GLOMERULAR FILTRATION RATE MEASUREMENT AND ESTIMATION: IMPROVEMENT AND VALIDATION OF EXISTING METHODS

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Declaration

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This dissertation includes **three** original papers published in peer-reviewed journals, **one** paper that has been accepted for publication, and **two** unpublished papers. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and, for each of the cases where this is not the case, a declaration is included in the dissertation indicating the nature and extent of the contributions of co-authors.

(Declaration with signature in possession of candidate and supervisor.)

Signature

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Abstract

Glomerular filtration rate (GFR) is regarded as the best measure of kidney function. It can either be measured or estimated. This dissertation aims to provide a better understanding of GFR measurement in order to improve its performance and interpretation. It also aims to validate GFR estimation in local populations and to demonstrate the utility of simple adaptations of existing equations to improve estimation.

On completion of a GFR measurement, various quality control (QC) checks are performed to ensure the accuracy of the result. However, this requires comparison with clearly defined reference ranges. In a study of healthy, potential kidney donors, reference data for two QC parameters were defined.

In a study analysing the effect of measurement errors on GFR, the single-sample method was found to be the most robust technique overall, although for all methods measurement error was generally insignificant compared to expected biological variation in GFR. However, at low GFR values measurement errors were shown to affect all methods significantly. Errors in measurement of the doses were found to have the greatest impact on accuracy.

Using nuclear medicine techniques ^{51}Cr-ethylenediaminetetra-acetic acid (^{51}Cr-EDTA) is the most commonly used and widely studied exogenous filtration marker. However, ^{99m}Tc-diethylenetriaminepenta-acetic acid (^{99m}Tc-DTPA) is gaining favour because of a few technical advantages it has over ^{51}Cr-EDTA, its lower cost, and recent issues with the availability of ^{51}Cr-EDTA. In response to a systematic review suggesting that GFR measurement from the plasma clearance of ^{99m}Tc-DTPA was inaccurate, a mini meta-analysis was performed that demonstrated excellent agreement between ^{51}Cr-EDTA and ^{99m}Tc-DTPA clearance, thus supporting the use of ^{99m}Tc-DTPA as a reliable alternative.

Where GFR cannot be routinely measured, it is frequently estimated using a creatinine-based equation. The use of any equation first requires validation in the population in which it will be used. In a study evaluating the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations in non-cancer, mixed ancestry adults, both equations were found to perform well. However, in a study that evaluated equations in adults with cancer, the GFR estimates were found to be biased and imprecise. This study highlighted the limitations of using estimated GFR for guiding management decisions in

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cancer patients. A further study evaluated 11 estimating equations in non-cancer and cancer populations of South African children. The accuracy of all estimates was poor, particularly in the cancer group. Given the extensive use of GFR estimates in South Africa, these findings have profound implications for their use in the management of children and adults with cancer in this country.

Developing new equations for a specific population requires large datasets and incurs costs that are impractical in most middle- or low-income countries. A simpler alternative is to *adapt* existing equations. This work demonstrates the application of a relatively simple approach to adapt existing equations, using modest amounts of data and a readily available Microsoft® Excel-based tool. While this approach is simple and likely to require further refinement, its utility was demonstrated in paediatric and adult cancer populations.

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Opsomming

Glomerulêre filtrasiespoed (GFS) word as die beste maatstaf van nierfunksie beskou. Dit kan gemeet of geskat word. Hierdie proefskrif se doel is om ‘n beter begrip van GFS meting te bewerkstellig teneinde die metode se verrigting en interpretasie te verbeter. Verder word gepoog om GFS skatting in plaaslike populasies te valideer en om die nut van eenvoudige aanpassings van bestaande vergelykings om skatting te verbeter, te demonstreer.

