (UNCST-#SS 4283). For Malawi, permission was granted from the Malawi National Health Sciences Research Committee (#1072). For South Africa, permission was obtained from the University of the Witwatersrand Human Research Ethics Committee (#160938).

Prior to this study, each country determined their population prevalence of Impaired kidney function based on estimating glomerular filtration rate (eGFR) using the CKD-EPI (creatinine) equation without ethnicity coefficient.[2,3] Then, we classified participants by eGFR stage (G1-5), and selected a subsample of 1000 adults from each country stratified by sex and eGFR stage. We intended to recruit 3000 participants in total, based on a power calculation for the number of study participants needed to examine the accuracy of eGFR equations for predicting iohexol (measured) GFR in each country. We specified this as having 90% power to detect whether eGFR is within 5% of iohexol GFR at an eGFR of 60mls/min, assuming a standard deviation of 25mls/mins and a two-sided α=0.05. This gives

an estimated required sample size of 730 participants, however, to allow for participants who do not wish to participate and technical and screening failures, we oversampled to 1000 participants in each country.

SECTION S2 – STUDY PROCEDURES

Potential participants, 18 years and older, were screened for contraindications to intravenous iohexol administration, namely pregnancy or breastfeeding in women, uncontrolled epilepsy, severe, uncontrolled hypertension, or any acute pyrexial illness.

If eligible, a trained fieldworker obtained written, informed consent in the participant's first language. On the day of iohexol measurement, we recorded blood pressure, temperature, height and weight, and trained nurses established two intravenous (IV) access points in the antecubital fossa (in most cases) of the dominant and contralateral arm. From the dominant arm, we drew a baseline serum sample for creatinine and cystatin C and flushed the cannula with 10ml normal saline. A single bolus of 5ml Omnipaque^{TM} (350mg iodine/ml) was administered intravenously over 30 seconds, followed by a 10ml normal saline flush the cannula was removed. Time zero (T0) was the start time of administration of the intravenous bolus of iohexol. In the contralateral arm, at 5, 120, 180, and 240 minutes from T0, 1ml of aspirated venous blood was discarded, followed by collection of 4mls of venous blood in heparinized tubes. For each sampling time, we recorded the exact time of sample collection. Using a calibrated scale, each syringe was weighed pre- and post-administration of iohexol in grams, to two decimal points. These weights were used to calculate the dose of iohexol administered.

To calculate iohexol GFR adjusted for body surface area (BSA)(mL/min/1.73m^2) from plasma clearance of iohexol, we used the slope-intercept method for three time points (using the exact time of measurement of the samples intended to be taken at 120, 180 and 240 minutes) in the second (slow) exponential phase of iohexol elimination.[4] The extrapolated y-

intercept is the calculated concentration of iohexol at time zero, and the coefficient, k, is the slope of the semilog plot of plasma iohexol concentration against time [$P(t)=P_0 \exp(-kt)$]. We adjusted for body surface area using the Haycock formula [$BSA = 0.024265*Wt^{0.5378} * Ht^{0.3964}$] followed by the Brochner-Mortensen (BM) correction for adults, to account for iohexol plasma clearance in the first (rapid) exponential phase [$BM = (0.9908*GFR) – (0.001218*GFR^2)$].[5,6]

SECTION S3 – LABORATORY METHODS AND TESTING

In this section we detail laboratory methods and quality control measures for (i) iohexol in South Africa; (ii) creatinine in Malawi, South Africa, and Uganda; (iii) cystatin C testing in South Africa and Uganda. To test for systematic differences in laboratory procedures between partner countries, we conducted a recalibration study with split sample testing, which was particularly relevant as we knew that Malawi and South Africa were using the modified Jaffe method for creatinine and Uganda was using an enzymatic method. For this recalibration, 20 serum samples from Malawi and 50 serum samples from Uganda were split, and re-assayed in South Africa. Agreement between paired measurements was analysed using Bland Altman plots and, where appropriate, Passing-Bablok regression was used to derive calibration equations to correct for systematic between-country differences.

S3.1 Iohexol

In each partner country, iohexol plasma samples were processed on the same day of collection and stored at -112°F. Iohexol measurement was centralized and each country shipped samples to the National Health Laboratory Services (NHLS) in Johannesburg, South Africa. The NHLS is accredited to the ISO 15189 standard and participates in the Equalis External Quality Assurance Programme for iohexol (Uppsala, Sweden).[7] Using a published method, all iohexol samples were analysed using ultra high performance liquid chromatography-tandem mass spectrometry ((SCIEX (Redwood, CA, USA) 5500 QTRAP

(LC-MS/MS)).[8] Certified reference materials for iohexol (CRM: USP H0J211), and ioversol (CRM: USP 34510F) were purchased from Industrial Analytical (Kyalami, South Africa) for the calibration curve and internal standard, respectively. Both compounds were lyophilised and weighed to make a stock standard of 10g/L in deionised water for iohexol, and methanol for ioversol. Working standards were then prepared with further dilutions to create a three-point calibration curve of 50mg/L, 500mg/L, and 1500mg/L for iohexol by spiking the stock standard into drug-free serum. The working solution for ioversol was prepared by diluting the stock standard in methanol to a final concentration of 20mg/L. Internal quality control (IQC) samples were prepared by spiking the certified reference material for iohexol (standard) to create final concentrations of 100 and 1000 mg/L for respective low and high internal quality control (IQC). Coefficients of variation for internal quality control with the iohexol standard at 100 and 1000mg/L were 4.1% and 4.2% respectively. Equalis samples were included in every run as an additional quality check. Iohexol and ioversol were eluted on a gradient profile using a 2.7µm Halo C18 (0.3 x 50mm) column purchased from SCIEX (Redwood, CA, USA). Mass spectrometry was carried out using electrospray ionisation in positive mode (ESI+) and multiple reaction monitoring was used for identification of iohexol and ioversol with transitions of 821.7>803.7m/z and 807.9>589m/z, respectively. Equalis samples run as quality controls within each batch were within the accepted calculated z-score of < 2.0.

Table S3.1: NHLS External Quality Assurance Compliance (Equalis AB)

Year	**Assigned value for iohexol concentration (mg/L)**	**Measured mean (SD) for iohexol concentration (mg/L)**	**CV (ratio SD/mean)%**
2018			
1a	54.4	55.0 (1.6)	2.9
1b	38.0	37.5 (1.1)	2.9
2a	98.7	99.3 (6.0)	6.0
2b	54.3	52.5 (2.3)	4.4

2019			
1a	38.0	38.7 (2.8)	7.3
1b	19.2	18.5 (0.6)	3.5
2a	56.8	56.7 (2.7)	4.7
2b	19.5	19.4 (1.0)	5.0

S3.2 Creatinine and Cystatin C

Malawi, South Africa, and Uganda each performed their own creatinine measurements. All partner countries standardised their creatinine assays using an isotope-dilution mass spectrometry (IDMS) assay traceable to a standard reference material (967) for creatinine. The modified Jaffe method was used in Malawi and South Africa, and the enzymatic method in Uganda. For cystatin C measurements, Uganda and Malawi samples were processed by the Uganda laboratory, with South African samples processed by the NHLS in Johannesburg. The South African and Ugandan laboratories each procured the Tina-quant® Cystatin C Gen.2 test kits from the same batch and ran all samples after completion of the study. The assay is an immunoturbidimetric assay standardised to the ERM-DA471/IFCC reference material (Roche Diagnostics, USA).

Table S3.2: ARK laboratory analytic methods for serum creatinine and cystatin C

Laboratory	Laboratory instrument	Laboratory assay
Malawi	Beckman Coulter BD AU480 chemistry analyser	Creatinine: modified Jaffe method, standardised to an isotope-dilution mass spectrometry (IDMS) assay
South Africa	Roche Cobas C501 (6000) analyser	Creatinine: modified Jaffe method, standardised to an isotope-dilution mass spectrometry (IDMS) assay
Uganda	Roche Cobas C501 (6000) analyser	Creatinine: enzymatic method, standardised to an isotope-dilution mass spectrometry (IDMS) assay
South Africa	Roche Cobas E602	cystatin C: immunoturbidimetric method, standardised using ERM-DA471/IFCC reference material
Uganda	Roche Cobas C501 (6000) analyser	cystatin C: immunoturbidimetric method, standardised using ERM-DA471/IFCC reference material

S3.3 Recalibration and split sample testing study for creatinine and cystatin C

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Systematic bias may arise between study site laboratories because of different pre-analytic and analytic methods for biomarker measurement. As far as possible, bias can be eliminated in the pre-analytic stages through standardising study protocols prior to initiation of the study. Bias during the analytic stage can be controlled by a recalibration study, where measurements from one study site are recalibrated to the measurements in a reference study site. Recalibration allows for correction of inter-site laboratory differences in time or space, which relate to the types of assay, the manufacturer, and the analytic platform.[9] Despite the inherent quality assurance of manufacturer assays and the availability of standardised reference materials to aid calibration, evidence suggests that some assays do not meet optimal bias limits and calibration differences persist, explaining the need for rigorous internal and external quality control procedures in each study laboratory. All these potential sources of analytic bias may impact research data. For epidemiological studies involving population-level data, these small systematic differences may result in a shift of the distribution of a biomarker potentially biasing estimates of mean values and the prevalence of dichotomously defined variables, for example, the presence or absence of kidney disease.[9] In this recalibration and split sample testing study, we assessed interlaboratory bias for serum creatinine and cystatin C biomarkers measured in the ARK study partner laboratories. If significant systematic differences were observed, we determined the recalibration equation to correct for the analytic bias. We assessed between-partner laboratory creatinine and cystatin C measurement variability by shipping stored, randomly selected split serum samples from Uganda (n=50) and Malawi (n=20) for repeat testing in South Africa as the reference laboratory.

Quality control procedures

Daily internal quality control was performed as per standard laboratory practice and inter-assay coefficients of variation for each laboratory biomarker were calculated based on

analyses of commercial controls. For external quality assurance, standard serum is distributed to the participating laboratory for testing of common analytes. For creatinine, the laboratory in Malawi complied with the requirements of the Thistle QA Laboratory Services (South Africa) requirements, and likewise, the Uganda and South Africa laboratories met the requirements of the College of American Physicians (CAP) Quality Assurance Program. For cystatin C, the South Africa laboratory complied with the requirements of the Equalis External Quality Assurance Program (Uppsala, Sweden). The Uganda and South Africa laboratories are accredited to the ISO 15189 standard.

Recalibration of creatinine

For Uganda and Malawi respectively, we compared the split sample results from the reference laboratory in South Africa using scatter and differential plots. Outliers were flagged for review by the study team and defined as creatinine values more than three standard deviations from the mean difference between paired values.[9] A single outlier for creatinine measurement from Uganda was excluded after confirming it was transcriptional error during data entry, and there were no further outliers. Agreement analysis was performed using Bland-Altman plots and Lin's concordance correlation coefficient.

Recalibration of cystatin C

We used a similar approach to the recalibration of creatinine. Cystatin C measurements for Malawi and Uganda were performed in the Uganda laboratory, so for this component of the study we compared split samples from Uganda to repeat samples in South Africa reference laboratory. Initially, scatter and differential plots were examined, no outliers were identified, and the agreement analysis proceeded. A cumulative sum (Cusum) test was performed to assess the linearity and regression analysis (Passing-Bablok) was used to determine the calibration function for the relationship between the paired cystatin C values - assuming the

regression equation Y= A(intercept) +B(slope)*X. Statistical calculations were performed in Stata/SE, version 16.1.

Calibration for serum creatinine

Compared to split samples for creatinine in the South Africa laboratory, the correlation was good, and the relationship linear for Malawi and Uganda creatinine values (Table S2, Figure S1). With South Africa as reference, the bias was -2.70µmol/L (95% CI -6.70 – 1.59) and +9.29 µmol/L (95% CI 7.25 – 11.33 µmol/L) for Malawi and Uganda creatinine samples, respectively. Since laboratories in Malawi and South Africa used the modified Jaffe method, and Uganda used the enzymatic method, the systematic bias was ascribed to these methodological differences. Ideally, KDIGO recommends the enzymatic method in preference to Jaffe, as the former is less biased and less prone to interference.[10] On this basis, we recalibrated creatinine values for Malawi and South Africa by a constant factor of +9.29µmol/L to align with the Uganda laboratory.

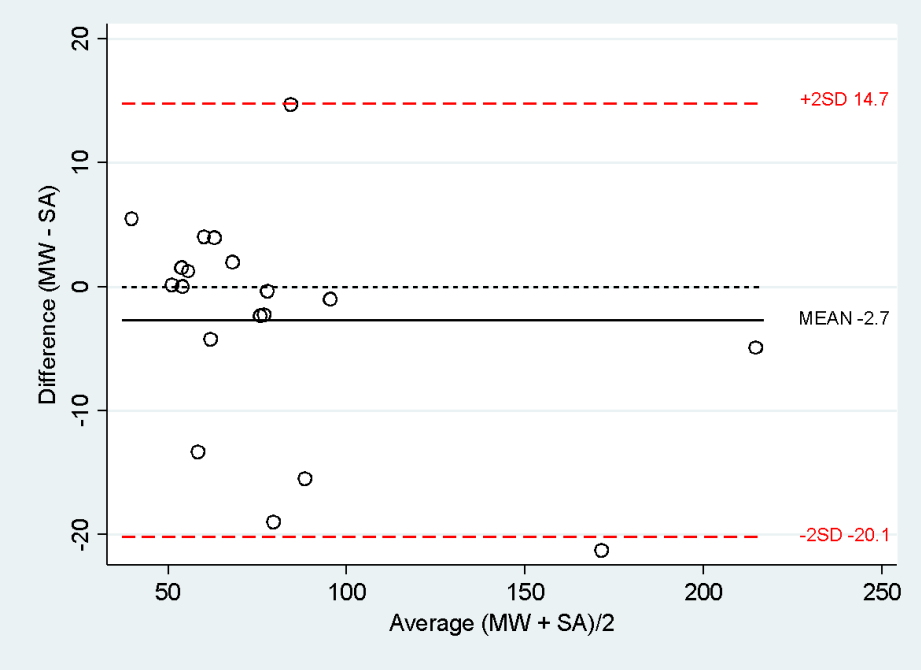
Calibration for cystatin C

When comparing Uganda cystatin C values to South Africa, the correlation was good, and the relationship linear with a bias of 0.10mg/dL (95% CI 0.36 – 0.17). With South Africa as the reference laboratory for the split sample, Passing-Bablok regression analysis was performed to determine the constants for the recalibration equation. (Table S2; Figure S2). The resulting regression coefficients (slope and intercept) were used to recalibrate the Uganda and Malawi cystatin C measurements for comparability to the South Africa reference laboratory values.

Table S3.3: Agreement and recalibration coefficients for creatinine and cystatin C

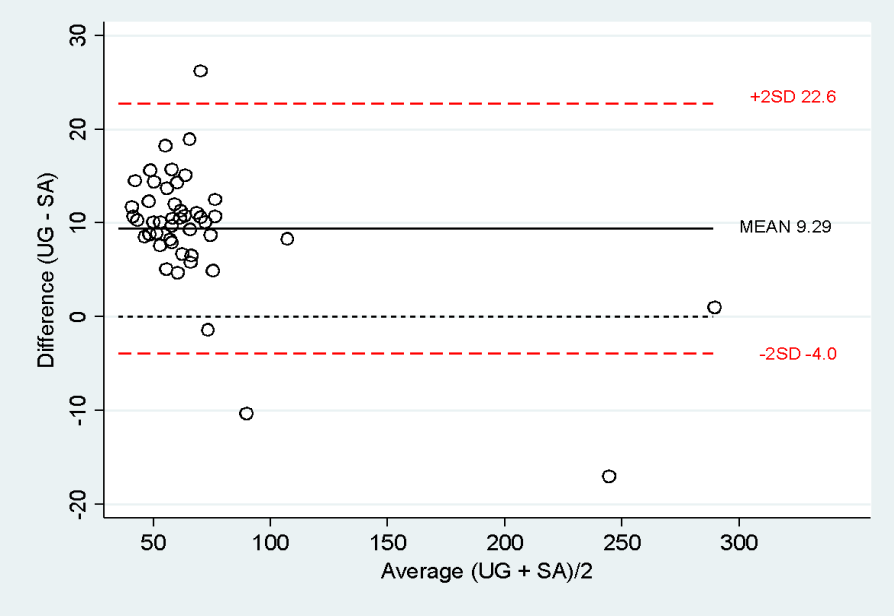
Country	Analyte	No of excluded outliers	R^2 Lin's Concordance Correlation Coefficient	Intercept	Slope
Malawi	creatinine(µmol/L)	0	0.977	n/a	n/a
Uganda	creatinine(µmol/L)	1	0.967	n/a	n/a
Uganda	Cystatin C (mg/dl)	0	0.942	0.178 (95% CI 0.090 – 0.349)	0.922 (95% CI 0.776 – 1)

Figure S3.1: Agreement: serum creatinine measurements for Malawi and South Africa



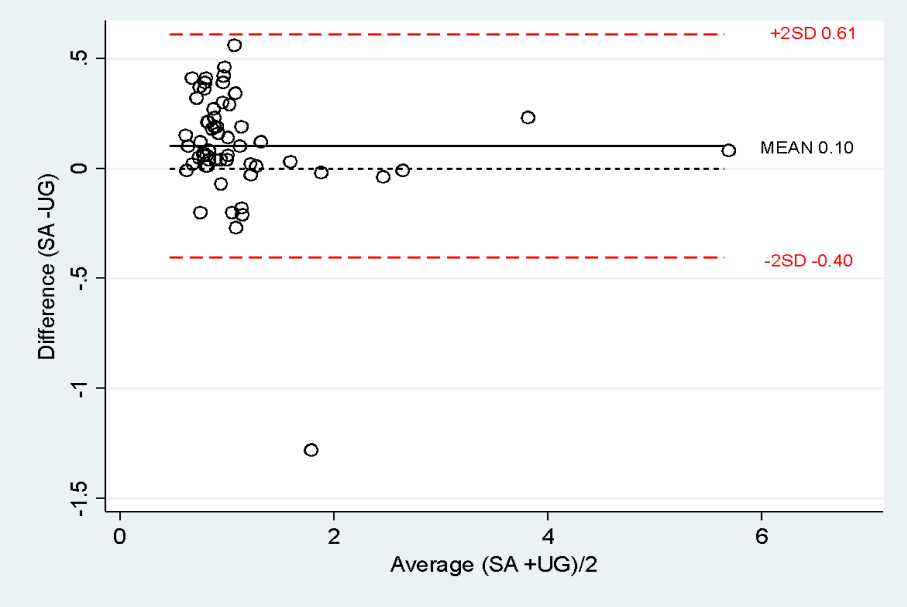
*Dashed red lines represent the 95% confidence intervals for creatinine in the calibration samples.

Figure S3.2: Agreement: serum creatinine measurements for Uganda and South Africa



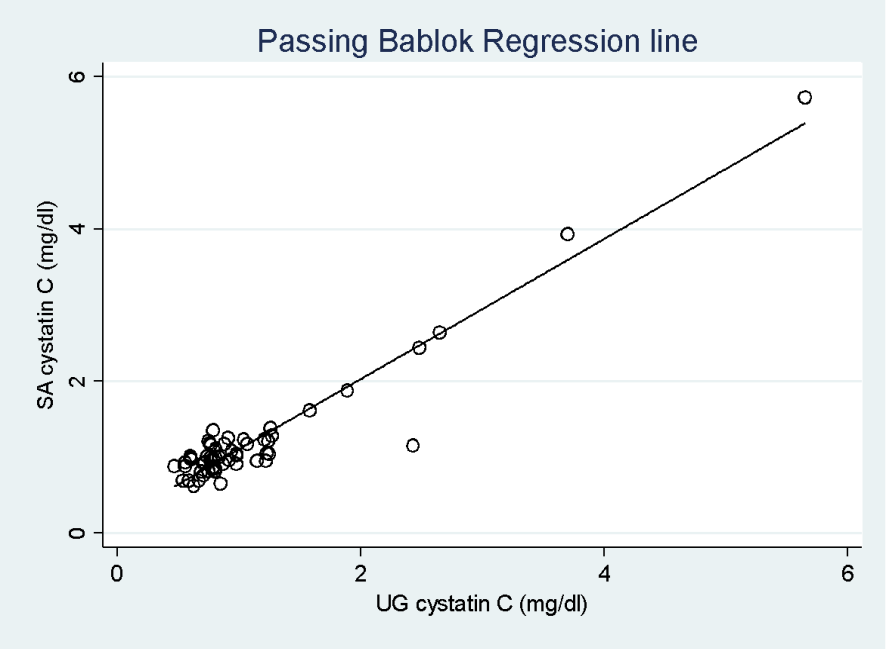
*Dashed red lines represent the 95% confidence intervals for creatinine in the calibration samples.

Figure S3.3: Agreement: serum cystatin C measurements in Uganda and South Africa



*Dashed red lines represent the 95% confidence intervals for cystatin C in the calibration samples.

Figure S3.4: Recalibration of cystatin C in South Africa and Uganda



SECTION S4 – GFR PREDICTION EQUATIONS

For all equations, we used standard conventional units (mg/dL) for serum creatinine (sCr) values rounded to the nearest 100th of a whole number. When sCr was expressed as standard international (SI) units (µmol/L), we rounded to the nearest whole number. The formula to convert sCr from conventional to SI units = [sCr (conventional units) x 88.4]. The units for serum cystatin C (scysC) are mg/l, and all equations were adjusted for body surface area (BSA) with units for GFR as mL/min/1.73m^2. We evaluated the performance of the following serum creatinine and serum cystatin C-based GFR prediction equations:

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Cockroft Gault equation adjusted for BSA

GFR = [140-age (years) x weight (kg) x (0.85 if female) x $1.73m^2$)] / [sCr x BSA^5 (m^2)]

In its original form, the Cockroft Gault equation did not adjust for body surface area (BSA).[11] However, this adjustment is required when comparing performance to other eGFR equations (all of which are adjusted for BSA). For our analyses we adjusted Cockroft Gault for BSA using the Haycock formula.[5]

4-variable Modification of Diet in Renal Disease (4-vMDRD) equation[12*]

GFR = 175 x sCr-1.154 x age-0.203(years) (x 0.742 if female) (x 1.1212 if African American)

*re-expressed for IDMS assays traceable to a standard reference material for creatinine

2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation[13]

$GFR = 141 \times \min(sCr/\kappa, 1)^{\alpha} \times \max(sCr/\kappa, 1)^{-1.209} \times 0.993^{age}$ (× 1.018 if female) (× 1.159 if African American)

κ is 0.7mg/dl (62 µmol/L) for females and 0.9mg/dl (80 µmol/L) for males
α is -0.329 for females and -0.411 for males

min indicates the minimum of sCr/κ or 1
max indicates the maximum of sCr/κ or 1

Equations expressed for specified sex and serum creatinine level[10]

Female ≤0.7 mg/dl (≤62 mmol/L) = 144 x $(sCr/62)^{-0.329}$ x 0.993^{age} [x 1.159 if African American]
Female >0.7 mg/dl (>62 mmol/L) = 144 x $(sCr/62)^{-1.209}$ x 0.993^{age} [x 1.159 if African American]
Male ≤0.9 mg/dl (≤80 mmol/L) = 141 x $(sCr/80)^{-0.411}$ x 0.993^{age} [x 1.159 if African American]
Male >0.9 mg/dl (>80 mmol/L) = 141 x $(sCr/80)^{-1.209}$ x 0.993^{age} [x 1.159 if African American]

2012 CKD-EPI cystatin C equation[14]

$GFR = 133 \times \min(scysC/0.8, 1)^{-0.499} \times \max(scysC/0.8, 1)^{-1.328} \times 0.996^{age}$ (x 0.932 if female)

min indicates the minimum of scysC/0.8 or 1

max indicates the maximum of scysC/0.8 or 1

Equations expressed for serum cystatin C level[10]

scysC ≤0.8: $133 \times (scysC/0.8)^{-0.499} \times 0.996^{age}$ [x 0.932 if female]

scysC >0.8: $133 \times (scysC/0.8)^{-1.328} \times 0.996^{age}$ [x 0.932 if female]

2012 CKD-EPI creatinine-cystatin C equation[14]

$GFR = 135 \times \min(sCr/\kappa, 1)^{\alpha} \times \max(SCr/\kappa, 1)^{-0.601} \times \min(scysC/0.8, 1)^{-0.375} \times \max(scysC/0.8, 1)^{-0.711} \times 0.995^{age}$ (x 0.969 if female) (x 1.08 if African American)

κ is 0.7mg/dl (62 µmol/L) for females and 0.9mg/dl (80 µmol/L) for males
α is -0.248 for females and -0.207 for males

min indicates the minimum of sCr/κ or 1
max indicates the maximum of sCr/κ or 1
min(scysC/0.8, 1) indicates the minimum of scysC/0.8 or 1
max(scysC/0.8,1) indicates the maximum of scysC/0.8 or 1

Equations expressed for specified sex, serum creatinine, and serum cystatin C level[10,15]

Female sCr≤62 cysC≤0.8 = $130 \times (sCr/62)^{-0.248} \times (scysC/0.8)^{-0.375} \times 0.995^{age}$ [x 1.08 if African American]
Female sCr≤62 cysC>0.8 = $130 \times (sCr/62)^{-0.248} \times (scysC/0.8)^{-0.711} \times 0.995^{age}$ [x 1.08 if African American]
Female sCr>62 cysC≤0.8 = $130 \times (sCr/62)^{-0.601} \times (scysC/0.8)^{-0.375} \times 0.995^{age}$ [x 1.08 if African American]
Female sCr>62 cysC>0.8 = $130 \times (sCr/62)^{-0.601} \times (scysC/0.8)^{-0.711} \times 0.995^{age}$ [x 1.08 if African American]
Male sCr≤80 cysC≤0.8 = $135 \times (sCr/80)^{-0.207} \times (/scysC/0.8)^{-0.375} \times 0.995^{age}$ [x 1.08 if African American]
Male sCr≤80 cysC>0.8 = $135 \times (sCr/80)^{-0.207} \times (scysC/0.8)^{-0.711} \times 0.995^{age}$ [x 1.08 if African American]
Male sCr≤80 cysC≤0.8 = $135 \times (sCr/80)^{-0.601} \times (scysC/0.8)^{-0.375} \times 0.995^{age}$ [x 1.08 if African American]
Male sCr≤80 cysC>0.8 = $135 \times (sCr/80)^{-0.601} \times (scysC/0.8)^{-0.711} \times 0.995^{age}$ [x 1.08 if African American]

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Revised Lund-Malmö Study equation[15]

GFR = eX–0.0158×Age+0.438×ln(Age)

Female sCr<150: X = 2.50+0.0121 × (150–sCr)
Female sCr≥150: X = 2.50–0.926 × ln(sCr/150)
Male sCr<180: X = 2.56+0.00968 × (180–sCr)
Male sCr≥180: X = 2.56–0.926 × ln(sCr/180)

Full Age Spectrum (FAS) creatinine equation[16]

$$GFR = \frac{107.3}{(sCr/Q)} \quad (\text{if} \leq 40 \text{ years})$$

$$GFR = \frac{107.3}{(sCr/Q)} \times 0.988^{(Age\ -40)} \quad (\text{if} > 40 \text{ years})$$

[sCr/Q (female) = 0.70mg/dL or 61.88µmol/L; sCr/*Q (male) = 0.90mg/dL or 79.56µmol/L.[17]]

Population reference creatinine for the FAS (creatinine) equation in the ARK Study

One of the variables needed to estimate GFR using the FAS equation (creatinine) is a population-specific reference serum creatinine measurement.[16,17] We used the population prevalence data from the baseline ARK studies to establish country specific population reference creatinine measures. To do this, we included all serum creatinine measures ≥30µmol/L from apparently healthy adults, defined as those without hypertension, HIV infection, diabetes, obesity (BMI >30kg/m^2), and we excluded all participants recruited for this iohexol measured GFR study to ensure independent datasets. The median creatinine for men and women, by country, was used to calculate the Q-value for the FAS equation.