Na voltooide GFS meting word verskeie gehaltetoetse gedoen om die akkuraatheid van die resultaat te verseker. Dit verg egter vergelyking met duidelik gedefinieerde verwysingswaardes. Verwysingsdata vir twee gehalteparameters is in ’n studie van gesonde moontlike nierskenkers daargestel.

In ‘n studie wat die effek van metingsfoute op GFS ontleed, is bevind dat die enkel-monster metode algeheel die mees robuste tegniek is, alhoewel metingsfout vir alle metodes oor die algemeen vergeleke met verwagte biologiese variasie in GFS nie betekenisvol was nie. By lae GFS waardes is egter aangetoon dat metingsfoute alle metodes betekenisvol beïnvloed. Daar is getoon dat foute in meting van die dosis die grootste impak op die akkuraatheid het.

^{51}Cr-etileendiamientetra-asynsuur (^{51}Cr-EDTA) is die mees algemeen gebruikte en bes nagevorste eksogeene filtrasiemerker in kerngeneeskundige tegnieke om GFS te bepaal. ^{99m}Tc-di-etileentriamienpenta-asynsuur (^{99m}Tc-DTPA) wen egter veld weens ‘n aantal tegniese voordele wat dit bo ^{51}Cr-EDTA het, soos laer koste en meer onlangse probleme met die beskikbaarheid van ^{51}Cr-EDTA. In antwoord op ‘n sistematiese oorsig wat daarop dui dat GFS meting gebaseer op plasma opruiming van ^{99m}Tc-DTPA onakkuraat sou wees, is ‘n mini meta-analise gedoen wat uitstekende ooreenkoms tussen ^{51}Cr-EDTA en ^{99m}Tc-DTPA opruiming getoon het, en sodoende die gebruik van ^{99m}Tc-DTPA as betroubare alternatief ondersteun.

Waar GFS nie roetinegewys gemeet kan word nie, word dit dikwels geskat met behulp van ‘n kreatinien-gebaseerde vergelyking. Die gebruik van enige vergelyking vereis eers validasie in die populasie waarin dit gebruik gaan word. ‘n Studie waarin die sogenaamde *Modification of Diet in Renal Disease (MDRD)* en *Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)* vergelykings in kankervrye volwssenes van gemengde afkoms evalueer is, het bevind dat beide vergelykings goed doen. ‘n Ondersoek wat die vergelykings in volwassenes met kanker evalueer het, is egter bevind date GFS skattings sydig en nie presies was nie.

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Hierdie studie het die beperkings van die gebruik van GFS skattings om oor kliniese hantering van kankerpasiënte te besluit, uitgelig. ‘n Verdere ondersoek het 11 skattings-vergelykings in populasies van Suid-Afrikaanse kinders met en sonder kanker evalueer. Die akkuraatheid van alle skattings was swak, veral in die groep met kanker. In die lig van die wye gebruik van GFS skattings in Suid-Afrika, het hierdie bevindings verregaande implikasies vir hul gebruik in die hantering van kinders en volwassenes met kanker in hierdie land.

Die ontwikkeling van nuwe vergelykings vir spesifieke populasies vereis groot datastelle en koste wat in die meeste middle- en lae-inkomste lande onprakties is. *Aanpassing* van bestaande vergelykings is ‘n eenvoudiger alternatief. Hierdie werk toon die toepassing van ‘n redelik eenvoudige benadering om bestaande vergelykings aan te pas met behulp van matige hoeveelhede data en geredelik beskikbare Microsoft® Excel-gebaseerde nutsprogrammatuur. Alhoewel die benadering eenvoudig is en waarskynlik verdere verfyning gaan vereis, is die nut daarvan in volwasse kanker- en pediatriese populasies getoon.

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List of abbreviations

All abbreviations have been defined in the text when used for the first time. A full list of all abbreviations used in this dissertation appears below.