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Table S4.1: Population reference serum creatinine measurements for the FAS equation, by country and sex

Country	Sex	Sample size (N)	Serum Creatinine (median, µmol/L)	Serum Creatinine (median, mg/dL)
Malawi	Female	1693	67	0.76
	Male	1542	84	0.95
South Africa	Female	269	64	0.73
	Male	337	81	0.92
Uganda	Female	2111	59	0.67
	Male	1585	72	0.81

FIGURES AND TABLES

Figure S4: Derivation of the ARK iohexol GFR (mGFR) study participants

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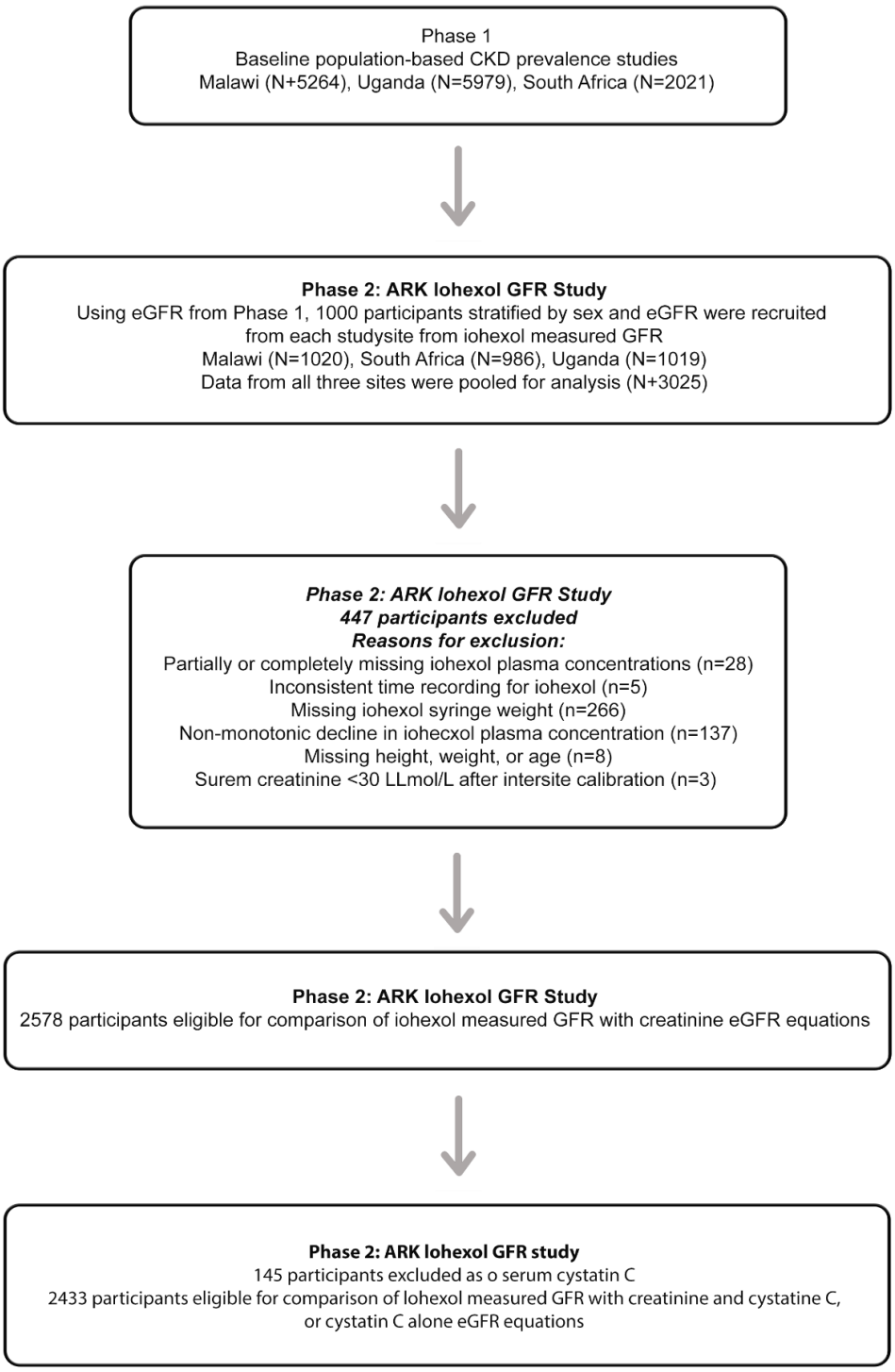

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Table S5: Characteristics of the ARK iohexol GFR study participants before (N=3025) and after exclusions (N=2578), by sex, by country, and overall

	Pooled Sample (N=3025)							Pooled Sample after exclusions (N=2578)						
Characteristic[1]	**Malawi**		**South Africa**		**Uganda**		**Overall**	**Malawi**		**South Africa**		**Uganda**		**Overall**
	Females	**Males**	**Females**	**Males**	**Females**	**Males**		**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	
	N=542	**N=477**	**N=664**	**N=322**	**N=561**	**N=458**	**N=3025**	**N=474**	**N=424**	**N=636**	**N=311**	**N=413**	**N=320**	**N=2578**
Age - yr	53.2 (13.4)	53.0 (16.3)	45.7 (14.4)	44.4 (16.3)	50.9 (14.4)	51.5 (15.2)	49.9 (15.2)	53.1 (13.5)	52.8 (16.3)	45.6 (14.4)	44.5 (16.3)	50.6(14.2)	51.5 (15.1)	49.5 (15.2)
Age group category														
<40 yr	77 (14.3%)	95 (19.9%)	251 (37.8%)	143 (44.4%)	126 (22.5%)	106 (23.1%)	798 (26.4%)	70 (14.8%)	87 (20.5%)	244 (38.4%)	138 (44.4%)	92 (22.3%)	74 (23.1%)	705 (27.4%)
40-60 yr	304 (56.6%)	235 (49.3%)	293 (44.1%)	113 (35.1%)	311 (55.4%)	219 (47.8%)	1475 (48.8%)	269 (56.8%)	209 (49.3%)	280 (44.0%)	109 (35.1%)	232 (56.2%)	158 (49.4%)	1257 (48.8%)
>60 yr	156 (29.1%)	147 (30.8%)	120(18.1%)	66 (20.5%)	124 (22.1%)	133 (29.0%)	752 (24.9%)	135 (28.5%)	128 (30.2%)	112 (17.6%)	64 (20.6%)	89 (21.6%)	88 (27.5%)	616 (23.9%)
Body mass index[2]	26.63 (5.78)	23.25 (3.98)	30.11 (6.36)	25.04 (5.10)	23.91 (4.49)	21.05 (3.09)	25.35 (5.87)	26.76 (5.86)	23.11 (3.86)	30.14 (6.41)	25.02 (5.08)	23.92 (4.47)	21.03 (3.12)	25.61 (5.98)
Body mass index category[3]														
<18.5 (underweight)	18 (3.3%)	27 (5.7%)	9 (1.4%)	15 (4.7%)	32 (5.7%)	89 (19.5%)	190 (6.3%)	14 (3.0%)	25 (5.9%)	9 (1.4%)	14 (4.5%)	21 (5.1%)	63 (19.7%)	146 (5.7%)
18.5-24.9 (normal)	224 (41.3%)	323 (67.9%)	146 (22.0%)	166 (51.6%)	344 (61.4%)	318 (69.7%)	1521 (50.4%)	194 (40.9%)	293 (69.1%)	140 (22.0%)	162 (52.1%)	259 (62.7%)	222 (69.4%)	1270 (49.3%)
25.0-29.9 (overweight)	165 (30.4%)	99 (20.8%)	190 (28.6%)	87 (27.0%)	130 (23.2%)	44 (9.7%)	715 (23.7%)	148 (31.2%)	86 (20.3%)	182 (28.6%)	84 (27.0%)	91 (22.0%)	32 (10.0%)	623 (24.2%)
>=30.0 (obese)	135 (24.9%)	27 (5.7%)	319 (48.0%)	54 (16.8%)	54 (9.6%)	5 (1.1%)	594 (19.7%)	118 (24.9%)	20 (4.7%)	305 (48.0%)	51 (16.4%)	42 (10.2%)	3 (0.9%)	539 (20.9%)
Weight (kg)	64.78 (15.28)	63.59 (12.08)	78.66 (17.28)	74.72 (16.81)	57.59 (11.81)	57.27 (9.57)	66.24 (16.41)	65.00 (15.48)	63.24 (11.82)	78.70 (17.37)	74.64 (16.71)	57.56 (11.89)	56.95 (9.64)	67.06 (16.72)
Height (cm)	155.78 (6.03)	165.26 (6.83)	161.59 (5.96)	172.58 (7.13)	155.07 (6.70)	164.81 (6.45)	161.57 (8.49)	155.67 (5.99)	165.28 (6.83)	161.56 (5.91)	172.60 (7.14)	154.98 (6.83)	164.42 (6.36)	161.72 (8.53)
Body Surface Area (m^2)[4]	1.68 (0.22)	1.71 (0.19)	1.90 (0.23)	1.89 (0.24)	1.58 (0.18)	1.61 (0.16)	1.73 (0.24)	1.68 (0.22)	1.70 (0.18)	1.90 (0.23)	1.89 (0.24)	1.58 (0.19)	1.61 (0.16)	1.74 (0.24)
Serum creatinine (mg/dL)[5]	0.73 (0.22)	0.94 (0.36)	0.61 (0.16)	0.83 (0.24)	0.74 (0.20)	0.88 (0.27)	0.77 (0.27)	0.73 (0.20)	0.92 (0.30)	0.61 (0.16)	0.83 (0.23)	0.74 (0.20)	0.89 (0.31)	0.77 (0.25)
Serum creatinine (µmol/L)[6]	64.3 (19.1)	83.0 (31.6)	54.2 (13.9)	73.7 (20.9)	65.8 (17.6)	77.7 (24.3)	68.3 (23.6)	64.7 (17.8)	81.6 (26.1)	54.1 (13.9)	73.4 (20.2)	65.5 (17.7)	78.6 (27.3)	67.8 (22.5)
Serum cystatin C (mg/dL)[7]	0.98 (0.40)	1.03 (0.34)	0.99 (0.28)	1.01 (0.29)	0.88 (0.23)	0.90 (0.28)	0.97 (0.31)	0.97 (0.34)	1.01 (0.30)	0.99 (0.27)	1.01 (0.28)	0.88 (0.23)	0.91 (0.31)	0.97 (0.29)
Iohexol GFR[8]	72.6 (25.7)	78.2 (29.3)	78.1 (33.4)	83.1 (37.3)	82.7 (35.8)	94.4 (43.7)	80.0 (33.7)	74.8 (20.1)	79.1 (20.4)	78.6 (25.0)	82.0 (26.4)	86.1 (26.3)	97.1 (31.2)	81.9 (25.6)
Iohexol GFR category[9]														
≥90 ml/min/1.73m²	104 (20.3%)	127 (28.2%)	211 (31.8%)	132 (41.0%)	164 (37.9%)	197 (56.6%)	935 (34.3%)	100 (21.1%)	124 (29.3%)	205 (32.2%)	130 (41.8%)	160 (38.7%)	190 (59.4%)	909 (35.3%)
60-89 ml/min/1.73m²	287 (56.1%)	225 (50.0%)	290 (43.7%)	117 (36.3%)	199 (46.0%)	94 (27.0%)	1212 (44.4%)	271 (57.2%)	220 (51.9%)	282 (44.3%)	113 (36.3%)	195 (47.2%)	87 (27.2%)	1168 (45.3%)
45-59 ml/min/1.73m²	86 (16.8%)	62 (13.8%)	95 (14.3%)	41 (12.7%)	45 (10.4%)	33 (9.5%)	362 (13.3%)	76 (16.0%)	61 (14.4%)	92 (14.5%)	40 (12.9%)	42 (10.2%)	29 (9.1%)	340 (13.2%)
30-44 ml/min/1.73m²	22 (4.3%)	24 (5.3%)	51 (7.7%)	22 (6.8%)	11 (2.5%)	12 (3.5%)	142 (5.2%)	21 (4.4%)	18 (4.3%)	49 (7.7%)	21 (6.8%)	10 (2.4%)	10 (3.1%)	129 (5.0%)
<30 ml/min/1.73m²	13 (2.5%)	12 (2.7%)	17 (2.6%)	10 (3.1%)	14 (3.2%)	12 (3.5%)	78 (2.9%)	6 (1.3%)	1 (0.2%)	8 (1.3%)	7 (2.3%)	6 (1.45%)	4 (1.3%)	32 (1.2%)
Estimated GFR[10]	83.3 (19.9)	85.7 (20.8)	99.4 (19.2)	98.4 (21.4)	93.3 (19.8)	98.2 (19.7)	92.9 (21.0)	82.6 (19.4)	86.2 (20.1)	99.7 (19.1)	98.5 (21.3)	93.7 (19.9)	97.8 (20.5)	93.2 (21.0)
Estimated GFR category[9]														
≥90 ml/min/1.73m²	205 (38.2%)	212 (44.4%)	486 (73.2%)	227 (71.0%)	331 (59.0%)	336 (73.4%)	1797 (59.5%)	174 (36.7%)	194 (45.8%)	468 (73.6%)	220 (70.7%)	241 (58.4%)	237 (74.1%)	1534 (59.5%)
60-89 ml/min/1.73m²	273 (50.8%)	216 (45.3%)	154 (23.2%)	74 (23.0%)	201 (35.8%)	107 (23.4%)	1025 (34.0%)	245 (51.7%)	189 (44.6%)	146 (23.0%)	71 (22.8%)	154 (37.3%)	69 (21.6%)	874 (33.9%)
45-59 ml/min/1.73m²	44 (8.2%)	29 (6.1%)	18 (2.7%)	14 (4.4%)	20 (3.6%)	6 (1.3%)	131 (4.3%)	41 (8.7%)	26 (6.1%)	17 (2.7%)	14 (4.5%)	13 (3.2%)	5 (1.6%)	116 (4.5%)
30-44 ml/min/1.73m²	12 (2.2%)	12 (2.5%)	5 (0.8%)	6 (1.9%)	6 (1.1%)	4 (0.9%)	45 (1.5%)	12 (2.5%)	10 (2.4%)	4 (0.6%)	5 (1.6%)	3 (0.7%)	4 (1.3%)	38 (1.5%)
<30 ml/min/1.73m²	3 (0.6%)	8 (1.7%)	1 (0.2%)	1 (0.3%)	3 (0.5%)	5 (1.1%)	21 (0.7%)	2 (0.4%)	5 (1.2%)	1 (0.2%)	1 (0.3%)	2 (0.5%)	5 (1.6%)	16 (0.6%)

[1]All data reported as mean (standard deviation) unless otherwise stated; categories reported as number (%); percentages may sum to +/-100 due to rounding; [2]Body mass index (BMI) calculated by dividing weight (kilograms) by height squared (metres); [3]BMI category: WHO classification for obesity[22]; [4]Body surface area calculated according to the Haycock formula[5]; [5] Serum creatinine in mg/dL (conventional units): to convert to µmol/L, multiply by 88.4; creatinine data unadjusted for the calibration study; [6]Serum creatinine in µmol/L (SI units): to convert to mg/dl, divide by 88.4; creatinine data unadjusted for the calibration study; [7]Serum cystatin C in mg/dL: cystatin C data unadjusted for the calibration study; [8]Iohexol GFR: ml/min/1.73m^2; [9]GFR category as per KDIGO[10]; [10]Estimated GFR: ml/min/1.73m^2 using CKD-EPI creatinine equation without ethnicity coefficient. *For the pooled sample (N=3025)*: Overall: N=3024 for sex; N=3019 for age, age category; N=3020 for height, weight, BMI, BMI category, BSA; N=2729 for iohexol GFR, iohexol GFR category; Iohexol GFR reported as median (IQR). Malawi females: N=537 for age and age category; N=529 for serum creatinine; N=511 for serum cystatin C; N=512 for iohexol GFR, iohexol GFR category. Malawi males: N=476 for height, weight, BMI, BMI category, BSA; N=467 for serum creatinine; N=457 for serum cystatin C; N=450 for iohexol GFR, iohexol GFR category. Uganda females: N=560 for height, weight, BMI, BMI category; N=465 for serum cystatin C; N=433 for iohexol GFR, iohexol GFR category. Uganda males: N=456 for height, weight, BMI, BMI category, BSA; N=385 for serum cystatin C. South Africa females: N=659 for serum cystatin C; N=348 for iohexol GFR, iohexol GFR category. *For the pooled sample after exclusions (N=2578):* Overall N=2433 for cystatin C.

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Figure S5: Distribution of iohexol GFR overall and by country

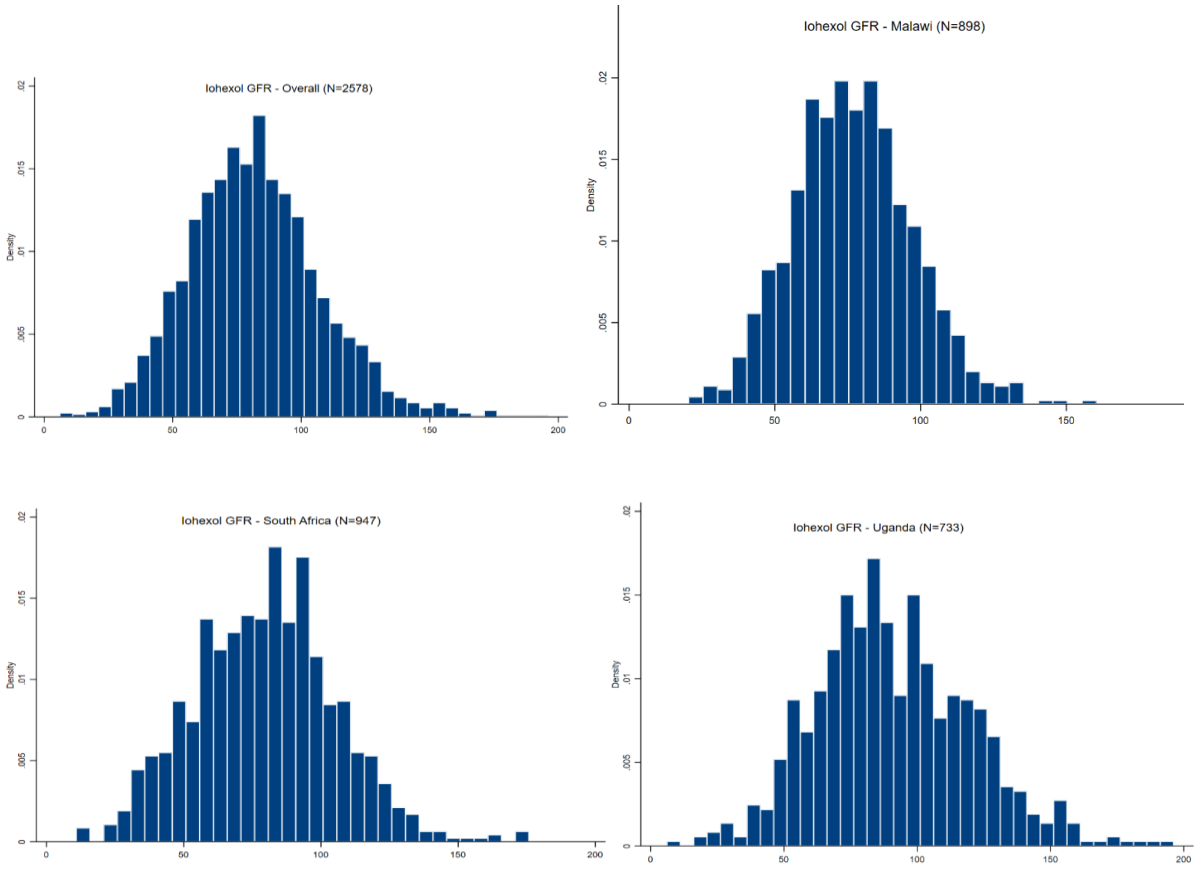

Figure S6: Volume of distribution overall and by country

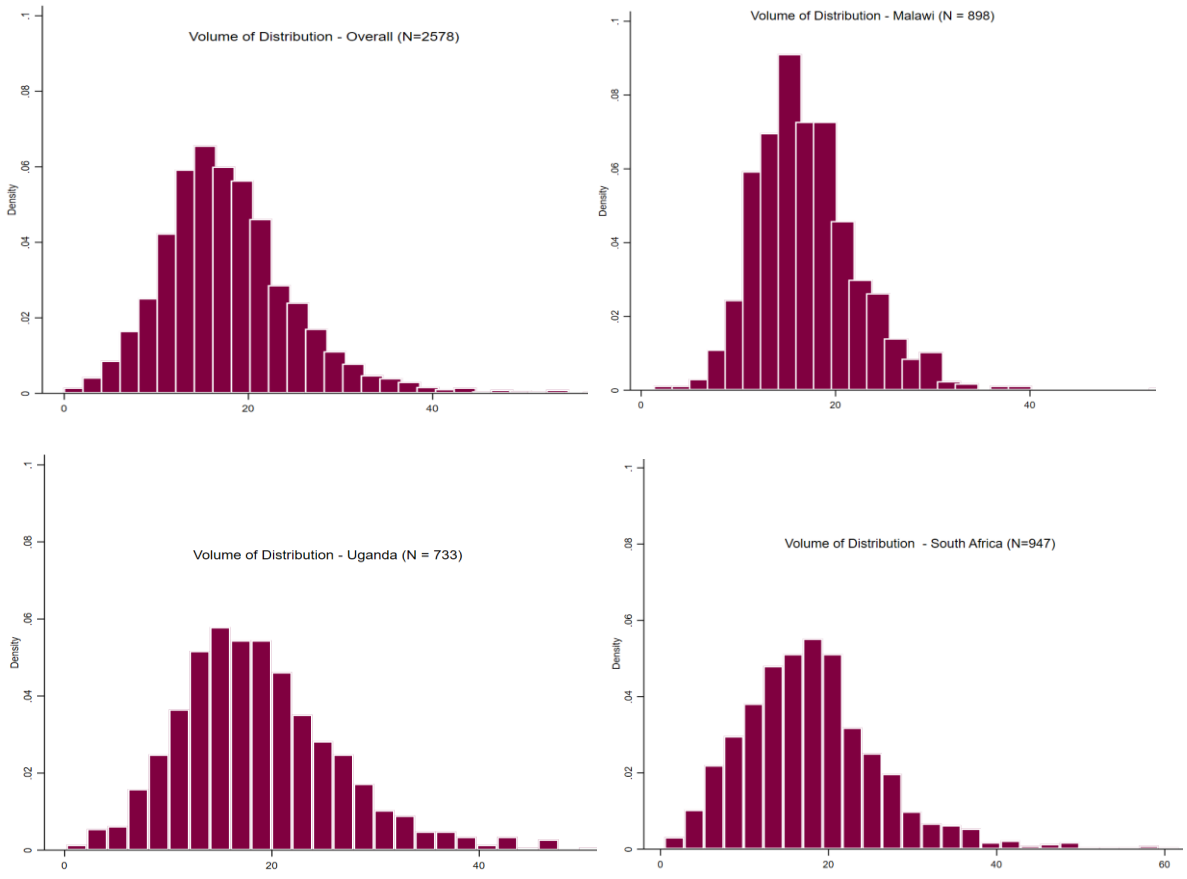

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Figure S7: Cumulative distribution plot: correlation coefficient (r) for the slope-intercept iohexol GFR derivation overall and by country

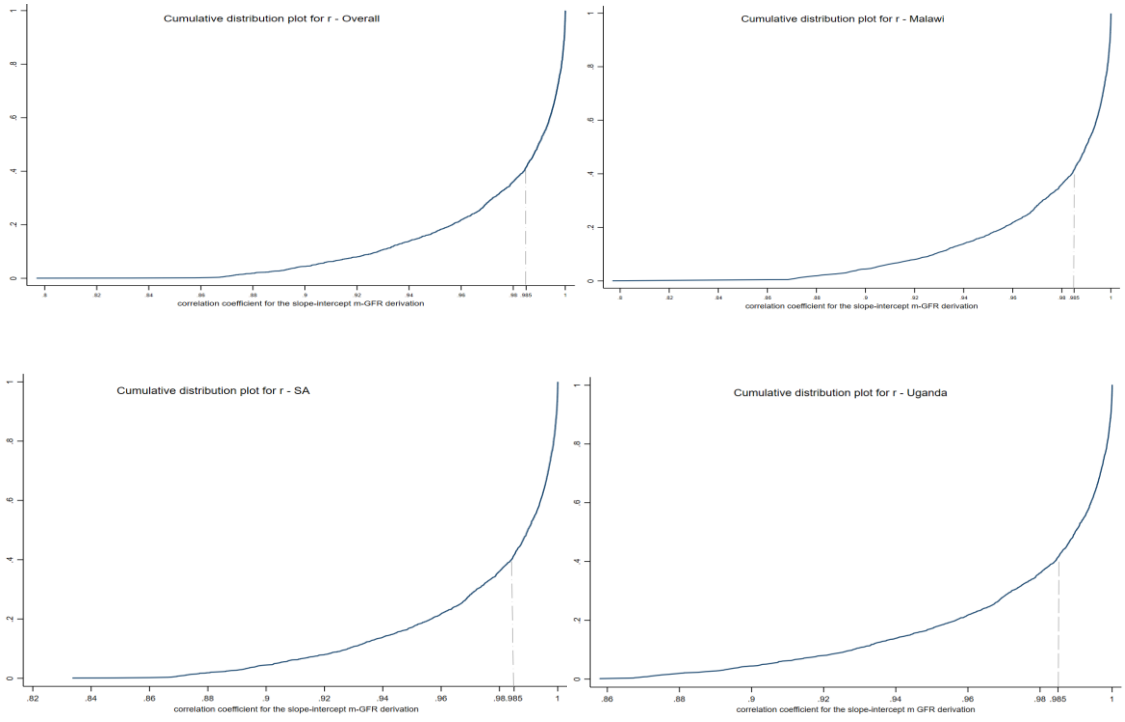

Figure S8: GFR distribution: comparing eGFR equations to iohexol GFR for the ARK Study

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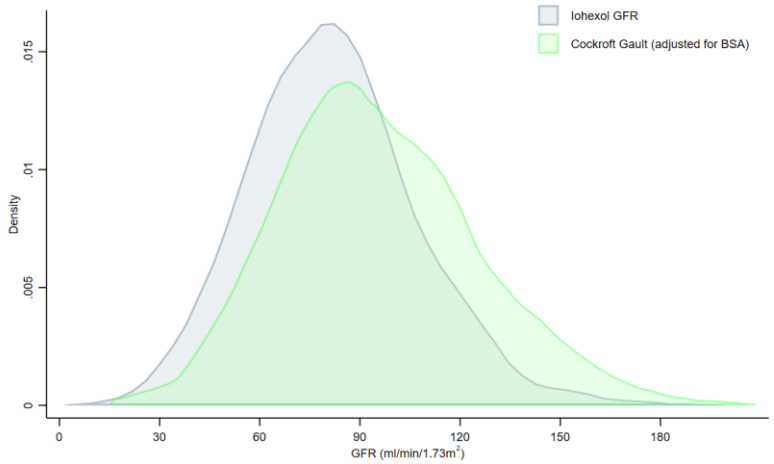

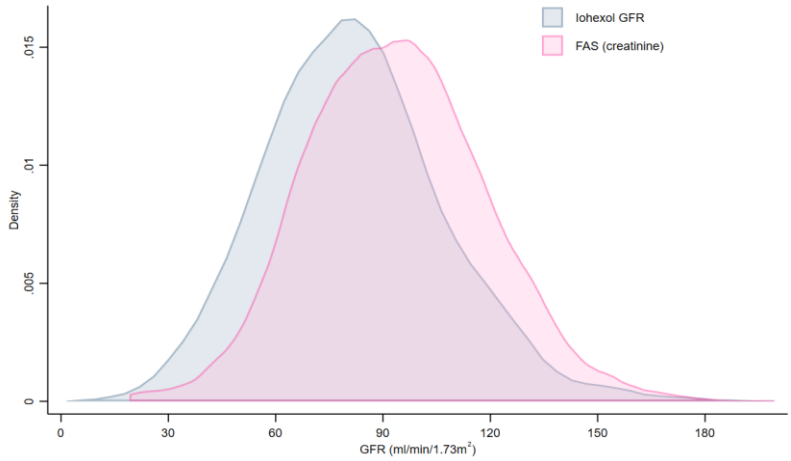

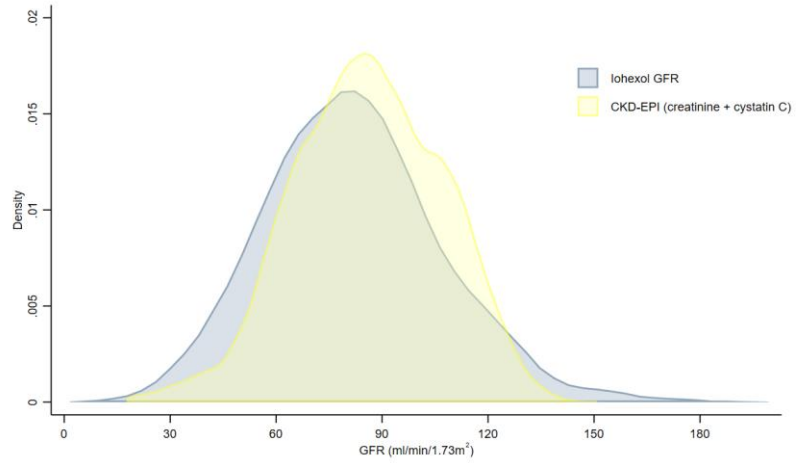

Data shown are from the pooled ARK Iohexol Study including Malawi, South Africa, and Uganda; N=2578 for creatinine-based equations; N=2433 for cystatin C-based equations