BNMS	British Nuclear Medicine Society
BSA	body surface area
CI	confidence interval
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CKiD	Chronic Kidney Disease in Children
CPM	counts per minute
CV	coefficient of variation
CV_{BV}	coefficient of variation of biologic variation
CV_{ME}	coefficient of variation of measurement errors
CV_{RM}	coefficient of variation of repeat measurements
EANM	European Association of Nuclear Medicine
ECV	extracellular fluid volume
eGFR	estimated glomerular filtration rate
FAS	full age spectrum
Gao's QF	Gao's quadratic formula
GFR	glomerular filtration rate
HIV	human immunodeficiency virus
IDMS	isotope dilution mass spectrometry
IQR	interquartile range
KDIGO	Kidney Disease Improving Global Outcomes
LOA	limits of agreement
MAE	median absolute error
MDRD	Modification of Diet in Renal Disease
mGFR	measured glomerular filtration rate
min cts	minimum counts
MPE	median percentage error
NHLS	National Health Laboratory Service
NPV	negative predictive value
PPV	positive predictive value

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QA	quality assurance
QC	quality control
RMSE	root mean square error
SAMRC	South African Medical Research Council
Scr	serum creatinine
SD	standard deviation
SHI	simple height-independent
SI-GFR	slope-intercept glomerular filtration rate
SO-GFR	slope-only glomerular filtration rate
SS-GFR	single-sample glomerular filtration rate
T½	half time
V_D	volume of distribution
WHO	World Health Organization

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Contributions

Declaration by the candidate:

With regard to the following chapters/articles, the nature and scope of my contribution were as follows:

Title of paper (page number)	Nature of contribution	Extent of contribution (%)
^{99m}Tc-DTPA volume of distribution, half-life and glomerular filtration rate in normal adults **(p.1-21)**	Literature review, data analysis and manuscript	70
Measuring GFR using the plasma clearance of ^{99m}Tc-DTPA **(p. 22-25)**	Literature review, data analysis and manuscript	75
Propagation of measurement errors in glomerular filtration rate determination: a comparison of slope-intercept, single-sample and slope-only methods **(p. 26-47)**	Literature review, data analysis and manuscript	60
Validation of equations to estimate glomerular filtration rate in South Africans of mixed ancestry **(p. 48-63)**	Literature review, data analysis and manuscript	70
Estimated glomerular filtration rate in children: evaluating and adapting existing equations for a specific population **(p. 64-82)**	Literature review, data analysis and manuscript	70
Can glomerular filtration rate estimation be adapted for local oncology patients? **(p. 83-102)**	Literature review, data analysis and manuscript	75

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Propagation of measurement errors in glomerular filtration rate determination: a comparison of slope-intercept, single-sample and slope-only methods **(p. 26-47)**			
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Introduction

Background

Glomerular filtration rate (GFR), defined as the rate at which the kidneys filter plasma, is widely regarded as the best measure of kidney function (Brenner and Levine 2008). In a young, healthy adult the average GFR is 120-130 ml/min, decreasing with age (Sherwood 2007). By convention, GFR measurements are scaled to body surface area (BSA) to allow comparison between individuals of different body sizes (Peters et al. 2000).

GFR determination is essential in many clinical scenarios. Foremost, it is used in patients with chronic kidney disease (CKD) for diagnosis, staging, and predicting prognosis (KDIGO 2013). GFR measurement is also required for the assessment of live kidney donors. Local practice, which is based on international guidelines, requires a GFR of ≥ 80 ml/min/1.73 m^2 for kidney donation (Sawinski and Locke 2018; BTS 2018). GFR determination is frequently required for evaluation of kidney function in patients with complex urological problems or prior to renal surgery (Burniston 2018). In cancer patients who will be treated with nephrotoxic chemotherapy, GFR is used to determine baseline kidney function and to monitor kidney function during treatment (Launay-Vacher et al. 2008). The GFR requirements differ depending on the type of chemotherapy, but in most instances a GFR of < 60 ml/min/1 73m^2 will either preclude the use of the drug or will necessitate a reduction in dose (UCLH 2009). For cancer patients being treated with carboplatin, GFR is used for the calculation of individual doses (Calvert 1989)..