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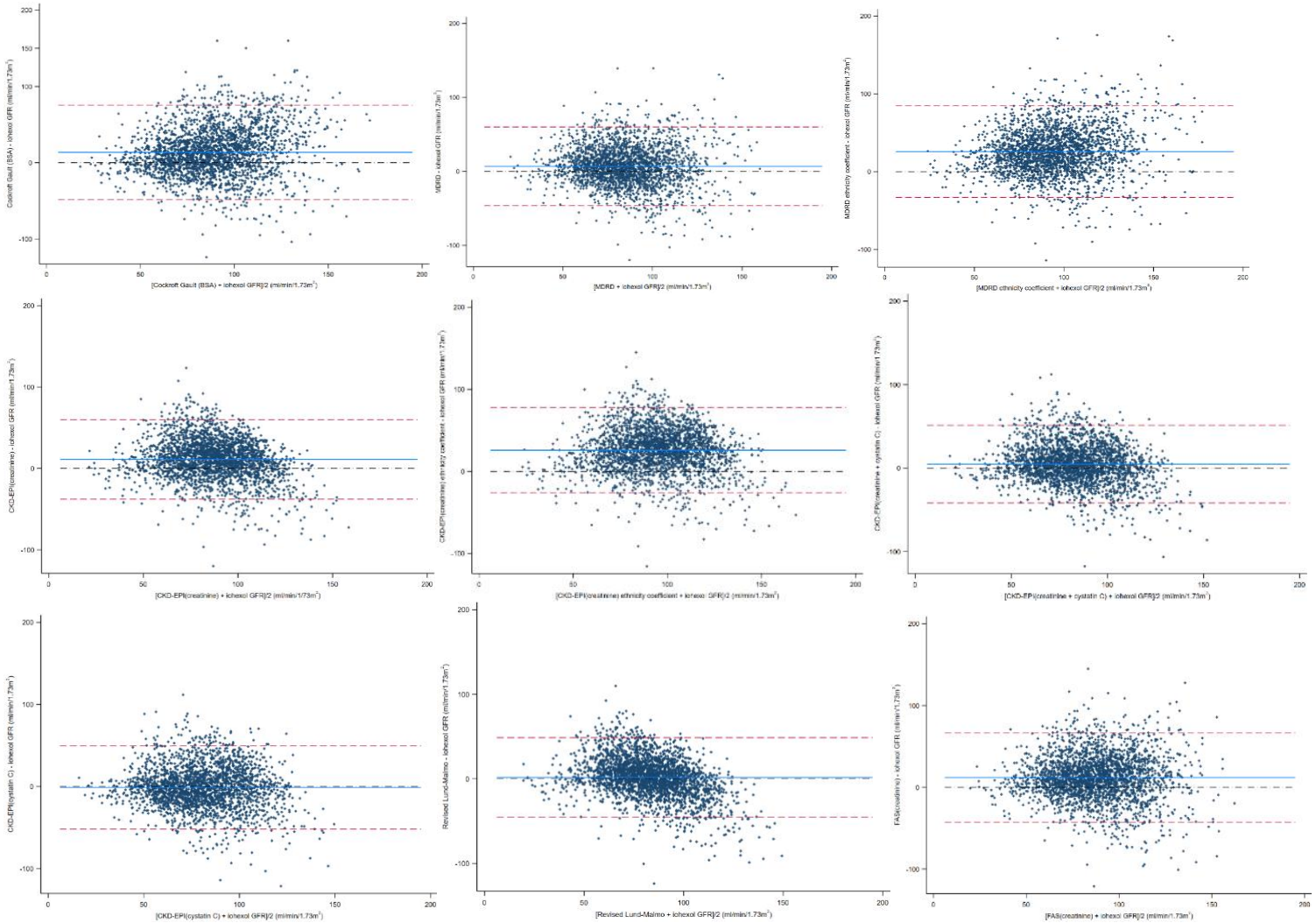
Figure S9: Agreement between eGFR equations and iohexol GFR overall



Red dashed lines indicate limits of agreement (+-2 SD); solid blue line indicates bias (ml/min/1.72m^2)

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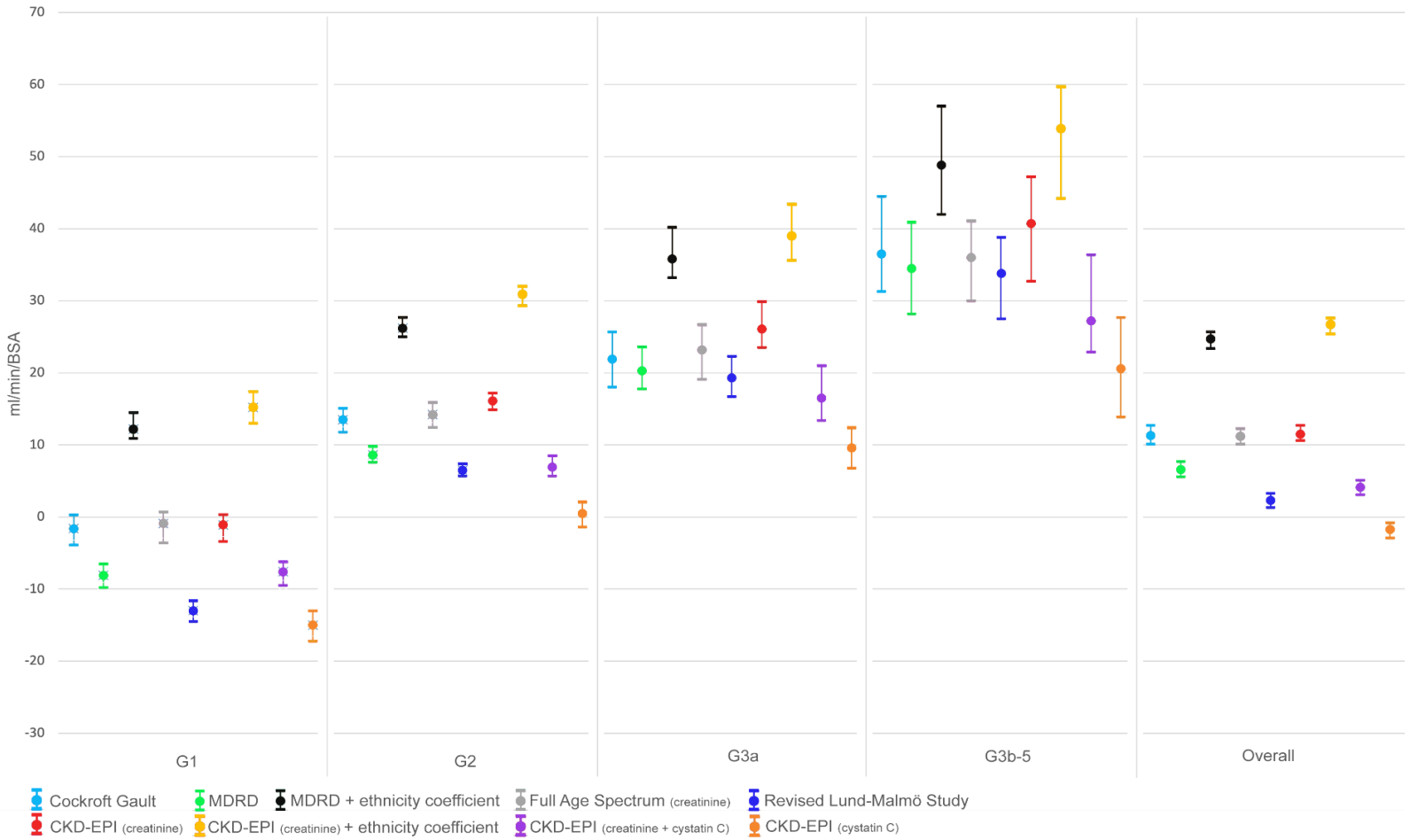
Figure S10 (a): Absolute Bias for each eGFR equation by GFR stage



*median difference (eGFR - mGFR) (95% CI)

Figure S10 (b): Precision for each eGFR equation by GFR stage

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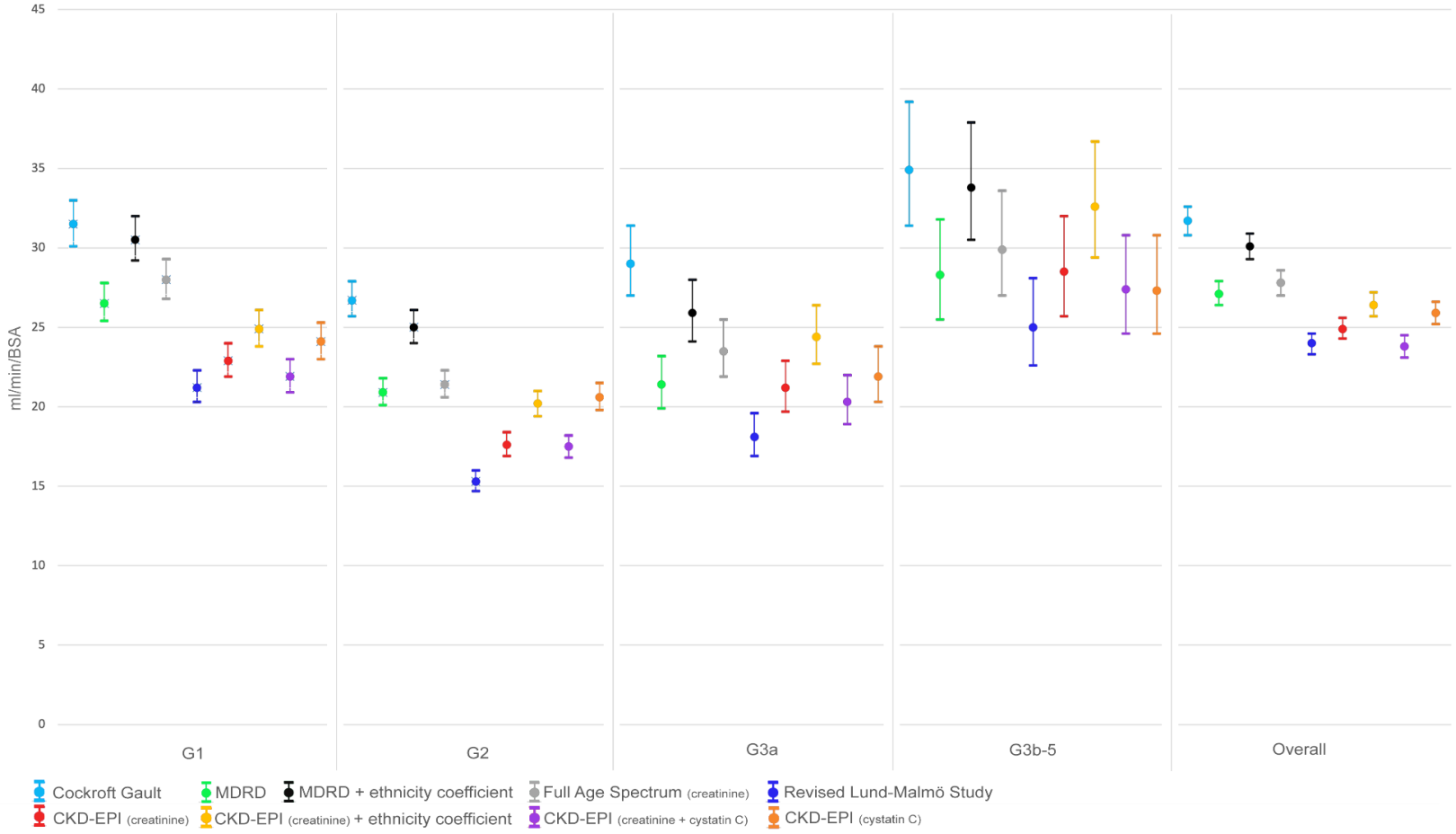
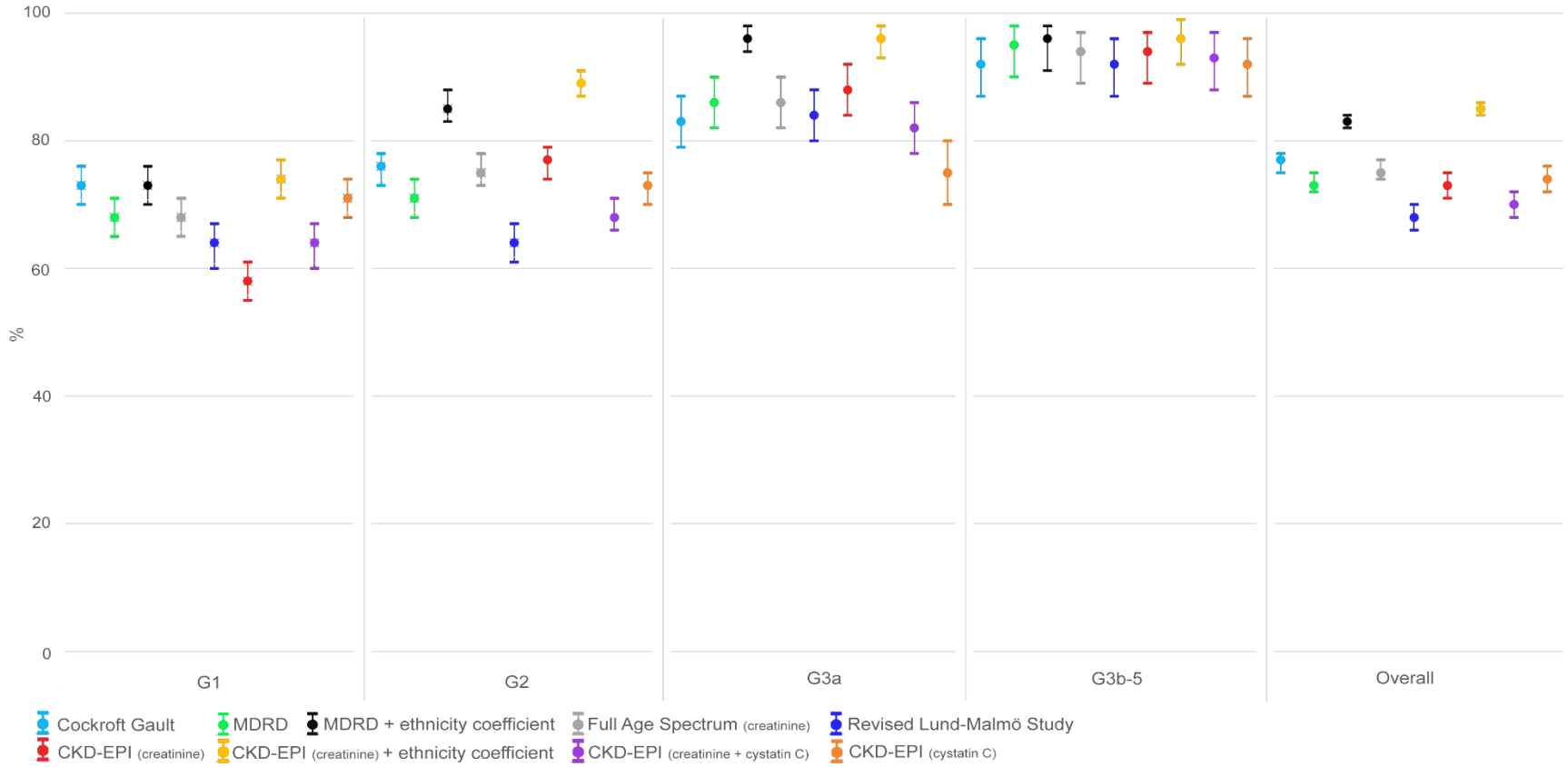


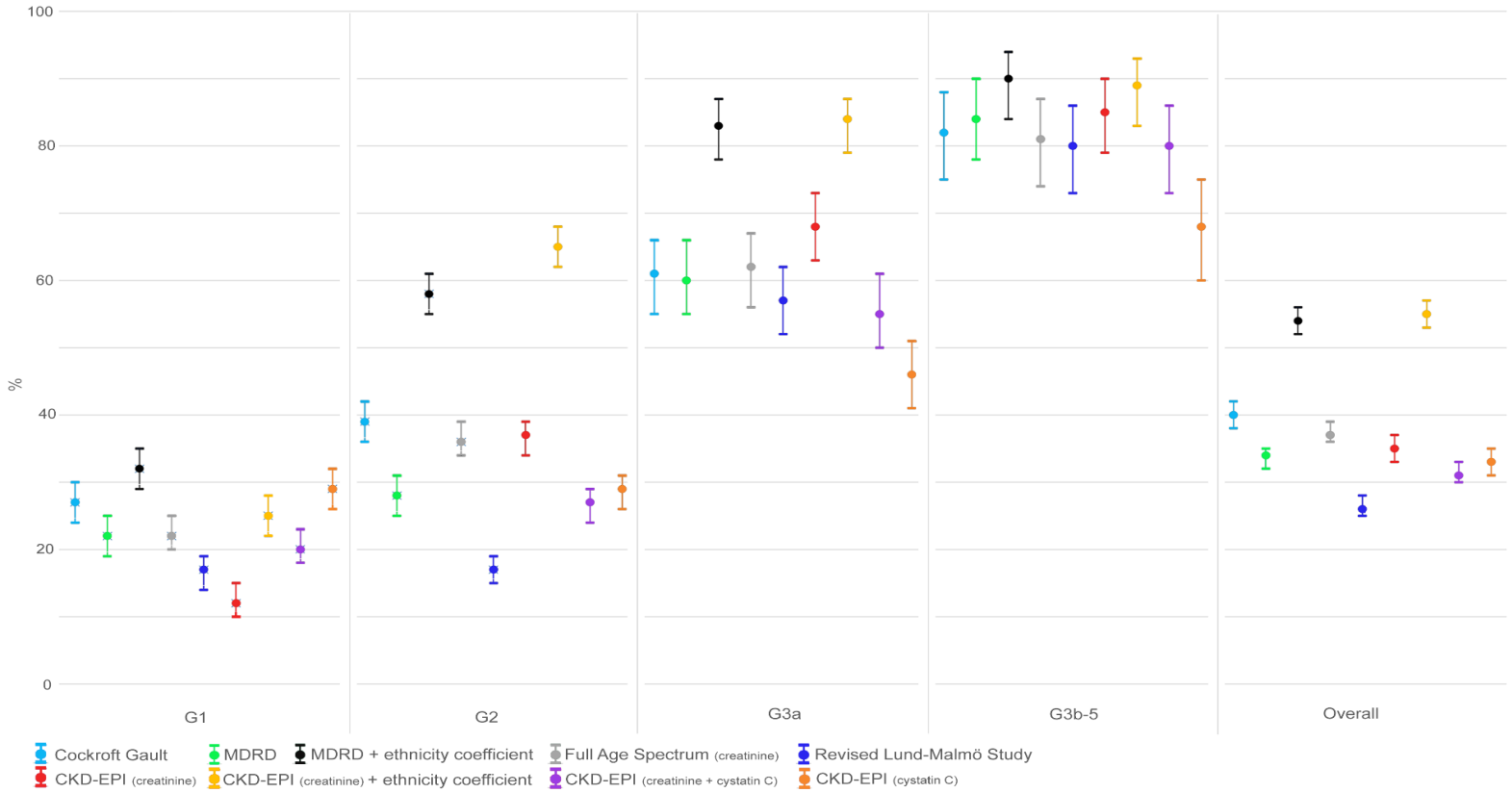
*standard deviation (eGFR - mGFR) (95% CI)

Figure S10 (c): Accuracy (1 - P_{10})* (95% CI) for each eGFR equation by GFR stage



*median (eGFR - mGFR) as the percent of mGFR for estimates that differed by more than 10% (1-P10) of mGFR

Figure S10 (d): Accuracy (1 - P_{30})* (95% CI) for each eGFR equation by GFR stage



*median (eGFR - mGFR) as the percent of mGFR for estimates that differed by more than 30% (1-P30) of mGFR

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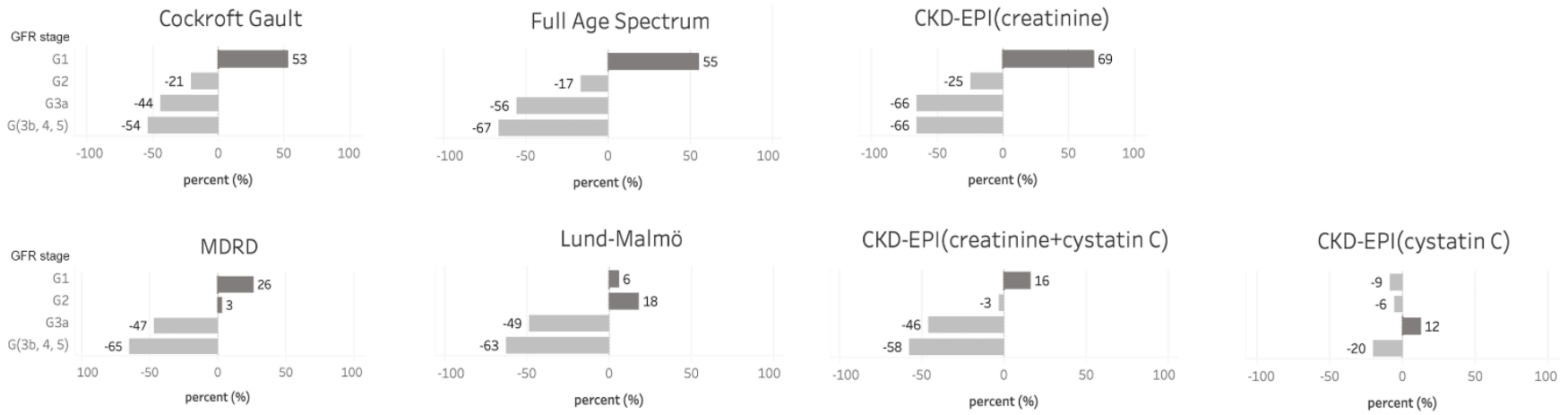
Figure S11: Schematic using relative bias to depict misclassification of iohexol GFR stage by various eGFR equations



[1]Relative bias (median percentage difference) = median of individual differences between estimated and iohexol GFR, expressed as a percent relative to iohexol GFR median [eGFR - iohexol GFR]/[iohexol GFR]%; Dark grey horizontal bands depict proportional addition of participants to GFR stage, light grey bands depict the proportional subtraction of participants to CKD for each eGFR equation compared to iohexol GFR

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Table S7: Overall – GFR stage comparing eGFR equations to iohexol GFR

GFR stage[1]	mGFR[2]	CG(BSA)[3]	MDRD[4]	MDRD (ec)[5]	FAS (Cr)[6]	Lund-Malmö[7]	CKD-EPI (Cr)[8]	CKD-EPI (Cr, ec)[9]	CKD-EPI (Cr+cysC)[10]	CKD-EPI (cysC)[11]
G1	909 (**35**)	1386(**54**)	1140 (**44**)	1871 (**73**)	1405 (**55**)	962 (**37**)	1534 (**60**)	1993 (**77**)	1052 (**43**)	826 (**34**)
G2	1168 (**45**)	928 (**36**)	1200 (**47**)	616 (**24**)	971 (**38**)	1382 (**54**)	874 (**34**)	493 (**19**)	1131(**47**)	1098 (**45**)
G3a	340 (**13**)	190 (**7**)	181 (**7**)	60 (**2**)	149 (**6**)	174 (**7**)	116 (**5**)	57 (**2**)	182 (**8**)	381(**16**)
G3b, G4, G5	161 (**6**)	74 (**3**)	57 (**2**)	31(**1**)	53 (**2**)	60 (**2**)	54 (**2**)	35 (**1**)	68 (**3**)	128 (**5**)
% classified correctly	reference category	1264 (**49**)	1318 (**51**)	1150 (**45**)	1297 (**50**)	1391 (**54**)	1265 (**49**)	1126 (**44**)	1304 (**54**)	1196 (**49**)
% classified as or more severe than mGFR stage	reference category	1633 (**63**)	1744 (**68**)	1292 (**50**)	1601(**62**)	1857 (**72**)	1520 (**59**)	1231 (**48**)	1688 (**69**)	1844 (**76**)

Data shown are number (%); [1]GFR (ml/min/1.73m^2)G1 >=90; G2 60-89; G3a 45-59; G3b 30-44; G4 15-29; G5 <15
[2]mGFR: iohexol GFR; [3]CG(BSA): Cockroft Gault equation adjusted for body surface area; [4]MDRD: MDRD equation no ethnicity coefficient;
[5]MDRD (ec): MDRD equation with ethnicity coefficient; [6]FAS (cr): Full Age Spectrum equation for creatinine; [7]Lund-Malmö: Revised Lund-Malmö Study equation; [8]CKD-EPI(Cr): CKD-EPI (creatinine) equation no ethnicity coefficient; [9]CKD-EPI (Cr, ec): CKD-EPI (creatinine) equation with ethnicity coefficient; [10]CKD-EPI (Cr+cysC): CKD-EPI (creatinine + cystatin C) equation no ethnicity coefficient;
[11]CKD-EPI (cysC): CKD-EPI (cystatin C) equation.
N=2578 for creatinine-based equations; N=2433 for cystatin C-based equations

Figure S12: Iohexol GFR stage compared to GFR stage estimated by the CKD-EPI (creatinine) equation without ethnicity coefficient, overall and by country

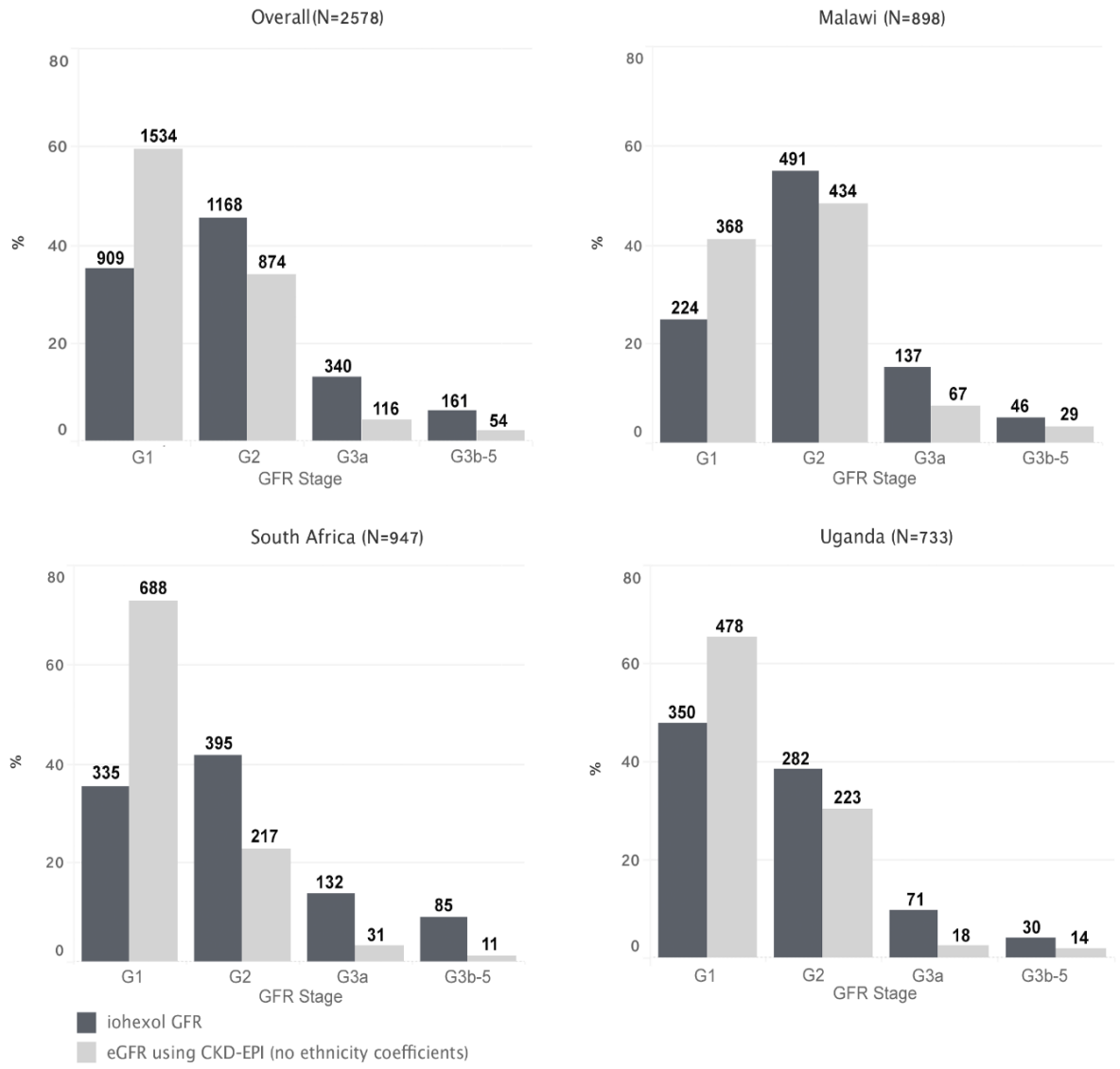

Table S8 (a): Malawi – Performance of eGFR equations compared to iohexol GFR

GFR estimating equation	[1]Absolute bias	[2]Relative bias	[3]Precision (RMSE)	[4]P_{10}	[5]P_{30}
Cockroft Gault (adjusted for BSA)	3.8	1.05	23.5	0.31	0.73
MDRD	1.8	1.02	22.3	0.30	0.75
MDRD ethnicity coefficient	18.0	1.24	25.2	0.21	0.56
FAS (creatinine)	10.2	1.14	22.0	0.28	0.70
Lund-Malmö (revised)	-0.5	0.99	18.4	0.36	0.81
CKD-EPI (creatinine)	7.6	1.09	19.4	0.32	0.74
CKD-EPI (creatinine) ethnicity coefficient	21.3	1.27	21.0	0.18	0.52
CKD-EPI (creatinine + cystatin C)	1.4	1.02	18.1	0.36	0.81
CKD-EPI (cystatin C)	-4.3	0.94	20.8	0.29	0.76

[1]Absolute bias: median of the difference between (estimated GFR - iohexol GFR)
[2]Relative bias: median of the difference between ([estimated GFR - iohexol GFR]/iohexol GFR)
[3] Precision RMSE (Root Mean Square Error): standard deviation of (estimated GFR - iohexol GFR)
[4]Precision IQR (Interquartile Range): IQR for (estimated GFR - iohexol GFR)
[5]Accuracy: proportion of eGFR results within 10% (P_{10}) and 30% (P_{30}) of iohexol GFR
N=898 for creatinine-based equations; N=871 for cystatin C-based equations

Table S8 (b): Malawi – GFR stage comparing eGFR equations to iohexol GFR

GFR stage[1]	mGFR[2]	CG(BSA)[3]	MDRD[4]	MDRD (ec)[5]	FAS (Cr)[6]	Lund-Malmö[7]	CKD-EPI (Cr)[8]	CKD-EPI (Cr, ec)[9]	CKD-EPI (Cr+cysC)[10]	CKD-EPI (cysC)[11]
G1	224 (**25**)	311 (**35**)	243 (**27**)	511 (**57**)	403 (**45**)	177 (**20**)	368 (**41**)	572 (**64**)	245 (**28**)	196 (**23**)
G2	491 (**55**)	438 (**49**)	518 (**58**)	336(**37**)	405 (**45**)	588 (**66**)	434 (**48**)	280 (**31**)	493 (**57**)	430 (**49**)
G3a	137 (**15**)	108 (**12**)	107 (**12**)	37 (**4**)	68 (**8**)	101 (**11**)	67 (**8**)	29 (**3**)	96 (**11**)	178 (**20**)
G3b, G4, G5	46 (**5**)	41 (**5**)	30 (**3**)	14 (**2**)	22 (**2**)	32 (**4**)	29 (**3**)	17 (**2**)	37 (**4**)	67 (**8**)
% classified correctly	reference category	476 (**53**)	478 (**53**)	398 (**44**)	467 (**52**)	517 (**58**)	455 (**51**)	386 (**43**)	498 (**57**)	434 (**50**)

Data shown are number (%); [1]GFR (ml/min/1.73m^2)G1 >=90; G2 60-89; G3a 45-59; G3b 30-44; G4 15-29; G5 <15
[2]mGFR: iohexol GFR; [3]CG(BSA): Cockroft Gault equation adjusted for body surface area; [4]MDRD: MDRD equation no ethnicity coefficient;
[5]MDRD (ec): MDRD equation with ethnicity coefficient; [6]FAS (cr): Full Age Spectrum equation for creatinine; [7]Lund-Malmö: Revised Lund-Malmö Study equation; [8]CKD-EPI(Cr): CKD-EPI (creatinine) equation no ethnicity coefficient; [9]CKD-EPI (Cr, ec): CKD-EPI (creatinine) equation with ethnicity coefficient; [10]CKD-EPI (Cr+cysC): CKD-EPI (creatinine + cystatin C) equation no ethnicity coefficient;
[11]CKD-EPI (cysC): CKD-EPI (cystatin C) equation.
N=898 for creatinine-based equations; N=871 for cystatin C-based equations

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Table S9 (a): South Africa – Performance of eGFR equations compared to iohexol GFR

GFR estimating equation	[1]Absolute bias	[2]Relative bias	[3]Precision (RMSE)	[4]P_{10}	[5]P_{30}
Cockroft Gault (adjusted for BSA)	28.3	1.36	31.0	0.16	0.42
MDRD	13.3	1.17	26.3	0.25	0.60
MDRD ethnicity coefficient	32.9	1.42	28.8	0.10	0.34
FAS (creatinine)	21.1	1.27	26.9	0.20	0.51
Lund-Malmö (revised)	8.2	1.10	23.9	0.31	0.68
CKD-EPI (creatinine)	19.4	1.24	24.7	0.23	0.54
CKD-EPI (creatinine) ethnicity coefficient	34.9	1.44	26.0	0.08	0.33
CKD-EPI (creatinine + cystatin C)	11.0	1.14	25.2	0.27	0.64
CKD-EPI (cystatin C)	4.4	1.06	27.9	0.26	0.64

[1]Absolute bias: median of the difference between (estimated GFR - iohexol GFR)
[2]Relative bias: median of the difference between ([estimated GFR - iohexol GFR]/iohexol GFR)
[3]Precision RMSE (Root Mean Square Error): standard deviation of (estimated GFR - iohexol GFR)
[4]Precision IQR (Interquartile Range): IQR for (estimated GFR - iohexol GFR)
[5]Accuracy: proportion of eGFR results within 10% (P_{10}) and 30% (P_{30}) of iohexol GFR
N=947 for creatinine-based equations; N=942 for cystatin C-based equations

Table S9 (b): South Africa – GFR stage comparing eGFR equations to iohexol GFR

GFR stage[1]	mGFR[2]	CG(BSA)[3]	MDRD[4]	MDRD (ec)[5]	FAS (Cr)[6]	Lund-Malmö[7]	CKD-EPI (Cr)[8]	CKD-EPI (Cr, ec)[9]	CKD-EPI (Cr+cysC)[10]	CKD-EPI (cysC)[11]
G1	335 (**35**)	715 (**76**)	520 (**55**)	785 (**83**)	671 (**71**)	479 (**51**)	688 (**73**)	816 (**86**)	519 (**55**)	414 (**44**)
G2	395 (**42**)	184 (**19**)	370 (**39**)	143 (**15**)	225 (**24**)	409 (**43**)	217 (**23**)	108 (**11**)	351 (**37**)	356 (**38**)
G3a	132 (**14**)	37 (**4**)	44 (**5**)	14 (**2**)	42 (**4**)	46 (**5**)	31 (**3**)	18 (**2**)	54 (**6**)	132 (**14**)
G3b, G4, G5	85(**9**)	11 (**1**)	13 (**1**)	5 (**1**)	9 (**1**)	13 (**1**)	11 (**1**)	5 (**1**)	18 (**2**)	40 (**4**)
% classified correctly	reference category	402 (**42**)	450 (**48**)	368 (**39**)	437 (**46**)	468 (**49**)	412 (**44**)	363 (**38**)	457 (**49**)	436 **4 6**)

Data shown are number (%); [1]GFR (ml/min/1.73m^2)G1 >=90; G2 60-89; G3a 45-59; G3b 30-44; G4 15-29; G5 <15
[2]mGFR: iohexol GFR; [3]CG(BSA): Cockroft Gault equation adjusted for body surface area; [4]MDRD: MDRD equation no ethnicity coefficient;
[5]MDRD (ec): MDRD equation with ethnicity coefficient; [6]FAS (cr): Full Age Spectrum equation for creatinine; [7]Lund-Malmö: Revised Lund-Malmö Study equation; [8]CKD-EPI(Cr): CKD-EPI (creatinine) equation no ethnicity coefficient; [9]CKD-EPI (Cr, ec): CKD-EPI (creatinine) equation with ethnicity coefficient; [10]CKD-EPI (Cr+cysC): CKD-EPI (creatinine + cystatin C) equation no ethnicity coefficient;
[11]CKD-EPI (cysC): CKD-EPI (cystatin C) equation.
N=947 for creatinine-based equations; N=942 for cystatin C-based equations

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Table S10 (a): Uganda – Performance of eGFR equations compared to iohexol GFR

GFR estimating equation	[1]Absolute bias	[2]Relative bias	[3]Precision (RMSE)	[4]P_{10}	[5]P_{30}
Cockroft Gault (adjusted for BSA)	0.0	1.00	32.2	0.24	0.65
MDRD	3.0	1.03	31.3	0.25	0.65
MDRD ethnicity coefficient	22.4	1.25	34.4	0.20	0.50
FAS (creatinine)	-0.4	0.99	29.7	0.26	0.68
Lund-Malmö (revised)	-3.0	0.97	27.5	0.27	0.72
CKD-EPI (creatinine)	5.9	1.07	28.0	0.27	0.68
CKD-EPI (creatinine) ethnicity coefficient	21.6	1.24	29.2	0.21	0.52
CKD-EPI (creatinine + cystatin C)	-0.5	0.99	25.8	0.32	0.74
CKD-EPI (cystatin C)	-6.7	0.92	26.9	0.29	0.72

[1]Absolute bias: median of the difference between (estimated GFR - iohexol GFR)
[2]Relative bias: median of the difference between ([estimated GFR - iohexol GFR]/iohexol GFR)
[3]Precision RMSE (Root Mean Square Error): standard deviation of (estimated GFR - iohexol GFR)
[4]Precision IQR (Interquartile Range): IQR for (estimated GFR - iohexol GFR)
[5]Accuracy: proportion of eGFR results within 10% (P_{10}) and 30% (P_{30}) of iohexol GFR
N=733 for creatinine-based equations; N=620 for cystatin C-based equations

Table S10 (b): Uganda – GFR stage comparing eGFR equations to iohexol GFR

GFR stage[1]	mGFR[2]	CG(BSA)[3]	MDRD[4]	MDRD (ec)[5]	FAS (Cr)[6]	Lund-Malmö[7]	CKD-EPI (Cr)[8]	CKD-EPI (Cr, ec)[9]	CKD-EPI (Cr+cysC)[10]	CKD-EPI (cysC)[11]
G1	350 (**48**)	360 (**49**)	377 (**51**)	575 (**78**)	331 (**45**)	306 (**42**)	478 (**65**)	605 (**83**)	288 (**47**)	216 (**35**)
G2	282 (**39**)	306 (**42**)	312 (**43**)	137 (**19**)	341 (**47**)	385 (**53**)	223 (**30**)	105 (**14**)	287 (**46**)	312 (**50**)
G3a	71 (**10**)	45 (**6**)	30 (**4**)	9 (**1**)	39 (**5**)	27 (**4**)	18 (**3**)	10 (**1**)	32 (**5**)	71 (**12**)
G3b, G4, G5	30 (**4**)	22 (**3**)	14 (**2**)	12 (**2**)	22 (**3**)	15 (**2**)	14 (**2**)	13 (**2**)	13 (**2**)	21 (**3**)
% classified correctly	reference category	386 (**53**)	390 (**53**)	384 (**52**)	393 (**54**)	406 (**55**)	398 (**54**)	377 (**51**)	349 (**56**)	326 (**53**)

Data shown are number (%); [1]GFR (ml/min/1.73m^2)G1 >=90; G2 60-89; G3a 45-59; G3b 30-44; G4 15-29; G5 <15
[2]mGFR: iohexol GFR; [3]CG(BSA): Cockroft Gault equation adjusted for body surface area; [4]MDRD: MDRD equation no ethnicity coefficient;
[5]MDRD (ec): MDRD equation with ethnicity coefficient; [6]FAS (cr): Full Age Spectrum equation for creatinine; [7]Lund-Malmö: Revised Lund-Malmö Study equation; [8]CKD-EPI(Cr): CKD-EPI (creatinine) equation no ethnicity coefficient; [9]CKD-EPI (Cr, ec): CKD-EPI (creatinine) equation with ethnicity coefficient; [10]CKD-EPI (Cr+cysC): CKD-EPI (creatinine + cystatin C) equation no ethnicity coefficient;
[11]CKD-EPI (cysC): CKD-EPI (cystatin C) equation.
N=733 for creatinine-based equations; N=620 for cystatin C-based equations

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Figure S13 (a): Iohexol GFR overall and by country, restricted for r>0.985

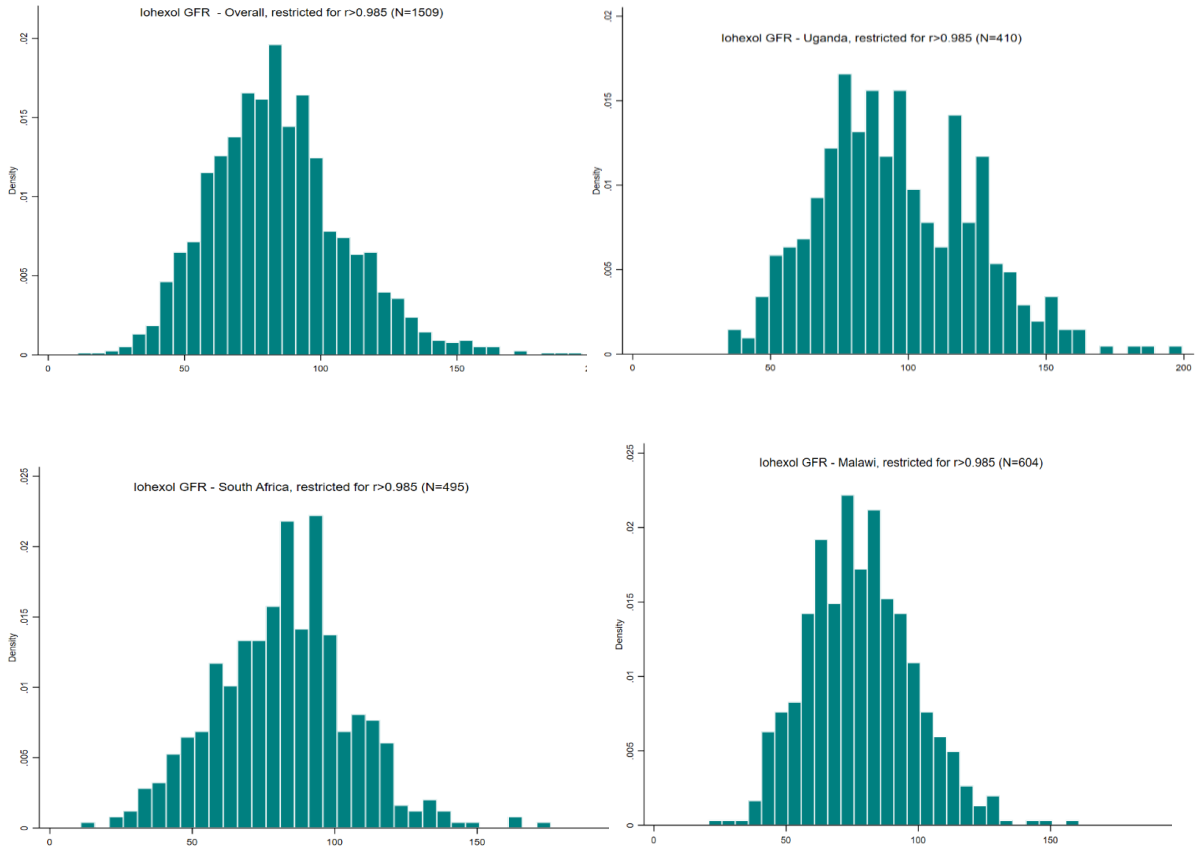
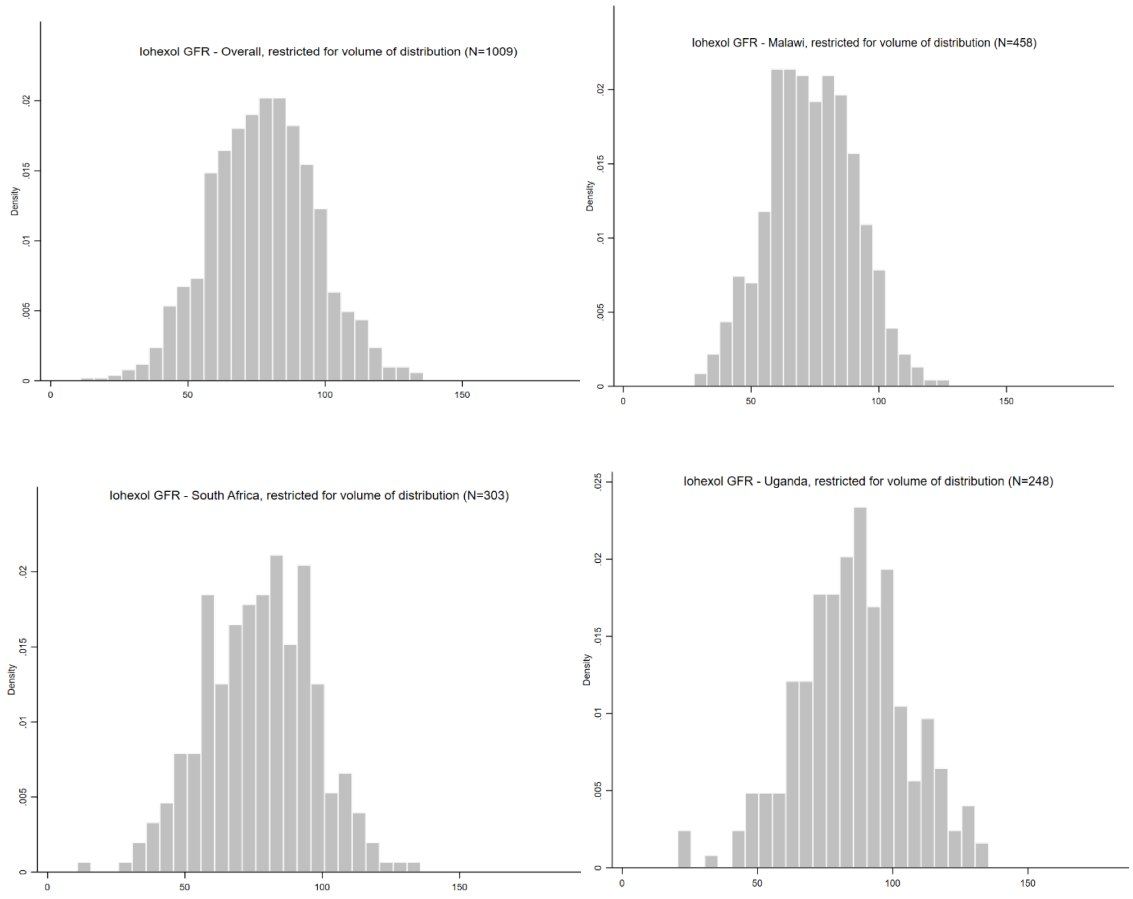
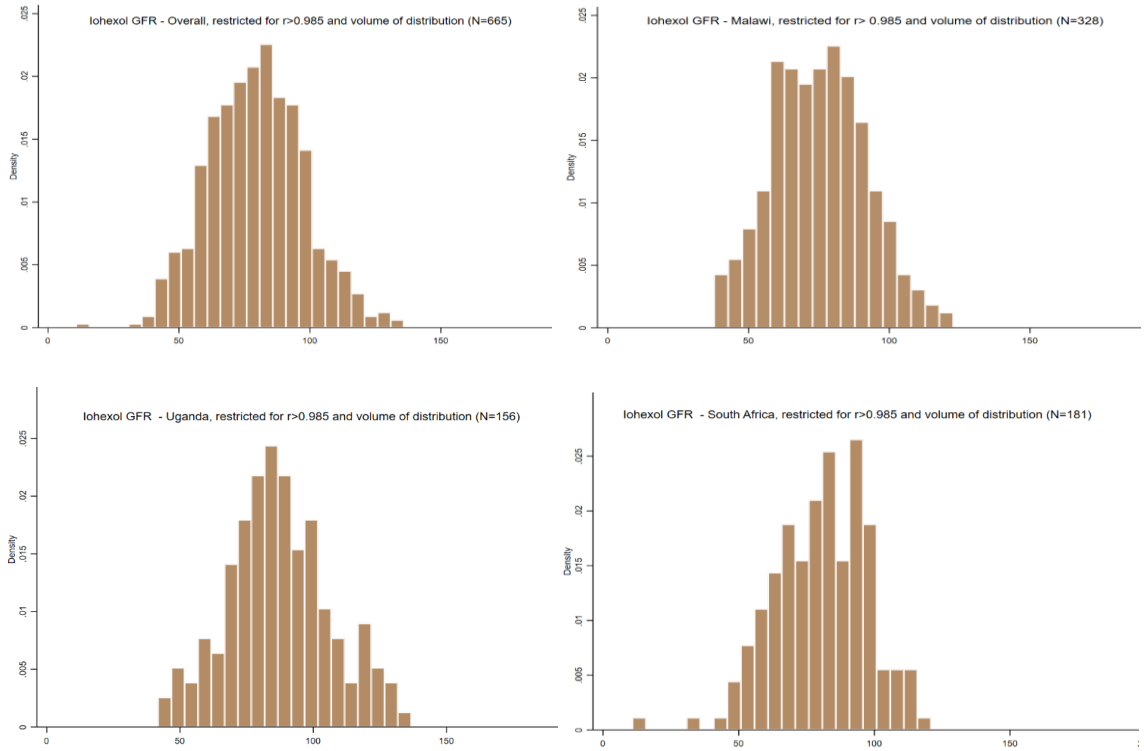


Figure S13 (b): Iohexol GFR overall and by country, restricted for Volume of distribution*



*Volume of Distribution: Normal ranges for sex-specific volumes of distribution were derived from the British Nuclear Medicine Society Guidelines defined as 11-17 litres for females; 13-30 litres for males.[4]

Figure S13 (c): Iohexol GFR overall and by country, restricted for r>0.985 + volume of distribution (VD)*



*Volume of Distribution: Normal ranges for sex-specific volumes of distribution were derived from the British Nuclear Medicine Society Guidelines defined as 11-17 litres for females; 13-30 litres for males.[4]

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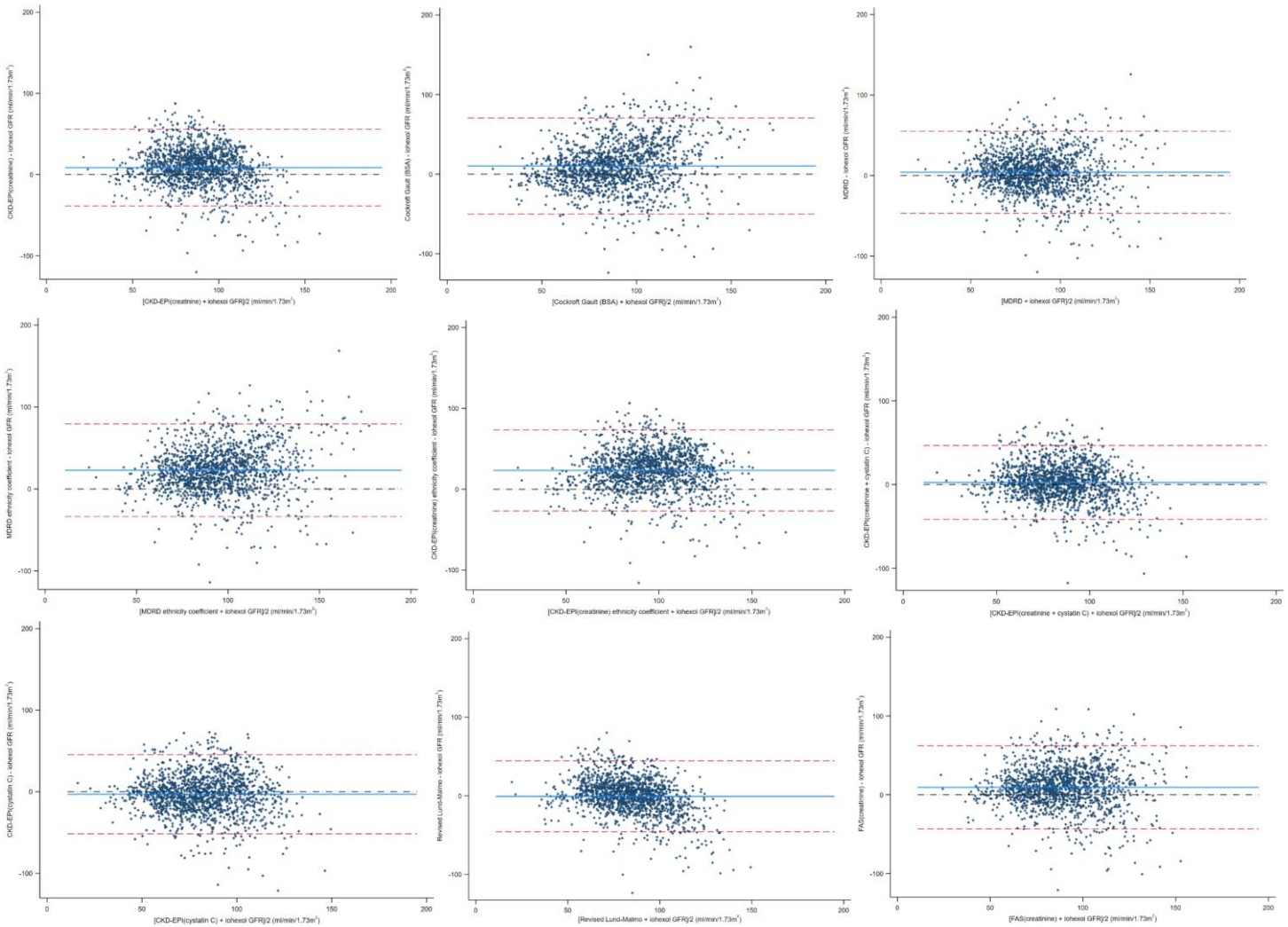
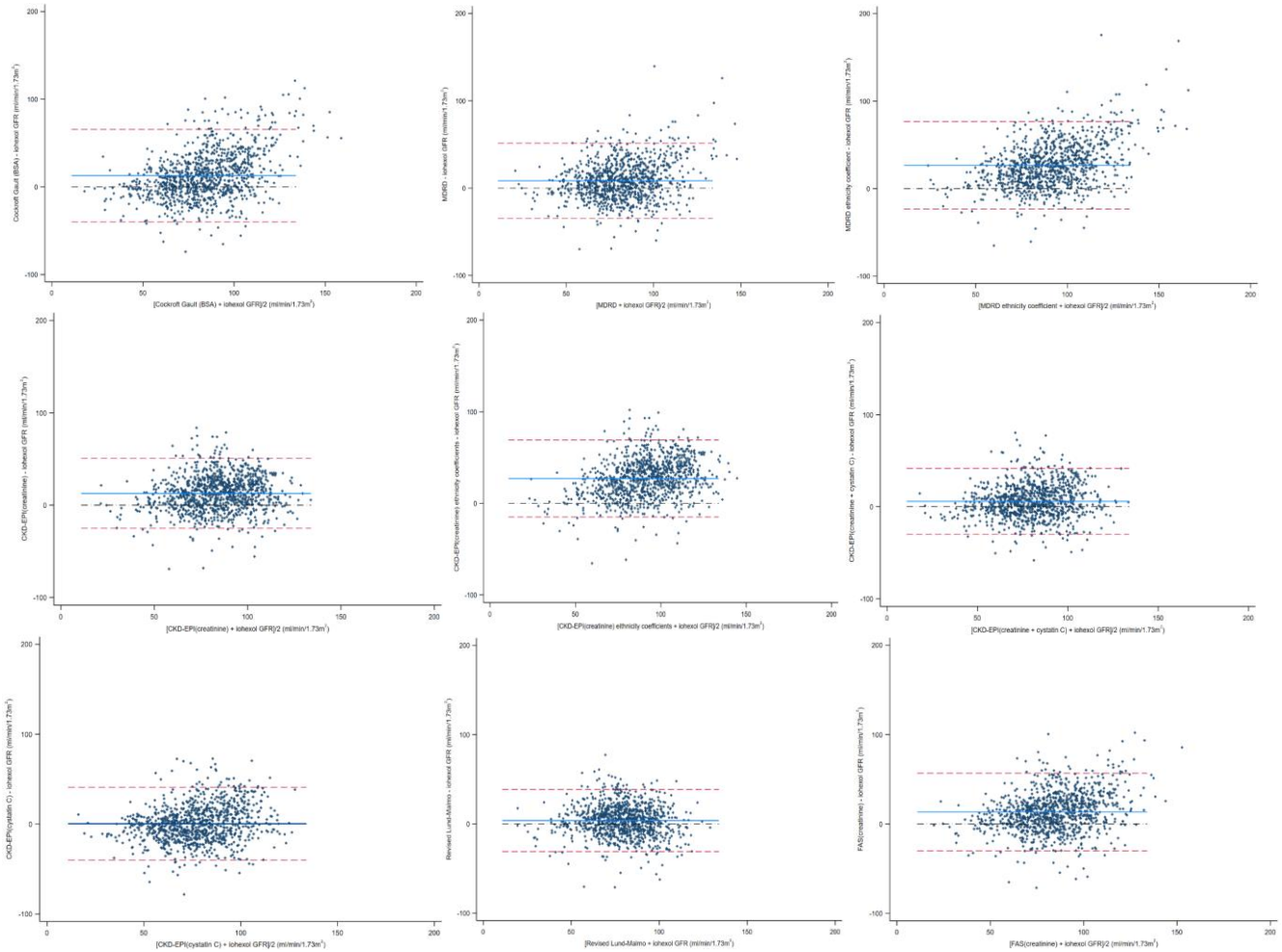
Figure S14 (a): Agreement: eGFR equations and iohexol GFR, restricted for r>0.985