As no test exists that can measure GFR directly, it requires an indirect approach, by measuring the urinary or plasma clearance and/or plasma concentration of another substance (Levey and Inker 2016). Urinary inulin clearance is regarded as the gold standard technique because inulin possesses characteristics closest to a perfect filtration marker, rendering its clearance closest to 'true' GFR (Brenner and Levine 2008). However, its measurement is complex as it requires a continuous intravenous infusion to maintain a constant plasma concentration, with carefully timed urine and blood collections (Sherwood 2007). For this reason, it is seldom used in the clinical setting and is generally reserved for research.

Measurement of the urinary or plasma clearance of other exogenous filtration markers provides an accurate, yet simpler alternative. The filtration marker is administered as an intravenous or

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subcutaneous bolus, obviating the need for a steady-state infusion. The most commonly used exogeneous filtration markers are ^{51}Cr-ethylenediaminetetra-acetic acid (^{51}Cr-EDTA), ^{99m}Tc-diethylenetriaminepenta-acetic acid (^{99m}Tc-DTPA), ^{125}I-iothalamate, non-radioactive iothalamate, and iohexol (Stevens and Levey 2009). Small systematic differences in the clearance of these markers and urinary inulin clearance exist, but provided standardized protocols are adhered to, the accuracy of all of these methods is good (Stevens and Levey 2009; Soveri et al. 2014). The choice of filtration marker and technique used largely depends on availability, cost and experience. British Nuclear Medicine Society (BNMS) and European Association of Nuclear Medicine (EANM) GFR guidelines recommend the use of either ^{51}Cr-EDTA or ^{99m}Tc-DTPA (Piepsz et al. 2001; Fleming et al. 2004; Burniston 2018) and consequently, these have become the preferred filtration markers in Europe. As South African nuclear medicine departments frequently base their practice on European guidelines, most also use these tracers.

Despite being methodologically simpler than urinary inulin clearance, measurement of the clearance of alternative exogenous filtration markers remains unavailable in many centres for a variety of reasons including lack of local expertise and its relatively time- and labour-intensive nature. It is thus not feasible to measure GFR in all patients. Generally, GFR measurement is reserved for the patient groups in which high accuracy is required, e.g. potential kidney donors. In the majority, however, an estimation of GFR is widely used.

The serum creatinine concentration provides an estimate of GFR because in the steady state it is related to the reciprocal of GFR (Stevens et al. 2006). The appeal of estimated GFR is that it only requires serum creatinine (Scr) measurement and is therefore simple, cheap and widely available. However, the Scr concentration is also affected by many non-GFR determinants including age, sex, ethnicity, body habitus, chronic illness, diet and certain medications (Stevens and Levey 2009). Various equations have been described to correct the Scr concentration for some of these factors, thus improving the accuracy of the GFR estimate. Despite this, few adaptations exist for the many different patient populations. This may be related to the resources required and relative complexity of developing these equations.

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Research objectives

The intention of any GFR measurement or estimation is to provide a value that is as close to the true GFR as possible, and thereby an accurate reflection of kidney function. The research objectives were thus:

- to increase our knowledge of specific aspects of GFR measurement in order to improve its performance and interpretation.
- to validate GFR estimation in South African populations and, where applicable, to develop tools to adapt existing equations in order to improve GFR estimation.

Significance and motivation

Many important clinical management decisions are based on GFR. For example, GFR must be above a certain level for a person to donate a kidney or to receive potentially curative but nephrotoxic chemotherapy for cancer. Certain patients, such as those with complex urological problems or on life-long immunosuppressive therapy, require long-term, serial GFR measurements to ensure timely detection of a deterioration in kidney function. GFR is also required to calculate individualised doses of certain chemotherapy drugs. In these challenging clinical scenarios, it is essential that GFR is measured as accurately as possible.