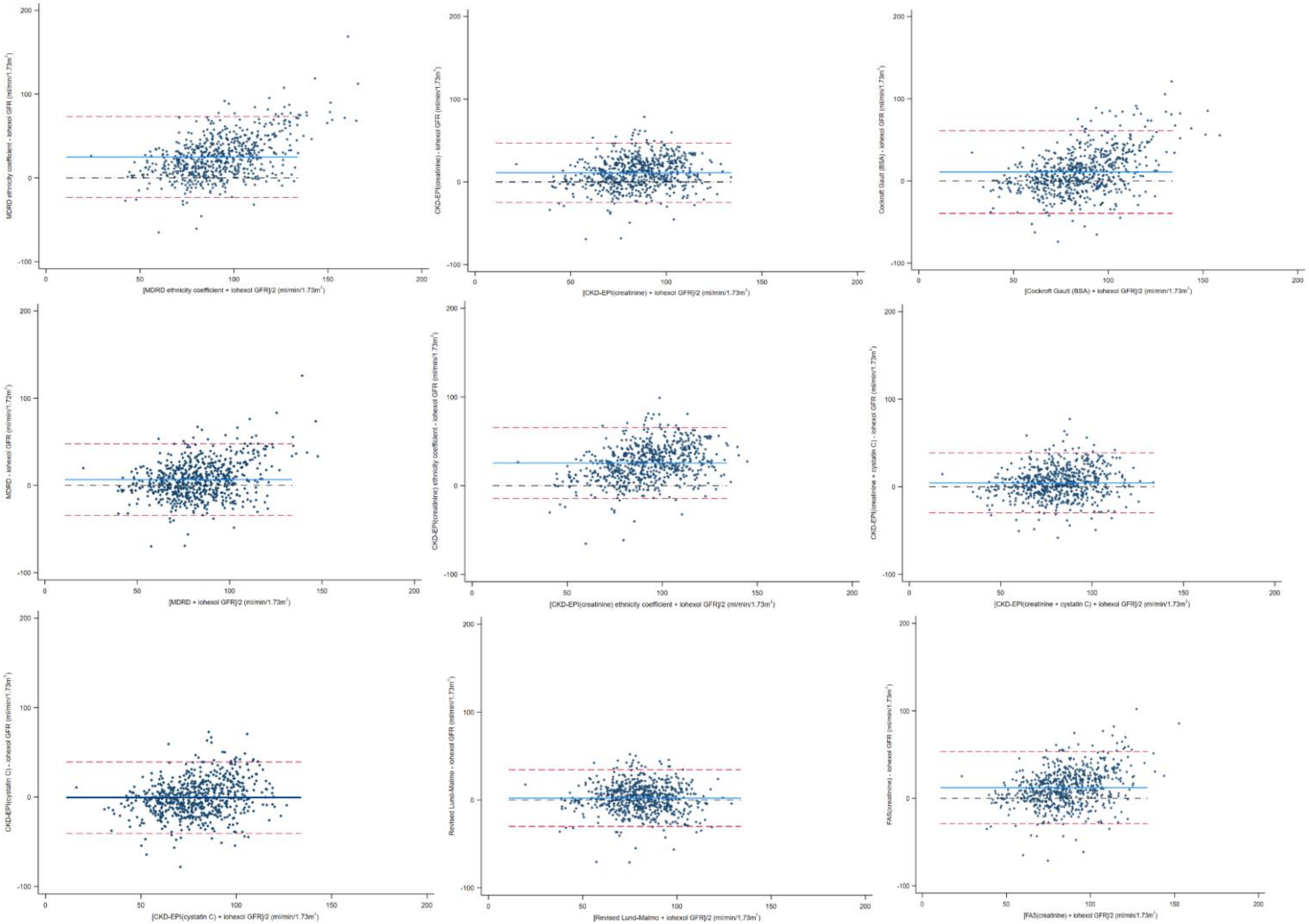
Red dashed lines indicate limits of agreement (+-2 SD); solid blue line indicates bias (ml/min/1.72m^2); normal ranges for sex-specific volume of distribution were derived from the British Nuclear Medicine Society Guidelines defined as 11-17 litres for females; 13-30 litres for males.[4]

Figure S14 (b): Agreement: eGFR equations and iohexol GFR restricted for Volume of Distribution*



Red dashed lines indicate limits of agreement (+-2 SD); solid blue line indicates bias (ml/min/1.72m^2); *Volume of Distibution: normal ranges for sex-specific volume of distribution were derived from the British Nuclear Medicine Society Guidelines defined as 11-17 litres for females; 13-30 litres for males.[4]

Figure 5.14 (c): Agreement: eGFR equations and iohexol GFR restricted for r>0.985 + Volume of Distribution*



Red dashed lines indicate limits of agreement (+-2 SD); solid blue line indicates bias (ml/min/1.72m^2); *Volume of Distribution: normal ranges for sex-specific volume of distribution were derived from the British Nuclear Medicine Society Guidelines defined as 11-17 litres for females; 13-30 litres for males.[4]

Table S11 (a): GFR stage: comparing eGFR equations to iohexol GFR restricted for r>0.985

GFR stage[1]	mGFR[2]	CG(BSA)[3]	MDRD[4]	MDRD (ec)[5]	FAS (Cr)[6]	Lund-Malmö[7]	CKD-EPI (Cr)[8]	CKD-EPI (Cr, ec)[9]	CKD-EPI (Cr+cysC)[10]	CKD-EPI (cysC)[11]
G1	566 (**38**)	783 (**52**)	660 (**44**)	1082 (**72**)	814 (**54**)	553 (**37**)	868 (**58**)	1157 (**77**)	618 (**43**)	481 (**34**)
G2	702 (**47**)	571 (**38**)	711 (**47**)	378 (**25**)	587 (**39**)	831 (**55**)	549 (**36**)	302 (**20**)	680 (**48**)	661 (**46**)
G3a	181 (**12**)	116 (**8**)	110 (**7**)	32 (**2**)	80 (**5**)	93 (**6**)	64 (**4**)	31 (**2**)	96 (**7**)	221 (**15**)
G3b, G4, G5	60 (**4**)	39 (**3**)	28 (**2**)	17(**1**)	28 (**2**)	32 (**2**)	28 (**2**)	19 (**1**)	37 (**3**)	68 (**5**)
% classified correctly	reference category	784 (**52**)	821 (**54**)	726 (**48**)	823 (**55**)	865 (**57**)	795 (**53**)	709 (**47**)	809 (**57**)	733 (**51**)

Data shown are number (%); [1]GFR (ml/min/1.73m^2)G1 >=90; G2 60-89; G3a 45-59; G3b 30-44; G4 15-29; G5 <15
[2]mGFR: iohexol GFR; [3]CG(BSA): Cockroft Gault equation adjusted for body surface area; [4]MDRD: MDRD equation no ethnicity coefficient;
[5]MDRD (ec): MDRD equation with ethnicity coefficient; [6]FAS (cr): Full Age Spectrum equation for creatinine; [7]Lund-Malmö: Revised Lund-Malmö Study equation; [8]CKD-EPI(Cr): CKD-EPI (creatinine) equation no ethnicity coefficient; [9]CKD-EPI (Cr, ec): CKD-EPI (creatinine) equation with ethnicity coefficient; [10]CKD-EPI (Cr+cysC): CKD-EPI (creatinine + cystatin C) equation no ethnicity coefficient;
[11]CKD-EPI (cysC): CKD-EPI (cystatin C) equation.
N=1509 for creatinine-based equations; N=1431 for cystatin C-based equations

Table S11 (b): GFR stage: comparing eGFR equations to iohexol GFR for Volume of Distibution*

GFR stage[1]	mGFR[2]	CG(BSA)[3]	MDRD[4]	MDRD (ec)[5]	FAS (Cr)[6]	Lund-Malmö[7]	CKD-EPI (Cr)[8]	CKD-EPI (Cr, ec)[9]	CKD-EPI (Cr+cysC)[10]	CKD-EPI (cysC)[11]
G1	259 (**26**)	473 (**47**)	395 (**39**)	681 (**68**)	493 (**49**)	324 (**32**)	541 (**54**)	732 (**73**)	372 (**38**)	283 (**29**)
G2	566 (**56**)	407 (**40**)	497 (**49**)	284 (**28**)	422 (**42**)	574 (**57**)	380 (**38**)	228 (**23**)	475 (**49**)	470 (**49**)
G3a	135 (**13**)	85 (**8**)	86 (**9**)	29 (**3**)	65 (**6**)	80 (**8**)	59 (**6**)	31 (**3**)	86 (**9**)	157 (**16**)
G3b, G4, G5	49 (**5**)	44 (**4**)	31 (**3**)	15(**2**)	29 (**3**)	31 (**3**)	29 (**3**)	18 (**2**)	36 (**4**)	59 (**6**)
% classified correctly	reference category	504 (**50**)	548 (**54**)	442 (**44**)	524 (**52**)	591 (**59**)	510 (**51**)	415 (**41**)	551 (**57**)	499 (**52**)

Data shown are number (%); [1]GFR (ml/min/1.73m^2)G1 >=90; G2 60-89; G3a 45-59; G3b 30-44; G4 15-29; G5 <15
[2]mGFR: iohexol GFR; [3]CG(BSA): Cockroft Gault equation adjusted for body surface area; [4]MDRD: MDRD equation no ethnicity coefficient;
[5]MDRD (ec): MDRD equation with ethnicity coefficient; [6]FAS (cr): Full Age Spectrum equation for creatinine; [7]Lund-Malmö: Revised Lund-Malmö Study equation; [8]CKD-EPI(Cr): CKD-EPI (creatinine) equation no ethnicity coefficient; [9]CKD-EPI (Cr, ec): CKD-EPI (creatinine) equation with ethnicity coefficient; [10]CKD-EPI (Cr+cysC): CKD-EPI (creatinine + cystatin C) equation no ethnicity coefficient;
[11]CKD-EPI (cysC): CKD-EPI (cystatin C) equation
N=1009 for creatinine-based equations; N=969 for cystatin C-based equations.
*Volume of Distribution: Normal ranges for sex-specific volumes of distribution were derived from the British Nuclear Medicine Society Guidelines defined as 11-17 litres for females; 13-30 litres for males.[4]

Table S11 (c): GFR stage: comparing eGFR equations to iohexol GFR restricted for r>0.985 + Volume of Distribution*

GFR stage[1]	mGFR[2]	CG(BSA)[3]	MDRD[4]	MDRD (ec)[5]	FAS (Cr)[6]	Lund-Malmö[7]	CKD-EPI (Cr)[8]	CKD-EPI (Cr, ec)[9]	CKD-EPI (Cr+cysC)[10]	CKD-EPI (cysC)[11]
G1	185 (**28**)	309 (**47**)	260 (**39**)	449 (**68**)	331 (**50**)	212 (**32**)	353 (**53**)	487 (**73**)	247 (**39**)	190 (**30**)
G2	386 (**58**)	276 (**42**)	331 (**50**)	189 (**28**)	280 (**42**)	388 (**58**)	261 (**39**)	149 (**22**)	321 (**50**)	322 (**50**)
G3a	79 (**12**)	57 (**9**)	57 (**9**)	21 (**3**)	38 (**6**)	47 (**7**)	35 (**5**)	21 (**3**)	51 (**8**)	97 (**15**)
G3b, G4, G5	15 (**2**)	23 (**4**)	17 (**3**)	6(**1**)	16 (**2**)	18 (**3**)	16 (**2**)	8 (**1**)	22 (**3**)	32 (**5**)
% classified correctly	reference category	355 (**53**)	387 (**58**)	313 (**47**)	378 (**57**)	423 (**64**)	362 (**54**)	293 (**44**)	379 (**59**)	344 (**54**)

Data shown are number (%); [1]GFR (ml/min/1.73m^2)G1 >=90; G2 60-89; G3a 45-59; G3b 30-44; G4 15-29; G5 <15
[2]mGFR: iohexol GFR; [3]CG(BSA): Cockroft Gault equation adjusted for body surface area; [4]MDRD: MDRD equation no ethnicity coefficient;
[5]MDRD (ec): MDRD equation with ethnicity coefficient; [6]FAS (cr): Full Age Spectrum equation for creatinine; [7]Lund-Malmö: Revised Lund-Malmö Study equation; [8]CKD-EPI(Cr): CKD-EPI (creatinine) equation no ethnicity coefficient; [9]CKD-EPI (Cr, ec): CKD-EPI (creatinine) equation with ethnicity coefficient; [10]CKD-EPI (Cr+cysC): CKD-EPI (creatinine + cystatin C) equation no ethnicity coefficient;
[11]CKD-EPI (cysC): CKD-EPI (cystatin C) equation.
N=665 for creatinine-based equations; N=641 for cystatin C-based equations.
*Volume of Distribution: Normal ranges for sex-specific volumes of distribution were derived from the British Nuclear Medicine Society Guidelines defined as 11-17 litres for females; 13-30 litres for males.[4]

Table S12 (a): eGFR equations compared to iohexol GFR restricted for r>0.985

GFR estimating equation	[1]Absolute bias	[2]Relative bias	[3]Precision (RMSE)	[5]P_{10}	[5]P_{30}
Cockroft Gault (adjusted for BSA)	8.9	1.11	30.8	0.25	0.64
MDRD	4.1	1.05	26.0	0.28	0.70
MDRD ethnicity coefficient	22.4	1.27	28.8	0.20	0.50
FAS (creatinine)	9.3	1.12	26.9	0.27	0.66
Lund-Malmö (revised)	0.5	1.01	23.0	0.34	0.78
CKD-EPI (creatinine)	9.2	1.11	24.1	0.30	0.69
CKD-EPI (creatinine) ethnicity coefficient	24.5	1.28	25.5	0.17	0.49
CKD-EPI (creatinine + cystatin C)	2.7	1.03	22.5	0.34	0.76
CKD-EPI (cystatin C)	-3.0	0.96	24.8	0.30	0.73

[1]Absolute bias: median of the difference between (estimated GFR - iohexol GFR)
[2]Relative bias: median of the difference between ([estimated GFR - iohexol GFR]/iohexol GFR)
[3]Precision RMSE (Root Mean Square Error): standard deviation of (estimated GFR - iohexol GFR)
[4]Precision IQR (Interquartile Range): IQR for (estimated GFR - iohexol GFR)
[5]Accuracy: proportion of eGFR results within 10% (P_{10}) and 30% (P_{30}) of iohexol GFR
N=1509 creatinine; N=1431 cystatin C

Table S12 (b): eGFR equations compared to iohexol GFR for VD*

GFR estimating equation	[1]Absolute bias	[2]Relative bias	[3]Precision (RMSE)	[4]P_{10}	[5]P_{30}
Cockroft Gault (adjusted for BSA)	10.0	1.13	26.9	0.27	0.66
MDRD	7.2	1.10	21.9	0.28	0.72
MDRD ethnicity coefficient	25.1	1.33	25.5	0.18	0.46
FAS (creatinine)	12.2	1.17	22.2	0.27	0.66
Lund-Malmö (revised)	4.1	1.05	17.7	0.37	0.82
CKD-EPI (creatinine)	12.8	1.17	19.3	0.29	0.67
CKD-EPI (creatinine) ethnicity coefficient	27.0	1.35	21.4	0.14	0.42
CKD-EPI (creatinine + cystatin C)	4.9	1.06	18.2	0.36	0.79
CKD-EPI (cystatin C)	-0.8	0.99	20.7	0.30	0.76

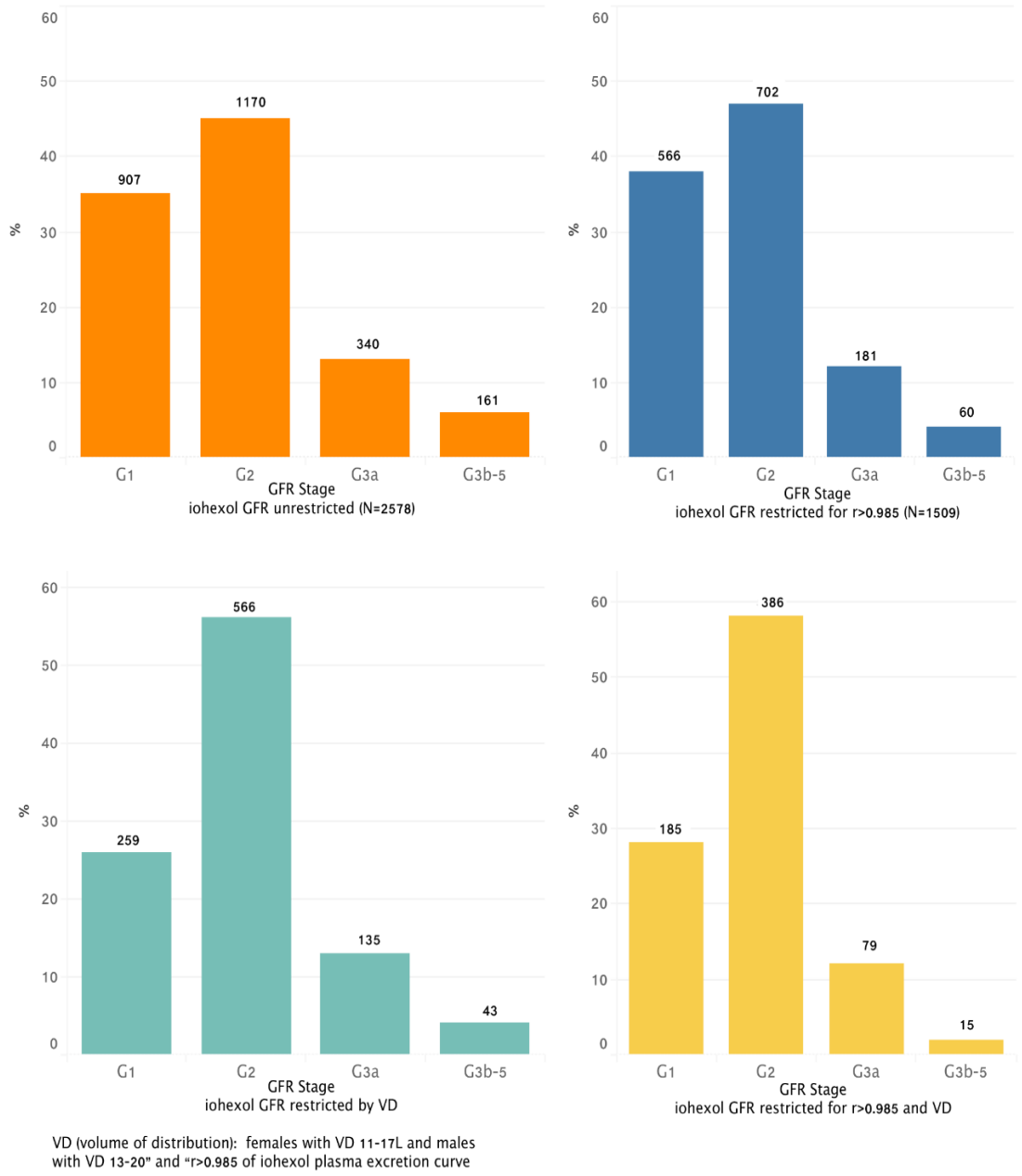
[1]Absolute bias: median of the difference between (estimated GFR - iohexol GFR)
[2]Relative bias: median of the difference between ([estimated GFR - iohexol GFR]/iohexol GFR)
[3]Precision RMSE (Root Mean Square Error): standard deviation of (estimated GFR - iohexol GFR)
[4]Precision IQR (Interquartile Range): IQR for (estimated GFR - iohexol GFR)
[5]Accuracy: proportion of eGFR results within 10% (P_{10}) and 30% (P_{30}) of iohexol GFR
N=1009 for creatinine; N=969 for cystatin C
*Volume of Distribution: Normal ranges for sex-specific volumes of distribution were derived from the British Nuclear Medicine Society Guidelines defined as 11-17 litres for females; 13-30 litres for males.[4]

Table S12 (c): eGFR equations compared to iohexol GFR for r>0.985 + VD[*]

GFR estimating equation	[1]Absolute bias	[2]Relative bias	[3]Precision (RMSE)	[4]P_{10}	[5]P_{30}
Cockroft Gault (adjusted for BSA)	8.4	1.11	25.7	0.28	0.70
MDRD	5.7	1.07	20.9	0.30	0.75
MDRD ethnicity coefficient	23.0	1.30	24.7	0.20	0.49
FAS (creatinine)	10.7	1.14	21.0	0.29	0.69
Lund-Malmö (revised)	2.5	1.03	16.5	0.40	0.87
CKD-EPI (creatinine)	11.4	1.15	18.3	0.31	0.71
CKD-EPI (creatinine) ethnicity coefficient	25.5	1.33	20.4	0.15	0.46
CKD-EPI (creatinine + cystatin C)	4.3	1.05	17.4	0.37	0.81
CKD-EPI (cystatin C)	-1.4	0.98	20.4	0.32	0.78

[1]Absolute bias: median of the difference between (estimated GFR - iohexol GFR)
[2]Relative bias: median of the difference between ([estimated GFR - iohexol GFR]/iohexol GFR)
[3]Precision RMSE (Root Mean Square Error): standard deviation of (estimated GFR - iohexol GFR)
[4]Precision IQR (Interquartile Range): IQR for (estimated GFR - iohexol GFR)
[5]Accuracy: proportion of eGFR results within 10% (P_{10}) and 30% (P_{30}) of iohexol GFR
N=665 creatinine; N=641 cystatin C
[*]Volume of Distribution: Normal ranges for sex-specific volumes of distribution were derived from the British Nuclear Medicine Society Guidelines defined as 11-17 litres for females; 13-30 litres for males.[4]

Figure S15: GFR stage for iohexol GFR, restricted for r<0.985, VD*, r<0.985 + VD*



*Volume of Distibution: Normal ranges for sex-specific volumes of distribution were derived from the British Nuclear Medicine Society Guidelines defined as 11-17 litres for females; 13-30 litres for males[4]

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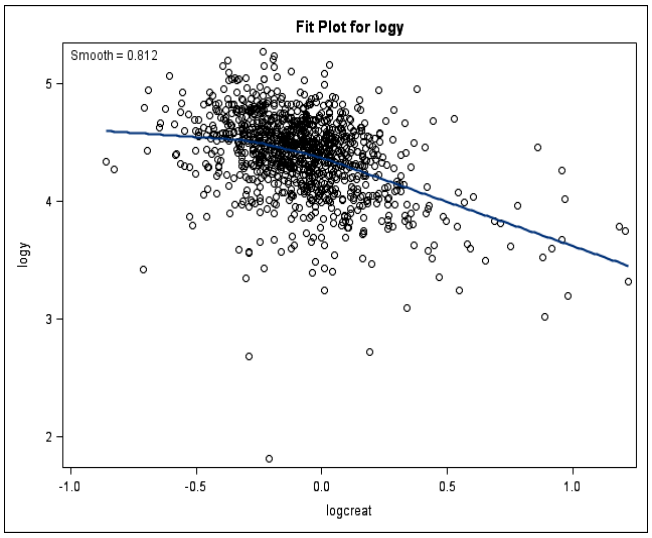
Figure S16: Lowess plot for log iohexol GFR (y axis) against log creatinine for men



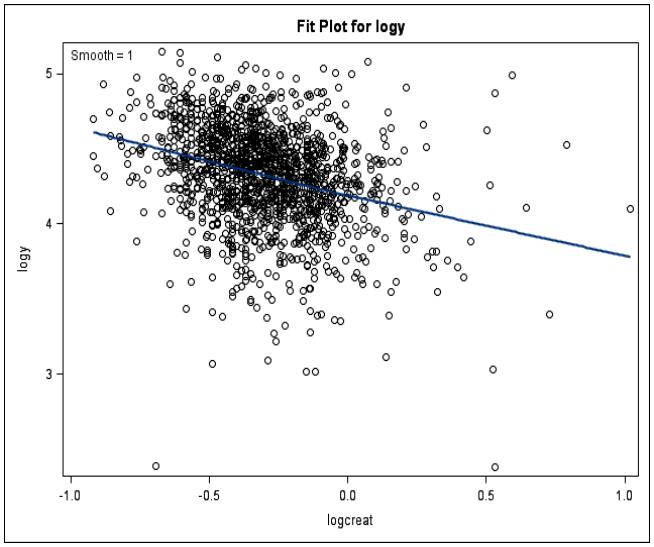


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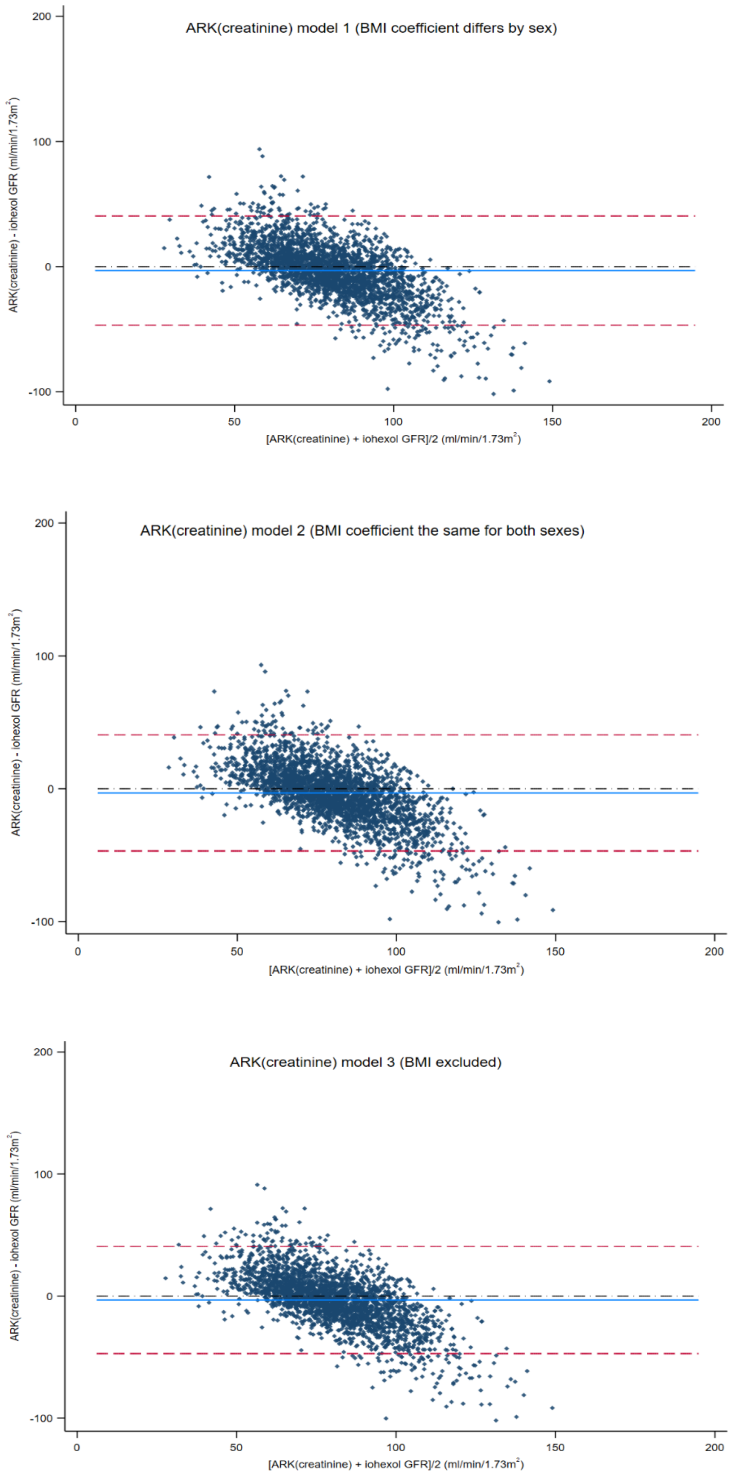
Figure S17: Agreement: models 1-3 for ARK (creatinine) eGFR equations and Iohexol GFR



Red dashed lines indicate limits of agreement (+-2 SD); solid blue line indicates bias (ml/min/1.72m^2)

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Table S14: Models for the ARK (creatinine) eGFR equations

ARK eGFR Model #1 – with bmi (allowed to differ by sex):

N	-2log likelihood'	df	R^2	adj_R^2	Note
2578	1074.3	5	0.2380	0.2365	highest adj-r2

If male: eGFR = 126 x $\min(1, SCr/0.82)^{-0.344}$ x $\max(1,SCr/0.82)^{-0.571}$ x 0.993^{age} x 0.999^{bmi}

If female: eGFR = 126 x $(SCr/0.82)^{-0.344}$ x 0.993^{age} x 0.992^{bmi}

ARK eGFR Model #1prime if forcing BMI coefficient among males to be 1 then:

N	-2log likelihood'	df	R^2	adj_R^2	Note
2578	1074.8	4	0.2378	0.236615	highest adj-r2

If male: eGFR = 124 x min(1, SCr/0.82)-0.345 x max(1,SCr/0.82)-0.578 x 0.993age
If female: eGFR = 124 x (SCr/0.82)-0.345 x 0.993age x 0.993bmi

ARK eGFR Model #1prime if forcing BMI coefficient among males to be 1 then:

N	-2log likelihood'	df	R^2	adj_R^2	Note
2578	1074.8	4	0.2378	0.236615	highest adj-r2

If male: eGFR = 124 x min(1, SCr/0.82)-0.345 x max(1,SCr/0.82)-0.578 x 0.993age
If female: eGFR = 124 x (SCr/0.82)-0.345 x 0.993age x 0.993bmi

ARK eGFR Model #2 – with BMI (common coefficient for women and men):

N	-2log likelihood	df	R^2	adj_R^2	Note
2578	1081.5	5	0.2359	0.234415	increased face validity

If male: eGFR = 142 x min(1, SCr/0.82)-0.340 x max(1,SCr/0.82)-0.559 x 0.993age x 0.994bmi
If female: eGFR = 121 x (SCr/0.82)-0.340 x 0.993age x 0.994bmi

ARK eGFR Model #3 – without bmi:

n	-2log likelihood'	df	R^2	adj_R^2	Note
2578	1112.7	4	0.2266	0.225398	simpler model without bmi

If male: eGFR = 124 x min(1, SCr/0.82)-0.339 x max(1,SCr/0.82)-0.574 x 0.993age
If female: eGFR = 103 x (SCr/0.82)-0.339 x 0.993age

For all models, we checked for evidence of interactions between sex*creatinine.

Table S15: Overall: comparing ARK (creatinine) models to eGFR equations with iohexol GFR as the reference

GFR estimating equation	[1]Absolute bias	[2]Relative bias	[3]Precision (RMSE)	[4]P_{10}	[5]P_{30}
ARK (creatinine) model 1[a]	-1.7	0.98	22.3	0.32	0.77
ARK (creatinine) model 2[b]	-1.7	0.98	22.3	0.32	0.77
ARK (creatinine) model 3[c]	-1.9	0.98	22.4	0.33	0.77
Cockroft Gault (adjusted for BSA)	11.3	1.15	31.7	0.23	0.60
MDRD	6.6	1.08	27.1	0.27	0.66
MDRD ethnicity coefficient	24.7	1.31	30.1	0.17	0.46
FAS (creatinine)	11.2	1.14	27.8	0.25	0.62
Lund-Malmö (revised)	2.3	1.03	24.0	0.32	0.74
CKD-EPI (creatinine)	11.5	1.15	24.9	0.27	0.65
CKD-EPI (creatinine) ethnicity coefficient	26.7	1.33	26.4	0.15	0.45
CKD-EPI (creatinine + cystatin C)	4.1	1.05	23.8	0.32	0.73
CKD-EPI (cystatin C)	-1.7	0.98	25.9	0.28	0.70

[1]Absolute bias: median of the difference between (estimated GFR - iohexol GFR)
[2]Relative bias: median of the difference between ([estimated GFR - iohexol GFR]/iohexol GFR)
[3]Precision RMSE (Root Mean Square Error): standard deviation of (estimated GFR - iohexol GFR)
[4]Precision IQR (Interquartile Range): IQR for (estimated GFR - iohexol GFR)
[5]Accuracy: proportion of eGFR results within 10% (P_{10}) and 30% (P_{30}) of iohexol GFR
N=2578 for creatinine-based equations; N=2433 for cystatin C-based equations
[a]ARK (creatinine) model 1: model based on age, sex, and sex specific coefficients for BMI
[b]ARK (creatinine) model 2: model based on age, sex, and the same coefficient for BMI for both sexes
[c]ARK (creatinine) model 3: model based on age and sex only, no BMI

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Table S16: Overall: comparing GFR stage by ARK (creatinine) models to eGFR equations with iohexol GFR

GFR stage[1]	mGFR[2]	ARK model 1[a]	ARK model 2[b]	ARK model 3[c]	CG(BSA)[3]	MDRD[4]	MDRD (ec)[5]	FAS (Cr)[6]	Lund-Malmö[7]	CKD-EPI (Cr)[8]	CKD-EPI (Cr, ec)[9]	CKD-EPI (Cr+cysC)[10]	CKD-EPI (cysC)[11]
G1	909 (**35**)	504 (**20**)	502 (**20**)	486 (**19**)	1386(**54**)	1140 (**44**)	1871 (**73**)	1405 (**55**)	962 (**37**)	1534 (**60**)	1993 (**77**)	1052 (**43**)	826 (**34**)
G2	1168 (**45**)	1920 (**75**)	1925 (**75**)	1949 (**76**)	928 (**36**)	1200 (**47**)	616 (**24**)	971 (**38**)	1382 (**54**)	874 (**34**)	493 (**19**)	1131(**47**)	1098 (**45**)
G3a	340 (**13**)	140 (**5**)	137 (**5**)	130 (**5**)	190 (**7**)	181 (**7**)	60 (**2**)	149 (**6**)	174 (**7**)	116 (**5**)	57 (**2**)	182 (**8**)	381(**16**)
G3b, G4, G5	161 (**6**)	14 (**1**)	14 (**1**)	13 (**1**)	74 (**3**)	57 (**2**)	31(**1**)	53 (**2**)	60 (**2**)	54 (**2**)	35 (**1**)	68 (**3**)	128 (**5**)
% classified correctly	reference category	1404 (**55**)	1401 (**54**)	1389 (**54**)	1264 (**49**)	1318 (**51**)	1150 (**45**)	1297 (**50**)	1391 (**54**)	1265 (**49**)	1126 (**44**)	1304 (**54**)	1196 (**49**)

Data shown are number (%); [1]GFR (ml/min/1.73m^2)G1 >=90; G2 60-89; G3a 45-59; G3b 30-44; G4 15-29; G5 <15
[2]mGFR: iohexol GFR; [3]CG(BSA): Cockroft Gault equation adjusted for body surface area; [4]MDRD: MDRD equation no ethnicity coefficient;
[5]MDRD (ec): MDRD equation with ethnicity coefficient; [6]FAS (cr): Full Age Spectrum equation for creatinine; [7]Lund-Malmö: Revised Lund-Malmö Study equation; [8]CKD-EPI(Cr): CKD-EPI (creatinine) equation no ethnicity coefficient; [9]CKD-EPI (Cr, ec): CKD-EPI (creatinine) equation with ethnicity coefficient; [10]CKD-EPI (Cr+cysC): CKD-EPI (creatinine + cystatin C) equation no ethnicity coefficient;
[11]CKD-EPI (cysC): CKD-EPI (cystatin C) equation.
N=2578 for creatinine-based equations; N=2433 for cystatin C-based equations
[a]ARK (creatinine) model 1: model based on age, sex, and sex specific coefficients for BMI
[b]ARK (creatinine) model 2: model based on age, sex, and the same coefficient for BMI for both sexes
[c]ARK (creatinine) model 3: model based on age and sex only, no BMI

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Table S18: Participant characteristics for external validation dataset (N=651)

Sample description	All participants Overall	Cohort Study HIV Positive Adults[18]	Clinical Sample Evaluating potential living kidney donors	Clinical Sample Evaluating kidney function for suspected CKD	Randomised Controlled Trial[19]	Cohort Study Adults with CKD[20]
Participant number N (%)	651 (100)	97 (14.9)	309 (47.5)	50 (7.7)	96 (14.8)	99 (15.2)
Radionuclide filtration marker		51Cr-EDTA[1]	51Cr-EDTA 99Tc-DTPA[2]	51Cr-EDTA 99Tc-DTPA	51Cr-EDTA	51Cr-EDTA
Dates of testing		2010-2011[3]	2007-2020[4]	2013-2020[5]	1994-1997[6]	2006[7]
Age – yr	43.3 (13.3)	37.0 (9.6)	40.3 (11.2)	49.3 (16.8)	52.5 (9.8)	46.5 (16.5)
Age category N (%)						
<40 yr	274 (42.1)	63 (65.0)	146 (47.3)	13 (26.0)	11 (11.5)	41 (41.4)
40-60 yr	310 (47.6)	33 (34.0)	149 (48.2)	26 (52.0)	64 (66.7)	38 (38.4)
60 yr	67 (10.3)	1 (1.0)	14 (4.5)	11 (22.0)	21 (21.9)	20 (20.2)
Female sex N (%)	365 (56.1)	40 (41.2)	185 (59.9)	16 (32.0)	76 (79.2)	48 (48.9)
Population Group						
Black	412 (63.3)	97 (100)	114 (36.9)	6 (12.0)	96 (100)	99 (100)
White	188 (28.9)	0 (0)	157 (50.8)	31 (62.0)	0 (0)	0 (0)
Indian/Asian	28 (4.3)	0 (0)	20 (6.5)	8 (16.0)	0 (0)	0 (0)
Mixed	13 (2.0)	0 (0)	12 (3.9)	1 (2.0)	0 (0)	0 (0)
Height (cm)	165.9 (9.6)	165.5 (8.5)	167.6 (9.2)	170.5 (10.5)	158.4 (8.0)	165.9 (9.0)
Weight (kg)	71.8 (14.9)	60.3 (12.4)	73.8 (14.3)	76.7 (15.6)	76.6 (13.6)	69.6 (13.8)
[9]Body mass index	26.2 (5.37)	22.1 (4.8)	26.2 (4.4)	26.3 (4.9)	30.6 (5.6)	25.5 (5.5)
BMI category N (%)						
<18.5 (underweight)	42 (6.5)	21 (21.7)	10 (3.2)	3 (6.0)	2 (2.1)	6 (6.1)
18.5-24.9 (normal)	229 (35.2)	53 (54.6)	99 (32.0)	13 (26.0)	13 (13.5)	51 (51.5)
25.0-29.9 (overweight)	210 (32.3)	11 (11.3)	124 (40.1)	25 (50)	28 (29.2)	22 (22.2)
>=30.0 (obese)	170 (26.1)	12 (12.4)	76 (24.6)	9 (18.0)	53 (55.2)	20 (20.2)
[10]Radionuclide GFR	82.0 (26.3)	90.2 (28.1)	90.3 (18.0)	56.2 (24.1)	82.2 (20.9)	60.7 (31.9)
[11]Serum creatinine (mg/dL)	1.08 (0.95)	1.00 (1.04)	0.87 (0.17)	1.60 (1.23)	0.85 (0.16)	1.80 (1.77)

All variables reported as mean (standard deviation); categories reported as frequency (percent); percentages might sum to +-100 due to rounding; for population group N=641; [1]51Cr-EDTA: chromium-51 labelled ethylene diamine tetra-acetic acid; [2]99Tc-DTPA: technetium-99 labelled diethylene triamine penta-acetic acid; [1,2]Before April 2019, the radionuclide testing comprised plasma excretion of 51Cr-EDTA, which was switched to plasma excretion of 99Tc-DTPA due to supply chain difficulties; [3]Multisample GFR testing at two and four hours; [4]Potential living kidney donors were evaluated using single sample GFR testing at three hours on the premise that potential donors are healthy with normal kidney function; [5]Those with suspected CKD were evaluated using multi-sample GFR testing at two and four hours; [6]Used single sample GFR testing at three hours; [7]Used multi-sample GFR testing at two and four hours if estimated GFR greater than 30ml/min/1.73m^2; and at two and five hours if estimated GFR less than or equal to 30ml/min/1.73m^2; [6]Body surface area calculated according to the Du Bois method[21]; Body mass index (BMI) calculated by dividing weight (kilograms) by height squared (metres); [8]Radionuclide GFR corrected for BSA (ml/min/1.73m^2); [9]To convert serum creatinine measurements from mg/dL to µmol/L, multiply by 88.4.All testing was performed in the Division of Nuclear Medicine, Department of Radiation Sciences, Charlotte Maxeke Johannesburg Academic Hospital, South Africa

Table S19: Performance of eGFR equations compared to radionuclide GFR (external validation)

GFR estimating equation	Absolute bias[1]	Relative Bias[2]	Precision[3]	Accuracy P_{10}[4]	Accuracy P_{30}[4]
CKD-EPI(creatinine)	5.5	1.10	18.6	0.34	0.77
Lund-Malmö (revised)	-3.3	1.00	17.4	0.37	0.85
ARK (creatinine)	-4.3	1.06	18.7	0.34	0.81

[1] Absolute bias: median of the difference (estimated GFR - radionuclide GFR)
[2] Relative bias: median of the difference (estimated GFR - radionuclide GFR/radionuclide GFR)
[3] Precision: RMSE (Root Mean Square Error): standard deviation of (estimated GFR - radionuclide GFR)
[4]Accuracy: the proportion of eGFR results that fall within 10% (P_{10}) and 30% (P_{30}) respectively, of radionuclide GFR

Table S20: GFR stage for eGFR equations compared to radionuclide GFR (external validation)

GFR stage[1]	Radionuclide mGFR	CKD-EPI(creatinine)	Revised Lund-Malmö	ARK(creatinine)
G1	256 (**39**)	324 (**50**)	206 (**32**)	115 (**18**)
G2	283 (**43**)	233 (**36**)	346 (**53**)	477 (**73**)
G3a	48 (**7**)	45 (**7**)	43 (**7**)	34 (**5**)
G3b	31 (**5**)	20 (**3**)	22 (**3**)	21 (**3**)
G4	27 (**4**)	14 (**2**)	23 (**4**)	4 (**1**)
G5	6 (**1**)	15 (**2**)	11 (**2**)	0 (**0**)
% participants classified correctly for all stages	Reference category	390 (**60**)	384 (**59**)	355 (**55**)

Data shown are number (%); [1]GFR (ml/min/1.73m^2)G1 >=90; G2 60-89; G3a 45-59; G3b 30-44; G4 15-29; G5 <15

6.0 Research Paper 4: Measurement of kidney function in sub-Saharan Africa

Table S21: Predicted population prevalence of impaired kidney function in ARK countries comparing imputation and GFR estimates

GFR staging	ARK Uganda (N=5715)			ARK Malawi (N=4719)			ARK South Africa (N=2020)		
GFR (ml/min/1.73m^2)	**Imputation[1]**	**CKD-EPI[2]**	**Lund-Malmö[3]**	**Imputation**	**CKD-EPI[2]**	**Lund-Malmö[3]**	**Imputation**	**CKD-EPI[2]**	**Lund-Malmö[3]**
Stage G1 (>=90)	3687 (65)	4510 (79)	3546 (62)	2328 (49)	3852 (82)	2999 (64)	903 (45)	1760 (87)	1432 (71)
Stage G2 (60-89)	1667 (29)	1101 (19)	2008 (35)	1829 (39)	808 (17)	1616 (34)	806 (40)	221 (11)	534 (26)
Stage G3a (45-59)	256 (4)	83 (1)	132 (2)	379 (8)	45 (1)	85 (2)	207 (10)	30 (1)	42 (2)
Stage G3b (30-45)	80 (1)	14 (0)	22 (0)	138 (3)	10 (0)	14 (0)	83 (4)	6 (0)	8 (0)
Stage G4 (15-29)	19 (0)	4 (0)	4 (0)	36 (1)	4 (0)	5 (0)	18 (1)	3 (0)	4 (0)
Stage G5 (<15)	6 (0)	3 (0)	3 (0)	9 (0)	0 (0)	0 (0)	4 (0)	0 (0)	0 (0)
Proportion with impaired kidney function (G3-G5) % (95% confidence interval)	6.32% (5.07-7.56)	1.77% (1.49- 2.20)	2.82% (2.40-3.28)	11.91% (10.13-13.69)	1.25% (0.95-1.61)	2.21% (1.80-2.66)	15.41% (13.30-17.53)	1.93% (1.38-2.63)	2.67% (2.01-3.47)

Data shown are number (**%**); Due to rounding percentages may sum to + or -100; Due to rounding the sum of people across the GFR categories may differ by +1 or -1 from the total N indicated at the top;
[1]Predictions based on 100 imputed datasets; [2]CKD-EPI (creatinine) equation without adjustment for ethnicity; [3]Revised Lund-Malmö Study Equation

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Table S22: Predicted population prevalence of impaired kidney function in AWI-Gen countries comparing imputation and GFR estimates

GFR staging	AWI-Gen Ghana (N=2011)			AWI-Gen Burkino Faso (N=2072)			AWI-Gen Kenya (N=2000)			AWI-Gen South Africa (N=5618)		
GFR (ml/min/1.73m^2)	**Imputation [1]**	**CKD-EPI[2]**	**Lund-Malmö[3]**	**Imputation**	**CKD-EPI[2]**	**Lund-Malmö[3]**	**Imputation**	**CKD-EPI[2]**	**Lund-Malmö[3]**	**Imputation**	**CKD-EPI[2]**	**Lund-Malmö[3]**
Stage G1 (>=90)	795 (40)	1510 (75)	993 (49)	881 (43)	1729 (83)	1315 (63)	831 (42)	1519 (76)	1087 (54)	2022 (36)	3401 (61)	2155 (38)
Stage G2 (60-89)	889 (44)	451 (22)	961 (48)	899 (43)	298 (14)	706 (34)	867 (43)	424 (21)	846 (42)	2514 (45)	1986 (35)	3177 (57)
Stage G3a (45-59)	219 (11)	33 (2)	38 (2)	199 (10)	31 (1)	37 (2)	201 (10)	39 (2)	47 (2)	688 (12)	181 (3)	225 (4)
Stage G3b (30-45)	82 (4)	14 (1)	13 (1)	70 (3)	10 (0)	9 (0)	76 (4)	13 (1)	13 (1)	280 (5)	34 (1)	42 (1)
Stage G4 (15-29)	21 (1)	2 (0)	5 (0)	17 (1)	2 (0)	4 (0)	20 (1)	4 (0)	7 (0)	83 (1)	6 (0)	9 (0)
Stage G5 (<15)	6 (0)	1 (0)	1 (0)	6 (0)	2 (0)	1 (0)	5 (0)	1 (0)	0 (0)	30 (1)	10 (0)	10 (0)
Proportion with impaired kidney function (G3-G5) % (95% confidence interval)	16.30% (13.96-18.63)	2.49% (1.85-3.27)	2.83% (2.15-3.66)	14.12% (11.83-16.42)	2.17% (1.59-2.90)	2.46% (1.84-3.22)	15.08% (12.70-17.46)	2.85% (2.17-3.68)	3.35% (2.61-4.24)	19.25% (17.48-21.03)	4.12% (2.61-4.66)	5.10% (4.53-5.70)

Data shown are number (**%**); Due to rounding percentages may sum to + or -100; Due to rounding the sum of people across the GFR categories may differ by +1 or -1 from the total N indicated at the top;
[1]Predictions based on 100 imputed datasets; [2]CKD-EPI (creatinine) equation without adjustment for ethnicity; [3]Revised Lund-Malmö Study Equation

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7.0 Chapter 7: Discussion, Recommendations and Conclusion

In this chapter I summarize the key findings from the PhD studies conducted, how these relate to the existing literature, my views on the implications of the findings to the field of nephrology in sub-Saharan Africa and suggestions for future work.

7.1 Conventional creatinine based eGFR methods identified low prevalence of impaired kidney function among rural Ugandan populations and traditional risk factors did not fully explain its occurrence: more studies are needed to clarify the risk factors for kidney disease in sub-Saharan Africa.

We found a relatively low prevalence of impaired renal function (measured by CKD-Epi without correction for ethnicity as GFR <60mls/min/1.73m^2) of 1.6% in the Kalungu rural population. This prevalence is low compared to that reported in a systematic review with a prevalence of 13.9% for sub-Saharan Africa (sSA) [1]. The difference could be explained by the criteria we used of eGFR without consideration of albuminuria. We did not measure albuminuria in our population-based studies where samples were collected in 2011-14 before kidney disease was a specific focus for MRC/UVRI & LSHTM. This could have led to underestimation of the prevalence of impaired renal function as well as the staging of the kidney disease in this population. In a study by Muiru, participants from rural areas (Uganda and Kenya) had much higher levels of urine abnormalities. Though the prevalence of GFR <60mls/min/1.73m^2 in the Muiru study of 1.7% was comparable to our GPC prevalence of 1.6%, the levels of proteinuria (urine dipstick >1+) were high at 5.4% and varied widely across the different regions in their study population[2]. The low prevalence of impaired renal function based on conventional eGFR in both these studies may be an indication that creatinine-based equations do not accurately predict the presence of kidney dysfunction in this population.

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Although it is recommended that kidney disease is properly defined according to the current KDIGO guidelines[3] for standardization, it is best to appreciate that majority of studies across Africa and globally are based on single creatinine GFR and/or albuminuria/proteinuria measurements [1, 4]. The use of both GFR estimation along with some measure of proteinuria/albuminuria with both measurements repeated at least 3 months apart is well appreciated, but may not be practical in sSA clinical and public health practice where this matters most. Moreover, the evidence for repeat measurements of both creatinine and albuminuria is not clear cut. Although there is good evidence to show that GFR <60mls/min/1.73m^2 and urine albumin-to-creatinine ratio (UACR) > 1.1 mg/mmol (10 mg/g) at any one time is associated with increased risk of cardiovascular complications and mortality[5, 6]. There is little data to support that repeat measurements of creatinine/albuminuria improve outcomes in CKD beyond the single measurements. Because of the time and resources needed to have the second measurement of creatinine and UACR, there is need for future robust studies to look into this issue. The key aspects to look at for sSA would be the absolute need for the second measurements and whether this has any incremental predictive value in terms of hard outcomes such as cardiovascular risk, need for renal replacement therapy and mortality. Until such a time, it may be better to consider using the currently acceptable terms of impaired kidney function even in the clinical settings. This would encompass all the three classifications of kidney disease namely; acute kidney injury, acute kidney disease and CKD[7, 8]. The need for a repeat measure of renal function to confirm CKD is potentially harmful in sSA since a good number of patients do not return for follow up visits until late in the disease process[9-11]. If a young person drops their GFR to <60mls/min/1.73m^2 they are likely to have significant issues so further classification is unlikely to be beneficial. If such individuals are not given full care early, they

may be more likely to present later to clinicians with advanced kidney disease and very few opportunities for intervention as often happens in sSA[12, 13]. Once such a person is identified full investigations and subsequent care may be more cost-effective than waiting for three months later (chronicity criteria) to confirm the diagnosis of CKD and thereby initiate treatment. This study also shows that creatinine may not be a good marker for determining kidney function among general populations of sSA and many cases may be missed with the current methods adopted from the Western world as we show from a more accurate method of iohexol clearance.

In our study we noted that some of the traditional risk factors such as diabetes mellitus and HIV-infections were not significantly associated with impaired renal function. This could be a result of our definition- which did not include proteinuria- often the defining presentation for diabetic nephropathy as well as HIV-associated nephropathy[14, 15]. It is possible that our study was not powered enough to investigate this association due to the low numbers of patients with diabetes mellitus. It is however, notable that several other population-based studies with inclusion of proteinuria as a marker of kidney disease have shown similar findings of lack of association with eGFR among this relatively young populations in East Africa and other parts of sSA[2, 16]. As matter-of-fact traditional risk factors like diabetes mellitus have quite a low prevalence in Uganda and the same goes for smoking and alcohol use[17]. Hyperfiltration in early stages of diabetes may be another explanation for lack of association between diabetes mellitus and low eGFR[18].

One could argue that the jury is still out on this issue of risk factors and we should not just accept what others have found in high income countries where the populations with CKD are much older with more co-morbidities[4, 19]. It is possible that in most of the young individuals seen in

sSA the causes and progression of kidney diseases are a different phenotype from that seen in the Western world. Certain predispositions like infections such as malaria, schistosomiasis, use of toxic medicines and herbs may play more central roles in the pathogenesis of kidney failure in these populations[20-22]. It is possible that these factors cause low grade acute kidney injury (AKI) that often goes undetected in the community only to manifest at a later stage when it is more advanced. Studies from countries with well characterized cohorts have demonstrated that repeated episodes of AKI predispose to rapid progression to ESRD and need for renal replacement therapy[23, 24]. Even after recovery, acute episodes of kidney injury continue to be a great risk for CKD and mortality many years later[24-26]. Moreover, there are indications that community acquired AKI commonly arises from single and treatable diseases like malaria in India with similar settings to sSA [27]. As we show in chapter 5, imputed data from the ARK cohorts suggests that the prevalence of renal impairment is two to three-fold higher compared to creatinine-based estimates in populations across seven countries in sSA. Therefore, the burden of kidney disease in sSA and associated risk factors may be grossly underestimated.

As regards HIV-infection (common in our study area but less common than in many other sSA countries) and the lack of association with renal impairment- it is possible that people with HIV infection do not have the high susceptibility genes of the APOL-1 which has been found to be common in West Africa[28, 29]. The APOL-1 genes confer increased risk for development of HIV-associated nephropathy (HIVAN) and cause rapid progression to ESRD [30]. The drivers of kidney disease in sSA need more research and the ongoing large-scale studies from H3-Africa may provide some answers related to the role of genetics and the environment in CKD from this part of the world [31]. Another possible explanation for lack of association with HIV, is that patients with HIV are now diagnosed early and are given treatment immediately (test and treat)

without waiting for decline in the immune system to very low CD4 counts, development of opportunistic infections and high viral load, all of which are associated with HIVAN[32, 33].

7.2 Baseline impaired renal function is associated with a graded increase in mortality in Uganda- bigger cohorts for follow-up of CKD patients for mortality and cardiovascular risk in sSA are needed

In spite of being a young population, we found that participants with a baseline eGFR of less than 45mls/min/1.73m^2 had six-fold higher mortality compared to those with eGFR greater than 90mls/min/1.73m^2 with strong evidence of a linear trend for risk of mortality as renal function declined.