The 2004 BNMS GFR guideline (Fleming et al. 2004), which was in effect until 2018, considered the volume of distribution and clearance half-time of the injected radiopharmaceutical to be important quality assurance tools to ensure the reliability of results of GFR measurement studies. However, the reference ranges provided for these parameters in the guideline were vague. This work sets out to better define them.

Interpretation of a GFR measurement requires comparison with reference data. However, most published reference ranges for GFR are based on the clearance of ^{51}Cr-EDTA, inulin, iohexol or iothalamate. Normative data for the plasma clearance of ^{99m}Tc-DTPA is scant. This has recently become significant as many departments have been compelled to change over to ^{99m}Tc-DTPA due to the global unavailability of ^{51}Cr-EDTA.

Genetically, socioeconomically and frequently also in terms of diet and pathology, local South African populations differ from the predominantly White northern hemisphere populations from which most GFR literature originates. There are also no locally derived formulae for

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estimating GFR, compounded by a paucity of studies that have validated GFR estimation in South African populations. Consequently, clinicians are frequently left with no alternative but to utilize existing estimation equations that are likely to be unsuitable for other patient populations.

Thesis, delineation, research questions

This dissertation has been compiled in one of the accepted hybrid formats, comprising both published and unpublished articles. Two articles describe prospective work, three describe retrospective work, and one is a letter to the editor containing a mini meta-analysis in response to a recently published review. The first two studies and letter address issues around GFR measurement. The latter three studies are more clinical and focus on GFR estimation in three different clinical populations. All studies were approved by the Stellenbosch University Health Research Ethics Committee. Approval from the University of Cape Town Human Research Ethics Committee was also obtained for the study that was conducted at the Red Cross War Memorial Children's Hospital.

The studies were designed around the following research questions:

- Are published reference ranges for GFR and two of its quality assurance parameters that were described for ^{51}Cr-EDTA valid for ^{99m}Tc-DTPA?
- Is the plasma clearance of ^{99m}Tc-DTPA a sufficiently accurate method to measure GFR?
- What is the statistical and clinical effect of measurement errors on GFR? Which measurement errors have the greatest effect? And which GFR calculation method is least sensitive to measurement errors?
- How accurate are published GFR estimation equations in a number of patient populations:
    - adult patients of mixed ancestry with and without chronic kidney disease
    - adult cancer patients
    - children with pathology
- In any of these populations, can the performance of the estimating equations be improved by adapting the equations to better fit the data?

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Definitions, assumptions and limitations

True GFR cannot be measured, so irrespective of the method used, the value obtained is an estimate of the true value (Levey and Inker 2016). However, for the purpose of clarity, in this dissertation 'measured GFR' or 'GFR measurement' refers to the measurement of the clearance of an exogenous filtration marker, specifically in this work the plasma clearance of ^{99m}Tc-DTPA or ^{51}Cr-EDTA. 'Estimated GFR' or 'GFR estimation' refers to the use of an empiric equation to estimate GFR from the serum creatinine concentration and sometimes additional patient-specific characteristics. These have been abbreviated mGFR and eGFR respectively.

In certain papers, the terms 'tracer' and/or 'radiopharmaceutical' have been used in place of 'exogenous filtration marker'.

The abbreviations '2-point SI-GFR' and '3-point SI-GFR' refer to GFR that is calculated using the slope-intercept method using 2 or 3 blood samples respectively.

The P_{30} of an estimated GFR equation is a measure of its accuracy and is defined as the proportion of estimates that are within 30% of the measured GFR (Stevens et al. 2006).

For the purposes of this dissertation, good accuracy has been defined as $P_{30} > 80\%$, moderate accuracy P_{30} of 60-80%, and poor accuracy $P_{30} < 60\%$.