Several studies from other countries have demonstrated the effects of microalbuminuria on mortality and adverse cardiovascular outcomes[5, 34]. We may also have misclassified some of the participants by not detecting all the individuals with low GFR (as we show in the GFR measurement paper). The consequences of this bias on mortality are hard to establish with our current study. Despite all these limitations with a bias towards the null of no association, our findings of a positive association of mortality suggest that GFR is a much more important physiological variable than has been recognized in sSA.

Our results suggest that kidney function plays a key role in overall health status and kidney status and assessment should be included within public health targets. There are a few simple steps that can easily be adapted. In most of the laboratories in Africa, laboratories do not concurrently report eGFR alongside the creatinine measurements. Since the main reason for testing for creatinine is to estimate kidney function, this can easily be adopted to help clinicians in the early detection of patients who may have eGFR <60mls/min/1.73m^2 where various

interventions may help to delay kidney disease progression. However, this should be taken in the context that use of the conventional creatinine-based equations may underestimate CKD as we in the subsequent studies with more accurate methods of determining GFR.

In the systematic review we conducted to understand how closely the current KDIGO recommendations are followed in classification of chronic kidney diseases, we found that over 80% of the 252 studies reviewed did not report whether creatinine measurements were isotope dilution mass spectroscopy (IDMSA) traceable, a major quality control for creatinine assays. Of those that reported the prevalence of CKD, only 14% fulfilled Kidney Disease: Improving Global Outcomes criteria further highlighting the challenges of determining the overall status of CKD in sSA. Prompted by these findings, we developed a simple tool that can help to ensure standardized measurements and reporting of GFR in sSA[35] see box 4 below

Box 4. Recommendations for reporting kidney function in sSA populations: the African Research of Kidney Disease (ARK) checklist for researchers

mGFR (gold standard reference method)—method and biomarker (51Cr-EDTA; 99mTc-DTPA; inulin, iohexol, iothalamate)
• Urinary clearance of biomarker, state which biomarker; OR
• Plasma clearance of biomarker, state which biomarker

Laboratory creatinine method—include all the following:
• Enzymatic
• Jaffe (alkaline picrate): modified or compensated
• IDMS traceable to a standard reference material
• The external quality control program used by the laboratory for creatinine

Estimating equations for GFR—state which equation was used:
4-v MDRD equation
- Original 4-v MDRD equation
- Use if laboratory method for creatinine was not IDMS-traceable
- State whether the coefficient for AA ethnicity was used

Re-expressed 4-v MDRD equation
- Use if laboratory method for creatinine was IDMS-traceable
- State whether the coefficient for AA ethnicity was used

CKD-EPI equation for creatinine
- Laboratory method for creatinine measurement must be IDMS-traceable
- State whether the coefficient for AA ethnicity was used

Cockcroft–Gault equation
- In its original form, this equation does not adjust for body surface area (BSA). To compare this equation to 4-v MDRD or CKD-EPI equations, which are adjusted for BSA, it is necessary to use the duBois formula and adjust for BSA.

Full Age Spectrum (FAS) equation for creatinine, have creatinine referenced from the general population

Diagnosis of CKD using KDIGO criteria—include the following:
True prevalence requires a randomized population-based sample: describe the sampling strategy
KDIGO Clinical Practice Guidelines (2012) are recommended for diagnosis of CKD and require testing for:
• Urine albumin/protein—if qualitative, confirm with quantitative test, preferably albumin: creatinine ratio AND
• Serum creatinine: use CKD-EPI equation for calculation of eGFR
• In the absence of prior testing or additional supporting evidence that confirms chronicity, demonstrate chronicity with a repeat of the abnormal diagnostic test after a minimum 12 weeks.

Recommendation: In sSA, for CKD-EPI equation—omit coefficient for AA ethnicity.

Acronyms: AA- African American; BSA-Body surface area; CKD- chronic kidney disease, CKD-Epi- chronic kidney disease epidemiology; eGFR-estimated glomerular filtration rate; IDMS-isotope dilution mass spectrometry; KDIGO- Kidney Disease-Improving Global Outcomes; MDRD-Modification of

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Diet in Renal Diet; mGFR- measured Glomerular Filtration Rate. Adopted from Fabian J et al 2019[35]

Assessment of kidney function should be included for patients who are at increased risk of developing kidney disease such as those with hypertension irrespective of their age. Kidney disease assessment and management needs to be integrated in the management of other non-communicable diseases and should be considered through the lens of the sustainable development goals[36].

7.3 Measuring glomerular filtration rate using iohexol is possible in Africa but not without challenges. The current eGFR equations overestimate glomerular filtration rate in sSA and the need for the ethnicity coefficient adjustments is not necessary- Cystatin C may provide a better measure of estimating GFR in sSA but cost is still a big issue.

For the third objective of the study, we had measured GFR as the starting point for fulfilling all the other objectives related to how to best measure kidney function in sSA. This objective presented us with several challenges that may be worth sharing. From the outset we had to develop the infrastructure to ensure that we could be able to measure the GFR using iohexol. Although the community was very familiar with blood draws and measurement of blood pressure and taking blood draws, they were less prepared to undergoing studies that would require them to stay at the clinic while having numerous needle pricks. This required regular and persistent community engagement from the start while explaining the purpose of the study. Part of the perceptions of the study population on kidney disease have been explored and published in a manuscript (see appendix at end of this chapter).

We struggled with the procurement of iohexol which was not readily available within the country and required importation for non-radiological use. The key aspects around iohexol measurement

were in the laboratory expertise and technology required for proper measurement of iohexol. At the time of initiating the study we only had three high-performance liquid chromatography machines in Uganda and they were not familiar with iohexol measurements. We had to abandon our earlier plans of building the capacity of iohexol measurement from within country and worked with colleagues from South Africa who had already built this capacity. We have now clearly established a technique of measuring plasma iohexol and using it to calculate the GFR which is a great achievement for our team. Because of the cost and complexity related to this procedure, there is need to carry on with explorative studies that can easily approximate measured eGFR in settings where certainty is required.

True GFR may be required in particular instances such as clinical trials that require renal safety as well as evaluation of potential kidney donors who need very accurate measurements of kidney function status [37].

Several small studies have looked at measured GFR in sSA but our study presents the largest prospective study done so far. We drew from the strength of existing community-based study cohorts with robust research management systems and had a substantial number of participants with lower levels of eGFR. We evaluated a number of eGFR estimating equations including newer equations such as the Full-age Spectrum (FAS) and Lund-Malmo equations. We used internationally approved standards in all our measurements and used a central laboratory for measurement of iohexol.

In conformity with other studies from across Africa[38, 39], we noted that the ethnicity correction co-efficient in all equations overestimated eGFR. There is an ongoing debate on the role of ethnicity and medical care. The use of creatinine as a marker of kidney disease may have

inherent challenges in this population. This is further supported by the notable similarities of the CKD prevalence stages measured by iohexol and estimated by Cystatin C, which has been noted to be more accurate in measuring GFR than creatinine[40]. Although cystatin C did not improve the performance of the estimating equations in a study from the Democratic Republic of Congo[39], other studies from high income countries have shown that cystatin C may a be better predictor of kidney function and mortality than creatinine among patients with GFR <60mls/min/1.73m^2 [41, 42]. This offers an opportunity to explore future use of point of care cystatin C tests in determining eGFR and looking at the incremental value it may offer in early diagnosis of patients with CKD in sSA.

Despite recruiting participants from three different countries from sSA it may be hard to generalize our findings due to the heterogeneity that exists across sSA populations. There are large genetic variations among individuals of Africa origin and this may also confer some differences in their body types as well as biological handling of creatinine[43-46].

Creatinine seems to be a poor marker of kidney dysfunction in sSA and this may be a result of the decreased body mass among majority of participants from sSA. In our study the African men and women had lower body mass than has been described elsewhere in black people living in high income countries [43]. This in part explains why the ethnicity coefficient correction for GFR as done for African Americans biases kidney function estimations in sSA.

Overall, our study suggests that the use of creatinine to predict GFR in sSA is biased with a tendency to overestimate GFR using the common existing equations. The use of the ethnicity coefficient further worsens this overestimation and may thus be a major reason for misclassification of these patients with antecedent negative consequences. For example,

initiation of interventions that delay disease progression such as low protein diet, use of less nephrotoxic agents as well as renal dosing of medicines may be delayed. Where there is a high index of suspicion or high stakes in decision making such as in kidney transplant donors, cystatin C may be a valuable confirmatory test instead of measured GFR which may be very complex. These alternative methods of confirming the true GFR or its best estimate need to be investigated and used to aid decision making among clinicians from low-income countries where measured GFR may not yet be feasible.

7.4 How do we best measure kidney function in sSA- some suggestions

We have now established that creatinine is not a good marker of estimating GFR in sSA making attempts to establish a new correction factor for well-established equations difficult. We are now left with the question of what should be done to improve the estimation of kidney function among individuals from sSA. This is not a unique question to us because the whole fraternity of nephrology is still grappling with the best way to determine kidney function. A recent review explored the key flaws with the current markers used for estimating GFR- and the authors concluded that endurance of the error in eGFR equations (in the presence of extensive research) means that the challenge lies with the use of cystatin C or creatinine as the key markers of renal function and this may not be improved by mathematical methods currently in use for GFR estimation[47]. Our findings strongly support this position. In contrast, some researchers from Europe still believe that creatinine can be improved as marker of eGFR. They studied eleven cohorts (5 CKD and 6 population based) with a total of 15,124 participants and found that particular attention should be given to the population and spectrum of patients in which the equation was developed before it is applied. They reported that the Lund-Malmö did better in patients with CKD while CKD-Epi performed better in patients with high GFR. They

recommend use of different estimation equations after putting into consideration the population under study (CKD vs healthy), comorbidities present along with the age, BMI and the prior likelihood of CKD. This will definitely be hard to apply in low resource settings because of the different measurements (height and weight) that will need to be collected on every patient with their associated errors. At present, the most pragmatic approach for most sSA countries is to continue using creatinine-based equations-without the ethnicity correction factor with an appreciation that there is a large margin of error in their eGFR. The eGFR should be enhanced by urine assessment for hematuria or proteinuria. In patients with a high index of suspicion and high prior likelihood of CKD, a repeat creatinine or better still a cystatin C measurement should be undertaken to confirm the diagnosis. Cystatin C is a much easier assay to set up and there are now several point of care cystatin C screening kits available for use [48]. Measured GFR though highly desirable may still be out of reach for any fruitful use in clinical practice.

Overall, the majority of patients present with urine abnormalities in sSA, combining eGFR estimates with urine analysis for protein and hematuria may be a good alternative in screening patients at increased risk for kidney disease. The role of diagnostic algorithms looking at different patient strata and populations may help improve the diagnosis of kidney disease.

7.5 Public Health implications of the key findings from the PhD studies and implications for the future of kidney disease in sSA.

From the GPC study we underestimated prevalence indicating that many more individuals may have kidney disease than what we found. This means that we need to develop better ways of stratifying patients at risk for both AKI and CKD. We need to enhance our healthcare systems to have a high index of suspicion among patients who present to the health care facilities even at

lower levels of health care. This can best be done through integration of CKD preventive messages into the existing framework of NCD management which currently exists in most of the sSA countries[49].

Impaired renal function is associated with increased risk of mortality among a young population of individuals in sSA. Clinicians need to take abnormalities in eGFR, particularly when <45mls/min/1.73m2 as seriously as they would take any other diagnosis of NCDs like diabetes and hypertension and institute individualized care for the patients. This may be as simple as advising and following up on lifestyle changes such as diet, salt restrictions and exercise to more complex decisions of initiating CKD medical care packages, adjustments of drugs and initiating regular follow-up of patients. This approach will need to be integrated in the existing framework of universal health care and also be supported from international and national levels. Understanding the burden of kidney disease better will focus on the need for treatment for advanced kidney disease in Africa with a more cost-effective lens.

The current use of ethnicity factor in eGFR estimating equations in sSA should be abandoned. Laboratories should strive to report eGFR along with the creatinine level and ensure that they have regular quality control for creatinine assays. This would help the clinicians to appropriately manage patients with kidney injury and appropriately refer those with advanced kidney disease for better care.

7.6 Future Research Recommendations

1. We remain with a big gap of understanding the specific risk factors for kidney disease in sSA, these need to be studied from within the existing cohorts. Traditional risk

factors such as hypertension, diabetes mellitus and HIV do not fully explain the occurrence of kidney disease in sSA.

2. Future studies including basic sciences should strive to establish if there are any peculiarities in the handling of creatinine by kidneys among people from sSA and the relationship of creatinine to body mass in this population. This may also explore the role of genetics in the production of creatinine and progression of CKD beyond APOL-1 genes.
3. A study exploring the role of repeated episodes of renal dysfunction (AKI) on progression and development of CKD in this relatively young population may be important in explaining part of the missing link in the CKD patients who report late for care.
4. Cystatin C should be integrated into the algorithm for determining kidney function in special populations where the true GFR determination is required.
5. There is need for larger prospective studies for establishing risk factors for CKD progression, cardiovascular disease and mortality in the African context.

7.7 Conclusion

Based on existing creatinine-based methods to estimate GFR, we found a relatively low prevalence of impaired renal function in the general population-most likely an underestimate. We also demonstrated that eGFR <45mls/min/1.73m^2 are associated with an increased all-cause mortality. However, using iohexol clearance, we showed that these creatinine-based measures over-estimate GFR and under-estimate CKD in sSA. This means a substantial proportion of people with kidney disease are missed by current eGFR equations which may have adverse

effects on the health of and care of patients with CKD in sSA. We recommend abandonment of use of the African-American ethnicity coefficient factor when estimating GFR in sSA.

Future studies should look into establishing the role of non-traditional risk factors for CKD in Africa as well as explore the role of cystatin C based GFR as a possible confirmatory diagnostic tool in patients with impaired renal function.

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Appendix: Understanding kidney disease in rural central Uganda; Findings from a qualitative study

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Understanding kidney disease in rural central Uganda – Findings from a qualitative study

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ABSTRACT

As part of a multicentre study on kidney disease (ARK) undertaken in Malawi, South Africa and Uganda we undertook a social science component in Uganda to gather information on people's understandings and perceptions of a diagnosis of kidney dysfunction, treatment and treatment seeking. We recruited 46 people who had been given information about kidney dysfunction and had been found to have some, usually early, signs of mild impairment. Data were collected during two in-depth interviews. Most participants had heard of the condition, but half denied knowledge of the health status of their kidneys or receiving results of tests from the clinic team. This response may have been linked to a lack of symptoms, for those with early stage kidney dysfunction. The treatment people reported receiving caused some uncertainty about condition severity. This may be because several people were treated for other conditions (such as urinary tract infections) and did not require treatment specifically for kidney disease. In our study, participants assessed illness severity based on symptoms and treatment and compared with the progression of other conditions.

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Introduction

Chronic kidney disease is a long-term condition with few symptoms until it is very advanced, and the symptoms are non-specific. The causes of kidney disease in sub-Saharan Africa are poorly understood, but it appears to be a consequence of a number of factors, including some genetic traits endemic to the region such as sickle cell disease, and other glomerulonephritides, and emerging non-communicable conditions (diabetes, hypertension and obesity) (Ene-Iordache et al., 2016; Jha et al., 2013; Kalyesubula et al., 2017). Infectious diseases, including HIV-associated kidney disease and other factors such as herbal remedies are also thought to be important in susceptibility to kidney disease (Jha et al., 2013; Naicker et al., 2015; Perico & Remuzzi, 2014).

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Public knowledge levels on the normal functioning of the kidney in places such as the United Kingdom and the United States are limited (Gaffney et al., 2014; Kazley et al., 2014; Slevin & Taylor, 2015). In a meta-analysis and systematic review of patient experience of a chronic kidney disease diagnosis Teasdale and colleagues (2017) documented limited understanding of kidney disease, with a wide-range of beliefs on the causes of disease and concerns about progression among patients participating in qualitative research studies in the United States, Australia, Brazil, Canada, the Netherlands, Taiwan and Sweden. A large cross-sectional survey in 12 countries spread across six regions also showed limited awareness of chronic kidney disease among people who were at risk of the disease (Ene-Iordache et al., 2016). Kazley and colleagues (2014) asked health care providers in the United States about the knowledge and response to diagnosis of their African American patients in South Carolina. Those providers reported that most of their patients denied having the condition and were reluctant to undergo treatment.

A systematic review, published in 2014, showed that the overall prevalence of chronic kidney disease in sub-Saharan Africa (sSA) was 13.9% (95% CI 12.2–15.7) (Stanifer et al., 2014). However, the true prevalence is unknown as these studies were drawn from convenience samples, often in hospitals or populations at high-risk for kidney disease, as well as uncertainty about how to estimate kidney function in sSA (Kalyesubula et al., 2018). The search for an accurate measure has over the last two decades resulted in several equations being developed to estimate Glomerular Filtration Rate (GFR) based on levels of serum creatinine, an endogenous breakdown product of creatine phosphate produced by muscle which provides information on kidney function. However, the equations to estimate GFR were developed among populations with a low proportion of ethnic Africans and these equations have had limited validation among people in sSA (Kalyesubula et al., 2018). The accuracy of these equations is likely to be poor since serum creatinine levels vary substantially with race, diet and nutritional status (Delanaye et al., 2011).

To address the gaps in knowledge regarding risk for kidney disease, kidney disease prevalence and optimal measurement of kidney function in sSA, a multicentre study, the African Research on Kidney Disease (ARK) Study (Kalyesubula et al., 2020) was recently undertaken in Malawi, South Africa and Uganda. All participating sites are Health and Demographic Surveillance sites, enabling a cross-sectional population-based determination of the prevalence of kidney dysfunction in adults from 20 to 80 years of age, followed by direct measurement of GFR using the excretion of intravenous iohexol to examine the accuracy of different estimating equations for GFR (eGFR) (Fabian et al., 2019). Previous studies using iohexol to measure GFR in sSA had been relatively small and included few people with impaired kidney function (Bukabau et al., 2018; Sagou Yayo et al., 2016; Wyatt et al., 2013).

In Uganda this survey was embedded within the General Population Cohort in a rural sub-county in Kalungu District, central Uganda, about 150 kilometres south of Kampala. The cohort was established 30 years ago, initially for the study of the epidemiology of HIV and AIDS, but in recent years the research focus has broadened to include other chronic communicable and non-communicable conditions. From 1990 the cohort participants were followed up annually, however since 2011 follow-up has been biennial (Asiki et al., 2013).

A social science component of the ARK Study was embedded within the Ugandan site with the aim of gathering information on people's understanding of information on kidney disease and perceptions of a diagnosis of kidney disease, treatment and treatment seeking. In this paper we report the findings from this social science component.

Most of the participants who were told that they were in the early stages of impaired kidney function in our study population had an asymptomatic condition, which did not at that time require specific treatment. We therefore focus in this paper on people's understanding of kidney disease and their response to the information they were given on disease, as well as treatment and management of kidney disease.

We draw on the work of Leventhal and colleagues (2016) and concepts from the 'Common-Sense Model of Self-Regulation' in our discussion of how the response to information on an asymptomatic chronic condition (at an early stage of potential disease) may be shaped by the experience of people's

knowledge of acute conditions, including an understanding of symptoms and expectations of a short time-line for the illness development. In this regard, the early stages of kidney disease may mirror the early experience of HIV-infection, with which one may live for months or years without experiencing ill-health, or when sickness occurs it is not necessarily linked to symptoms that identify a specific condition (Willard et al., 2009). So, a person may not realise that they have the condition unless tested and diagnosed by a health-worker, which may not happen at a primary health centre if symptoms are non-specific. The reference here to HIV is also relevant because this study took place in an area that was at one stage the epi-centre of the HIV epidemic; this may in itself influence expectations about disease progression and treatment approaches.

Methods

As noted above, the study area is in rural central Uganda. The local livelihoods are based mainly around agriculture with families growing crops such as coffee, tobacco, bananas, beans, maize, potatoes and cassava. Migration by some family members (younger women and men) to urban areas and fishing sites for wage work is common. Income is also made from making bricks, growing eucalyptus (and some pine) for sale and jobs in shops and bars in trading centres. The main ethnic group in the study area is the Baganda with some immigrants originating from Rwanda and Tanzania. Most of the population are Roman Catholics and the rest are Protestants and Muslims. Luganda is the main language spoken.

The study focused on people who as part of the ARK study had been given information about kidney dysfunction and had been found to have some, usually early, signs of impairment (most people in the sample were asymptomatic), exploring their reaction to being given this information, and their understanding of kidney dysfunction and (possible future) treatment. We recruited a random sample of 46 people from among those who were found to have estimated GFR <90 mls/min/1.73 m^2 during the recruitment of the 1000 participants for the iohexol component of the ARK Study (Kalyesubula et al., 2018). We did not confirm that kidney function remained at this level or below after three months which would be required to formally diagnose Chronic Kidney Disease (Kalyesubula et al., 2018). Data were collected during two in-depth interviews with each participant. The first interview was used to ask about day to day life, recent life history events and begin to talk about health seeking behaviour. The second interview built on this information and probed more extensively for information around health seeking, use of traditional treatments and awareness of kidney impairment prior to the screening for this study.

Most interviews were conducted at the participants' homes. Some participants chose to be interviewed at the clinic. The interviews were conducted by two Ugandan interviewers (a man and a woman) with experience in qualitative data collection methods. The interviews were conducted in the local language (Luganda) and lasted up to one hour. Besides audio-recording the interviews, interviewers took field notes. The interviewers transcribed the interviews and translated them into English. The transcripts were reviewed throughout the course of the study by a senior social scientist, to ensure quality and provide continuous feedback on interview content and format.

Data analysis

Data from 46 participants were included in the final analysis for this paper. A manual thematic content analysis approach, guided by the research questions listed above and constructs emerging from the data, was used to distil key concepts from the interviews. At the first level of analysis, the interviews were read repeatedly by the interviewers (EK and JSs) and a senior social scientist (DB), and then coded by EK and JSs, in consultation with DB and JS. Following the coding, EK and JSs prepared detailed analytical memos on reactions to information on kidney disease; being given information on their own kidney dysfunction; offers of treatment and their own treatment seeking. From these narratives JS, DB, EK and JSs distilled the findings for this paper.

Ethical considerations

Ethics approvals were obtained from the Uganda Virus Research Institute, Research Ethics Committee (UVRI-REC) and the Uganda National Council for Science and Technology (UNCST).

If medical conditions were diagnosed through study screening, the participants were referred to appropriate medical facilities including the local study clinic which had enhanced resources as a result of the ongoing research work. With regards to this study, where most participants were found to have only mild impairment of kidney function, those in need of follow-up were referred to the local clinic, while participants found to have advanced impairment were referred to appropriate nephrology services as directed by the study physicians (Kalyesubula et al., 2020).

Results

The majority of participants in the social science study sample were classified as having Stage 2 kidney dysfunction according to the staging given by Kidney Disease Improving Global Outcomes (KDIGO) CKD Work Group (Kidney Disease Improving Global Outcomes (KDIGO) CKD Work Group, 2013). People with Stage 2 kidney dysfunction do not usually require treatment unless there are complications because of another condition. Any treatment provided depends on risk factors (linked to diabetes or hypertension) and whether specific investigations are needed for kidney disease. Table 1 provides an overview of the kidney disease staging, age and sex of participants in this study.

We present the results in three parts: the reactions participants had to information on kidney dysfunction, their reactions to information on kidney disease and then their views on any treatment they may have been offered. Many of those who participated in the study agreed to take part, not because they had symptoms, but because they saw the survey as an opportunity to have their health assessed for free by health workers from an organisation which they had known for many years. This is summed up by one 40–49 year old man who said:

> I have grown up seeing the organization treating people who were down because of HIV and enabling them to become healthy once again although not being healed completely. Now hearing that those kidney disease researchers were health workers coming from the same organization, I could not hesitate to take part.

Reactions to being given information on kidney dysfunction

Nearly all the people who were told that they have some signs of kidney dysfunction said that they had heard about the condition before the study began. This level of knowledge was unexpected but could be explained by people having been told about the condition when seeking care for other conditions or knowing of relatives who had been given information on the condition or conditions, which sounded similar. One woman, for example, who was living with HIV, said that she had been diagnosed with kidney problems in the past and had learnt of the condition through that experience. However, knowing the name of the condition did not, for most people, mean that they had a detailed knowledge of the condition:

> I never knew that being diabetic or hypertensive could also lead to having the kidney disease, […] I am not aware and that persuaded me to take part. (60–69 year old man – Stage 2)

The information provided on kidney disease during study mobilisation within the GPC led a few people to join the study because of health concerns that they had:

> It (kidney disease) is linked to severe back ache and due to what I heard about the sickness and I was also disturbed by the back pain I decided to test and find out if I have a kidney problem. (80+ year old man – Stage 2)

> What mostly made me accept to participate is the swelling of the legs I experience at times which make me suspect that I could be a kidney patient too. I was even forced to ask the health worker about it but he (the health worker) comforted me not to worry and that I would get to know if the swelling was connected to that after the results come back. (40–49 year old woman – Stage 2)

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Table 1. Kidney disease staging, age range and sex of participants.

	30–39 years		40–49 years		50–59 years		60–69 years		70–79 years		80 years and over		
Chronic kidney disease stage[a]	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	female	Total
2	2	2		8	2	10	2	6	2	3	3	1	41
3a						1			1				2
3b							1						1
4					1								1
5			1										1
Total	2	2	1	8	3	11	3	6	3	3	3	1	46

[a]The staging level of kidney dysfunction was based on one measure of eGFR. The five stages of CKD, GFR and treatment for each stage are:

- Stage 1 – with normal or high GFR (GFR > 90 ml/min/1.73 m^2). No treatment necessary
- Stage 2 – mild CKD (GFR = 60–89 ml/min/1.73 m^2). Advice on healthy lifestyle: maintaining a healthy weight, smoking cessation, exercise levels. Monitoring and treating blood pressure, diabetes and other comorbidities.
- Stage 3A – moderate CKD (GFR = 45–59 ml/min/1.73 m^2). As Stage 2
- Stage 3B – moderate CKD (GFR = 30–44 ml/min/1.73 m^2). As Stage 2
- Stage 4 – Severe CKD (GFR = 15–29 ml/min/1.73 m^2). As Stage 2. Management of fluid balance, blood pressure, anaemia, bone health and dietary changes may be required.
- Stage 5 – End stage CKD (GFR = <15 ml/min/1.73 m^2). Management of fluid balance, blood pressure, anaemia, bone health and dietary changes commonly needed. Dialysis or kidney transplant may be required to continue living.

The information provided to the participants made it clear that if someone had chronic kidney disease it was a serious condition. About half of the participants said that when kidney dysfunction was first described to them, and participation in the study offered, they were very concerned that if they were found to have it, they would not be able to finance their own treatment. Some said that they joined the study to access free treatment for that reason.

> I looked at my health and economic status; I am not all that well. I am disturbed by sickness yet I am poor. What if I have the kidney disease and I refuse to participate? Then later I get to know I am affected. Where do I begin from since even the money for just the daily commodities is hard for me to obtain! I decided to take part because of that! (50–59 year old woman – Stage 2)

The information on the seriousness of chronic kidney disease, shared by the study team, was in some cases conflated with information that people had received from other sources, which raised the concern of some participants who perceived the condition as an acute health threat:

> I had feared very much to be among those whose kidneys are affected because one of my relatives had already told me that once the kidney or the liver become infected, chances are very limited for one to survive. I know death comes to everyone but I fear dying from such a serious illness! (70–79 year old woman – Stage 2)

Participants worried not only about themselves receiving a diagnosis of kidney impairment, but that their relatives and friends may have similar health problems. Concerns about the prevalence of the condition, as well as the fears over the condition being incurable, mirror continued anxiety about HIV in this population (Bukenya et al., 2017).

Reactions to being told of evidence that a person had kidney dysfunction

All 46 participants told that they have some level of impaired kidney function, were given this information by study clinic staff and the condition explained to them. However, when talking to the social science interviewers, more than half of the participants (26/46) said that they did not have kidney dysfunction. Of these, 14 people said that they had not received their results and a further 10 said that the results they received had not indicated that they had such a condition. Two participants said that they had not taken part in the kidney disease screening exercise at all. Both men and women expressed these views:

> They brought some information on a piece of paper. Unfortunately, I was away for my work then they handed the paper to mzee (meaning his father) […] I currently do not know where he placed that piece of paper. (40–49 year old man – Stage 5)

Interestingly this man also commented that his father had told him his kidneys 'were fine'.

> The health workers are the ones who know what they found. They are the ones who know what they are doing but have not disclosed to me what they found. Whether I am sick (with kidney problems) or not, I do not know. (70–79 year old woman – Stage 2)

The health worker team confirmed that all the people sampled for the social science component had received information on the stage of kidney disease they had been identified with and results were only given to the participant, not to family members, which suggests the denial of receiving and knowing about this information was linked to worry about the condition.

A fear of the condition was expressed by others who acknowledged being told that they had kidney dysfunction, but they said that the diagnosis was unexpected. Some of these participants recalled that they had been told that the condition was not curable, and they worried about the high cost of treatment and how family members would manage if they became very ill.

> After getting the results, I am just there, I am completely losing strength. I am worried. You know each time you learn you have an illness you never anticipated; you become worried, your peace is as if taken away. (60–69 year old man – Stage 3b)

Some wished they could have had their condition verified in a different health facility. A 50–59 year old woman mentioned others who sought such support:

> I did not have money for I would have gone to meet health workers elsewhere and find out what they would tell me about the same [kidney] issue because even my co- wife down there, who was invited in the same group, her well off children took her to Kampala for further investigation. (Stage 3a)

Six people said that they had been diagnosed with kidney dysfunction but they were doubtful that the diagnosis was correct. The reasons they doubted the diagnosis was because the test had been 'very simple', so they did not understand how such a test could diagnose a kidney disease. But also, they did not have any symptoms like pain around the lower abdomen where the kidneys are located. This suggests that the way the condition was diagnosed led to people questioning what had actually been tested. They also said that the health workers had provided limited advice on what to do to manage progression, which they would have expected if the disease was severe.

> If I was sick with the kidneys, why did the health workers at your place [study clinic] not prevent me from taking salt? They have not even told me about other things I should avoid if they found out that my kidney was sick. If they have not done so, have they not just put me in a worrying situation? (70–79 year old woman – Stage 3a)

However, 10 people did react positively to news of their condition. Of these, eight reported that they accepted the information and had linked to care at a local health facility where their condition could be monitored and treatment provided if required, as advised by the health workers. If more specialist care was needed, health facility staff could refer patients to specialists at district and regional hospitals. In addition to linking to formal health care, five of the ten people said that they had disclosed their reduced kidney function diagnosis to their spouses, adult children or siblings. The two people, both older men (in their 60s and 80s respectively), who had not linked to care said that they were contemplating the possibility of doing so 'one day'.

Reactions to advice on treatment

Given that half of the participants who had been found with kidney dysfunction had denied their diagnosis, it is not surprising that they also said that they were not accessing treatment. However, it is also very likely that they were participants who had only mild to moderate kidney impairment, and no treatment had been recommended for them. The lack of treatment may have led to doubts about their diagnosis, and hence a view that they were not affected. In addition, some participants were offered treatment for other conditions (urinary tract infections for example), rather than directly for kidney dysfunction, although they considered it to be treatment for kidney disease.

The differences in treatment offered, referred to above, was confusing for some people. A few participants (four people) expressed their doubts about whether the treatment given in the study clinic was effective because of the short duration of treatment relative to the severity of the condition:

> They were just minor tablets which I took for only a week. I then kept wondering, how someone they have said has kidney disease can be given such minor treatment, yet they say the kidney disease is very bad, I was not satisfied!' She continued; 'just such tablets to treat a serious disease! (70–79 year old woman – Stage 2)

Given that this woman did not require any treatment for kidney dysfunction it is likely that this woman had received treatment for a urinary tract infection; if she had kidney disease she would have had follow-up visits to the clinic and if the condition was severe, referral to another facility.

The short duration treatment for other conditions resulted in some people being very positive about 'kidney disease treatment'. Five people were happy about the limited treatment they received and said that they had adhered to the treatment.

> They told me 'your illness is about the kidney! Your kidneys have lots of fluids!' They started giving me drugs […] The drugs I got from [the study clinic] were for two weeks and I took them' (60–69 year old man – Stage 3b)

Nine of those who did disclose their condition to the social science interviewers said that they were seeking alternative forms of treatment in combination with the medication they received from the study clinic. The decision to use alternative medicine, herbs for example, was mostly due to the influence of friends and relatives. The same man who had received the treatment for two weeks, went on to say:

> I was taking tablets from the study clinic but then a friend directed/advised me to take some of that older woman's herbs because she is well known for treating many illnesses. What I did was to take the tablets first then after two hours I would take the herbal medicine so that it does not interfere with the tablets. (Stage 3b)

A 60–69 year old woman, who was assessed as 'Stage 2' so would not have required specific treatment for kidney dysfunction explained her use of herbs for a condition treated at the clinic:

> Since I completed the dosage [unspecified] I obtained from the study clinic, I have been taking powder made from rosemary adding it to hot water. I learnt from that young person (indicating someone nearby) that the herb has a lot of benefits which include brain boosting.

To sum up, we found that among people told that they had impaired kidney function, the majority had heard of the condition, but half denied knowledge of the health status of their kidneys or, in some cases, receiving any results of tests from the clinic team. The denial of the condition and delay or refusal to link to care may have been linked to a lack of symptoms for those with early stage kidney dysfunction, which may have led them to assume the information on kidney dysfunction to be incorrect. The range of treatments given to people who did report both being given information and treatment, caused some uncertainty about whether the condition was indeed serious. This was probably because several people were treated for other conditions (such as urinary tract infections) and did not require treatment specifically for kidney disease.

Discussion

Our findings suggest that there was limited understanding of kidney dysfunction among those told about the condition, a result which mirrors the findings of studies from elsewhere (Ene-Iordache et al., 2016; Kazley et al., 2014; Slevin & Taylor, 2015; Teasdale et al., 2017; Wright Nunes et al., 2016). The lack of symptoms experienced by many of those who were told that they had impaired kidneys led them to doubt that they had the condition, a finding also corroborated elsewhere (Kazley et al., 2014). Receiving a diagnosis provides a label for a condition, affording 'permission' to be ill (Jutel, 2009) and the packaging of symptoms with a label for treatment (Rosenberg, 2002). But when the condition is asymptomatic, a diagnosis may not provide confirmation of a condition, instead it can promote anxiety and disbelief (Walker & Rogers, 2017; Wright Nunes et al., 2016). A desire on the part of medical practitioners not to alarm a patient or overburden someone with other non-communicable conditions with a diagnosis of asymptomatic kidney disease may result in non or partial disclosure (Crinson et al., 2010; Daker-White et al., 2015; Nash et al., 2018). It is also important to note that there is some uncertainty over the interpretation of kidney dysfunction in older people (Hart & Anderson, 2018; Nguyen & Goldfarb, 2012). Many of our respondents were over 60 years of age and mild kidney impairment may have been part of the ageing process and the staging provided during the study was not confirmed with repeat testing for those with early stage kidney dysfunction.

Other research in the UK and Australia shows that perceptions of chronic kidney disease, informed by the information that patients have on the condition, shapes patients wellbeing and their response to treatment (Kazley et al., 2014), as well as influencing health outcomes (Chilcot, 2012). Indeed, Nash and colleagues (Nash et al., 2018) argue for greater attention in primary care facilities to the process of diagnosis to improve the quality of care, based on their findings from Canada.

In our study setting in Uganda, improving the rates of early diagnosis and treatment is important given access to, and affordability of, dialysis treatment for later stage kidney disease. Based on our findings several other factors affect the ways in which people react to a diagnosis, particularly a diagnosis which may be symptomless and 'chronic'. Leventhal and colleagues (2016) in their 'Common-sense model of illness self-management' describe how a model of acute illness shapes the way in which people respond to being diagnosed with a chronic condition. An acute illness is generally characterised by symptoms which cause someone to seek care, from a health service provider or to self-treat. In contrast, many chronic conditions are asymptomatic but 'the acute model leads patients to expect symptoms and a short time-line' (p. 939) (Leventhal et al., 2016), which is an approach that does not fit with the long-term management of asymptomatic progression. A delay in seeking care is often attributed to the lack of symptoms for conditions such as hypertension and diabetes (Musinguzi et al., 2018; Nnko et al., 2015; Rutebemberwa et al., 2013) but also for people living with HIV, before they experience significant illness as a result of the infection (Kawuma et al., 2018; Seeley et al., 2019). Many infectious diseases, common in the Ugandan setting (malaria for example), provide symptoms which lead the person affected to seek immediate treatment (Ladner et al., 2017). As Leventhal and colleagues (2016) note 'patients will not take drugs unless they perceive a need to do so, particularly if asymptomatic' (p. 939), in addition they may not, as we show in our findings, acknowledge that they have been told they have a condition, if they have no symptoms: a self-assessment which may be supported in the view of the patient by the 'failure' of the health services to offer treatment. This 'common-sense' assessment of health needs to be understood if people are to be encouraged to be screened for kidney disease and, if found affected but asymptomatic, supported to engage with follow-up and the monitoring of their condition.

Conclusion

Kidney disease causes both morbidity and mortality in rural Uganda, but in our study most people had a limited understanding of the condition, including of the asymptomatic nature of early stage disease. Our findings point to the importance of understanding the context in which people receive information on conditions which are new to them, the comparisons they may make with the progression of other conditions, and the ways in which they may interpret the type of treatment offered for conditions which they are told are serious.

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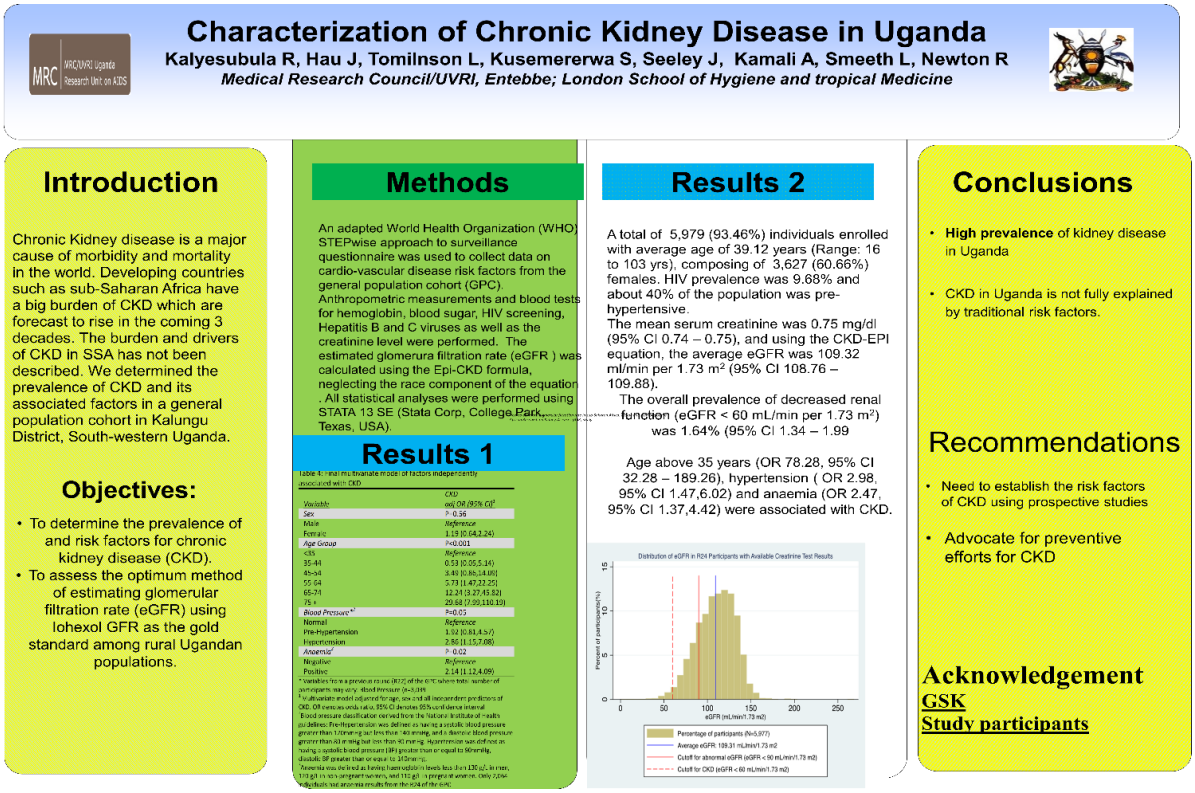
Chapter 7: Discussion, Recommendations and Conclusion

Appendix for Abstracts presented to national and international conferences.



Characterization of Chronic Kidney Disease in Uganda

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Introduction

Chronic Kidney disease is a major cause of morbidity and mortality in the world. Developing countries such as sub-Saharan Africa have a big burden of CKD which are forecast to rise in the coming 3 decades. The burden and drivers of CKD in SSA has not been described. We determined the prevalence of CKD and its associated factors in a general population cohort in Kalungu District, South-western Uganda.

Objectives:

- To determine the prevalence of and risk factors for chronic kidney disease (CKD).
- To assess the optimum method of estimating glomerular filtration rate (eGFR) using Iohexol GFR as the gold standard among rural Ugandan populations.

Methods

An adapted World Health Organization (WHO) STEPwise approach to surveillance questionnaire was used to collect data on cardio-vascular disease risk factors from the general population cohort (GPC). Anthropometric measurements and blood tests for hemoglobin, blood sugar, HIV screening, Hepatitis B and C viruses as well as the creatinine level were performed. The estimated glomerura filtration rate (eGFR) was calculated using the Epi-CKD formula, neglecting the race component of the equation . All statistical analyses were performed using STATA 13 SE (Stata Corp, College Park, Texas, USA).

Results 1

Table 4: Final multivariate model of factors independently associated with CKD

Variable	CKD aOR (95% CI)[1]
Sex	P- 0.36
Male	*Reference*
Female	1.19 (0.64,2.24)
Age Group	P=0.001
<35	*Reference*
35-44	0.53 (0.05,5.14)
45-54	3.48 (0.86,14.08)
55-64	5.73 (1.47,22.25)
65-74	12.24 (3.27,45.82)
75 +	29.44 (7.09,110.10)
Blood Pressure[2]	P=0.03
Normal	*Reference*
Pre-Hypertension	1.92 (0.81,4.57)
Hypertension	2.85 (1.15,7.08)
Anaemia[3]	P- 0.02
Negative	*Reference*
Positive	2.14 (1.12,4.09)

[1] Multivariate model adjusted for age, sex and all independent predictors of CKD. OR versus odds ratio, 95% CI denotes 95% confidence interval

[2] Blood pressure classification derived from the National Institute of Health guidelines. Pre-Hypertension was defined as having a systolic blood pressure greater than 120mmHg but less than 140 mmHg, and a diastolic blood pressure greater than 80 mmHg but less than 90 mmHg. Hypertension was defined as having a systolic blood pressure (BP) greater than or equal to 90mmHg, diastolic BP greater than or equal to 140mmHg.

[3] Anaemia was defined as having haemoglobin levels less than 13% g/L in men, 11% g/L in non-pregnant women, and 11% g/L in pregnant women. Only 7,054 individuals had anaemia results from the 924 of the 6,941

Results 2

A total of 5,979 (93.46%) individuals enrolled with average age of 39.12 years (Range: 16 to 103 yrs), composing of 3,627 (60.66%) females. HIV prevalence was 9.68% and about 40% of the population was pre-hypertensive.

The mean serum creatinine was 0.75 mg/dl (95% CI 0.74 – 0.75), and using the CKD-EPI equation, the average eGFR was 109.32 ml/min per 1.73 m^2 (95% CI 108.76 – 109.88).

The overall prevalence of decreased renal function (eGFR < 60 mL/min per 1.73 m^2) was 1.64% (95% CI 1.34 – 1.99

Age above 35 years (OR 78.28, 95% CI 32.28 – 189.26), hypertension (OR 2.98, 95% CI 1.47,6.02) and anaemia (OR 2.47, 95% CI 1.37,4.42) were associated with CKD.

Conclusions

- **High prevalence** of kidney disease in Uganda
- CKD in Uganda is not fully explained by traditional risk factors.

Recommendations

- Need to establish the risk factors of CKD using prospective studies
- Advocate for preventive efforts for CKD

Acknowledgement

GSK

Study participants

Abstract for protocol- presented to World Congress of Nephrology in Melbourne, Australia-2019 and Montreal, Canada-2021

13 (28%) patients had CKD stage 3. 6/13 had low predicted risk of progression at 5 years of whom 4/6 progressed unexpectedly. 1/13 was identified as having high risk at 5 years and that 1 patient progressed to CKD5D/T.

6/18 CKD 3/4 patients with predicted low risk progressed to CKD5D/T unexpectedly. 1/6 had emergency abdominal surgery, 1/6 patient had unexplained rapid progression and 4/6 had acute upper gastro-intestinal haemorrhage causing terminal decline of kidney function.

Conclusions: The number of patients analysed was small. The 8-variable equation accurately predicted high risk of progression to CKD5D/T in 7/9 CKD3/4 patients.

Conversely, 6/18 patients with predicted low risk progressed to CKD5D/T. Acute medical events including upper gastro-intestinal bleed accounted for most instances of unexpected progression.

SAT-194

PLASMA PEPTIDOMICS BASED MULTIVARIABLE MODEL FOR THE CLASSIFICATION OF HYPERTENSIVE FROM NORMOTENSIVE SUBJECTS IN CKD

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Introduction: Hypertension is a major risk factor for cardiovascular disease and is also a risk factor for chronic renal failure. Despite of advancements in lowering blood pressure, the best approach to lower it, remains controversial due to the lack of information on its development. We therefore, performed plasma proteomics to identify the markers discriminating hypertensive from normotensives.

Methods: Plasma samples from hypertensive (n=118) and normotensive subjects (n=85) from the "InGenious Hypercare" cohort were used for the study. We performed liquid chromatography online coupled to electrospray ionization quadrupole ion trap mass spectrometry for analysis of the plasma samples. Hypertension specific plasma peptides were identified and a model was developed using least absolute shrinkage and selection operator logistic regression. The underlying peptides were identified and sequenced offline using matrix assisted laser desorption ionization mass spectrometry. Further, to get an insight in to the mechanisms, pathway analysis was performed using KEGG and GO databases.

Results: By comparison of plasma samples, 27 biomarkers were identified discriminating hypertensives from normotensives. 70% of the features selected were found to occur less likely in hypertensive patients. A cross-validated predictor model was developed with the overall R square of 0.434 and the area under the ROC curve was 0.891 with 95% confidence interval 0.8482 to 0.9349, P<0.0001. The mean value of the cross-validated predictor score of normotensive and hypertensive patients was found to be -2.007 ± 0.3568 and 3.383 ± 0.2643, respectively. Phosphtidylinositol 3 kinase regulatory, humanin, anoctamin 10, NIK related protein kinase, Mannose-6- phospho isomerase, tryptophan, erythrocyte membrane glycopeptide, transcription factor Dp-2, pleckstrin homology domain-containing family O member 1, cardiac phospholamban, osteocalcin or sarcolipin, ras-related protein Rab-13, protein prune homolog, nexilin and palladin were the identified peptides. The pathway analysis revealed that these proteins had mostly cardiac related functions.

Conclusions: Plasma proteomics model was able to predict the hypertensive-normotensive status based on 27 molecular features. After validation in other cohorts for reproducibility, the identified markers may be useful to clarify the causes of hypertension and to predict the development of hypertension and hence of cardiovascular events.

SAT-195

DIAGNOSTIC UTILITY OF WHOLE-EXOME SEQUENCING IN A CHRONIC KIDNEY DISEASE COHORT

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Introduction: Genomic technologies enable the rapid and cost-effective sequencing of DNA and have demonstrated a definitive diagnosis in several patient groups. The clinical utility of whole exome sequencing (WES) in a kidney disease cohort is not yet well established. We describe the patient characteristics and diagnostic yield of a cohort of 200 patients with suspected genetic kidney disease referred for WES via a multidisciplinary renal genetics clinic.

Methods: 200 sequential patients were recruited into a prospective observational cohort study through five tertiary academic centres in Victoria, Australia. Patients were referred by their treating nephrologist to a dedicated renal genetic service funded by the Melbourne Genomics Health Alliance. Following review by a multidisciplinary team, consisting of a nephrologist, clinical geneticist and genetic counsellor, patients were recruited for genomic sequencing, with analysis for a pre-determined list of genes of interest. We measured the diagnostic yield and its effect on short term clinical management.

Results: Singleton WES was performed on 123 adult patients and 83 paediatric patients. Majority were female (118) and median age was 27 years (range 0-73 years). 100 of these patients were isolated cases (77 had a known positive family history). From 104 exome results available to date (38 paediatric and 66 adults), 43 patients received a positive molecular diagnosis (41%) and of these 22 (51%) resulted in a change from the original clinical diagnosis. The diagnostic yield was greater in the paediatric cohort (53%) compared to the adult cohort (35%). The effects of genomic testing on clinical management is currently being analysed.

Conclusions: Singleton WES resulted in a substantial number of positive diagnoses in both adult and paediatric patients. The ongoing analysis of this cohort will allow delineation of the sensitivity of exome sequencing to current diagnostic methods, enable health economic analysis of testing and facilitate identification of the clinical predictors of a positive diagnosis. To our knowledge, this is the largest prospective kidney disease cohort to undergo whole exome sequencing with integrated utility analysis to date.

SAT-196

HOW TO ESTIMATE GLOMERULAR FILTRATION RATE IN SUB-SAHARAN AFRICA: DESIGN AND METHODS OF THE AFRICAN RESEARCH ON KIDNEY DISEASES (ARK) STUDY

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Introduction: Chronic kidney disease (CKD) is a substantial cause of morbidity and mortality worldwide with disproportionate effects in sub-Saharan Africa (SSA). The optimal methods to accurately estimate glomerular filtration rate (eGFR) and therefore facilitate identification of CKD among African populations are uncertain. We plan to measure iohexol excretion and correlate measured GFR with existing equations to determine the optimal methods to estimate GFR and determine the prevalence of CKD in Malawi, South Africa and Uganda

Methods: The African Research on Kidney Disease (ARK) study is a three country study embedded within existing cohorts. We seek to enrol 3,000 adults >18 years stratified by eGFR using baseline serum creatinine. Study procedures include questionnaires on socio-demographics and potential risk factors for kidney disease, anthropometry, body composition, blood pressure, blood chemistry and urine microscopy and albuminuria. All participants will have a measured GFR (mGFR) by plasma clearance of iohexol at 120, 180 and 240 minutes. Blood and urine samples will be bio-banked.

pre-existing symptoms. 233 (28.5%) used telemedicine facility and sought telephonic advice from (private) physicians, while 149 (18.2%) were able to undertake an in-person visit to their regular healthcare provider. Among those with ESKD, seven subjects were undergoing regular in-centre haemodialysis. Four of them reported missing their scheduled sessions. One developed severe breathlessness and died despite receiving dialysis.

Conclusions: This is the first study conducted in India to assess the effect of the ongoing COVID 19 pandemic on risk perceptions and access to health services for persons with CKD. Our findings provide insights into the risk perceptions, and practices prevailing in a high CKD burden setting in rural India. We highlight the urgent need for comprehensive guidelines that address continuum of care for NCDs/CKD during the current and future disruptions to routine healthcare service delivery. Prioritization by governments to ensure uninterrupted essential primary healthcare services would be key to preparing for future pandemics.

Conflict of Interest: STOP CKDu study is funded by the Government of Andhra Pradesh under a grand challenges research programme in partnership with Indian Council of Medical Research.

POS-331

ASSOCIATION OF IMPAIRED KIDNEY FUNCTION WITH MORTALITY IN RURAL UGANDA: RESULTS OF A GENERAL POPULATION COHORT STUDY

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Introduction: The burden of kidney disease in sub-Saharan Africa is currently poorly understood. Very limited monitoring and treatment is available for people affected. The association with other diseases and with mortality is unknown in this setting. We sought to determine the association between kidney function and subsequent all-cause mortality.

Methods: In a general population cohort with detailed measurement of health-related parameters in rural Uganda, we estimated the baseline glomerular filtration rate (GFR) between 2011-2014 in 5,678 participants. We followed participants up to March 2019 with regular ascertainment of mortality and migration. Using multivariable cox regression, we determined associations between baseline eGFR and mortality.

Results: The median age of the participants at baseline was 36 years (IQR 24-50), 60.7% were female, 14.6% hypertensive, 9.7% HIV-positive and 1.8% diabetic. We registered 140 deaths with a median follow-up of 5.0 years. Adjusting for age and sex, HIV, hypertension, diabetes, BMI, marital status, and alcohol and tobacco use participants with eGFR ≤45 mls/min/1.73m^2 had six-fold higher mortality compared to those with eGFR ≥90mls/min/1.73m^2 (HR 6.12 (95% CI 2.27-16.45)) with strong evidence of a linear trend for risk of mortality as renal function declined (P<0.001).

Conclusions: In a prospective cohort with high rates of follow-up we found that baseline kidney function was associated with subsequently increased mortality in a graded manner. Improved understanding of the determinants of kidney disease and its progression are needed in order to inform interventions for prevention and treatment.

No conflict of interest

POS-332

QUALITY OF LIFE IN PATIENTS WITH DIABETIC NEPHROPATHY: FINDINGS FROM THE KNOW-CKD (KOREAN COHORT STUDY FOR OUTCOME IN PATIENTS WITH CHRONIC KIDNEY DISEASE) COHORT

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Introduction: Diabetic nephropathy (DN) is a major cause of end-stage renal disease, and can affect quality of life (QoL) because it requires arduous lifelong management. This study analyzed QoL differences at baseline and after 5 years between DN anDiabetic nephropathy (DN) is a major cause of end-stage renal disease, and can affect quality of life (QoL) because it requires arduous lifelong management. This study analyzed QoL differences at baseline and after 5 years between DN and non-DN patients with other chronic kidney disease (CKD).d non-DN patients with other chronic kidney disease (CKD).

Methods: The analysis included subjects (n=1766) from the KNOW-CKD (KoreaN cohort study for Outcome in patients With Chronic Kidney Disease) cohort who completed the Kidney Disease Quality of Life Short Form (KDQOL-SF). The factors that influenced the QoL of participants with DN (n=390) were first analyzed, and differences in QoL between DN and non-DN participants was examined. To maintain homogeneity, most factors that influenced the QoL of participants with DN were controlled by propensity score-matched pair sampling using the greedy matching technique. In total, 239 DN and 239 non-DN subjects were finally selected, and differences in the mean KDQOL-SF scores between the 2 groups were then analyzed.

Results: In the multivariate linear regression model, higher QoL scores were found for taller DN subjects and lower QoL scores were found for those who were unemployed or unmarried, received Medical Aid, had lower economic status, had higher platelet counts and alkaline phosphatase levels, and used clopidogrel or insulin. Patient satisfaction (59.9 vs. 64.5, P=0.022) and general health (35.3 vs. 39.1, P=0.041) were significantly lower in the DN group than in the non-DN group. Scores generally decreased in both groups during the 5-year follow-up, and the scores in the work status, sexual function, and role-physical domains were lower among patients with DN than among non-DN patients, but the differences were not statistically significant.

Conclusions: In conclusion, among the DN subjects, socioeconomic factors were found to be strong risk factors for impaired QoL, as well as high platelet counts, high alkaline phosphatase levels, and clopidogrel and insulin use. The DN subjects showed lower QoL than the non-DN subjects in the domains of patient satisfaction and general health. In conclusion, we confirmed that DN itself affected QoL more strongly than other types of CKD.

No conflict of interest

POS-333

THE INDIAN CHRONIC KIDNEY DISEASE STUDY: DETAILED DESCRIPTION OF BASELINE CHARACTERISTICS

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Introduction: The Indian Chronic Kidney Disease (ICKD) study is an ongoing, nationwide, multi-centric prospective cohort study recruiting participants with mild to moderate CKD that aims to identify risk factors for CKD development and progression and implement effective therapies. Here, we report the baseline socio demographic, etiology of CKD, risk factors and laboratory parameters in the inception cohort.

Methods: Patients with confirmed CKD between 18-70 years of age and estimated glomerular filtration rate (eGFR) of 15-60ml/min/1.73m^2 or eGFR >60ml/min/1.73m2 and proteinuria/albuminuria with stable clinical course for at least 3 months have been recruited. Organ transplant recipients, those with malignancy for last 2 years, non-Indian ethnicity, pregnancy in case of females, on immunosuppressive therapy, life expectancy <1 year and with poor functional status are excluded. Socio-demographic details, history related to kidney diseases, traditional and indigenous risk factors, CVD and other co-morbidities are recorded. Blood and urine samples are being collected at baseline and annually. Primary outcome of the study is time to ESRD/RRT, 50% decline in eGFR and any new cardiovascular event

Results: Total 4056 CKD subjects has been enrolled. The mean age of the cohort was 50.3 +/-11.8 years with 67.2% males. Median eGFR was