Measurement of creatinine clearance is an established technique to estimate GFR (Sherwood 2007). However, because it systematically overestimates GFR, and is frequently erroneous due to incomplete urine collections, it has been superseded by more robust methods and its measurement is largely historical (Perrone et al. 1992; Stevens et al. 2006). It has thus not been addressed in this dissertation.

The serum concentration of cystatin-C provides an additional method for estimating GFR (Stevens et al. 2006), but at present, there are no laboratories in South Africa than have the facilities to measure it. It was thus also not included in this work.

A general limitation of four of the studies is that, being based on populations that are demographically representative of the population of the Western Cape province, the results may not be generalizable to other South African or international populations.

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Theory base, general literature review

Using nuclear medicine techniques, GFR is usually measured from the plasma clearance of either ^{99m}Tc-DTPA or ^{51}Cr-EDTA. Both tracers are considered acceptable in current BNMS and EANM GFR guidelines (Piepsz et al. 2001; Burniston 2018). Interpretation of a GFR measurement requires comparison with normative data and for this purpose the previous BNMS guideline cited a ^{51}Cr-EDTA reference range described by Granerus and Aurell (Granerus and Aurell 1981; Fleming et al. 2004). Subsequently, ^{51}Cr-EDTA reference ranges have been described in many other larger populations (Hamilton et al. 2000; Grewal and Blake 2005; Peters et al. 2012b; Soares et al. 2013; Blake et al. 2013). However, reference ranges for ^{99m}Tc-DTPA are lacking. In a 2014 systematic review, which compared the plasma and urinary clearance of various tracers to urinary inulin clearance, some doubt was cast over the use of ^{99m}Tc-DTPA. Its plasma clearance was concluded to be too inaccurate for routine use whereas plasma clearance of ^{51}Cr-EDTA was found to be sufficiently accurate (Soveri et al. 2014). These results contradicted the finding of good agreement between ^{99m}Tc-DTPA and ^{51}Cr-EDTA in earlier studies (Rehling et al. 1984; Fleming et al. 1991; Biggi et al. 1995). This was the motivation for the letter and mini meta-analysis in chapter 2.

When measuring the plasma clearance of ^{99m}Tc-DTPA or ^{51}Cr-EDTA, calculation of the area under the full (bi-exponential) clearance curve provides optimal accuracy. However, this is labour-intensive and invasive as it requires at least 10 blood samples to be taken from the patient (Fleming et al. 2004). Its complexity led to the development of simpler alternatives. Of these, the slope-intercept (Chantler et al. 1969), single-sample and slope-only methods (Peters 1992) are most commonly used. Using the slope-intercept method, only the terminal exponential of the plasma clearance curve is measured. Two to four blood samples are taken at timed intervals after equilibration of the tracer. The counts of each sample are first expressed in terms of the standard counts. Then, the natural logarithms of these values are plotted against time, linear regression is used to fit the points to a single curve, and the area under the curve is calculated. Finally, a correction is made to account for the missing early exponential (Fleming et al. 2004).

Single-sample GFR uses an equation to calculate GFR from a single plasma sample that is taken, in most cases, between 3 and 4 hours after administration of the tracer. It is based on the premise that at any fixed time point, there is an inverse relationship between GFR and the

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plasma concentration of the tracer (Fleming et al. 2004). Consequently, many single-sample equations have been described with varying degrees of accuracy (McMeekin et al. 2016a).

The slope of the terminal exponential of the plasma clearance curve represents the rate at which glomerular filtration turns over the extracellular fluid (Peters 1992). Using the slope-only method, only this rate constant (α_2) is measured, and GFR is thus expressed in terms of extracellular fluid volume rather than BSA. At least 3 plasma samples are required, all taken after equilibration of the tracer. The natural logarithms of the background-corrected plasma counts are plotted against time, and linear regression is used to fit the points to a single curve (Peters 1992).

International and the current BNMS guidelines recommend a single-sample method (Blaufox et al. 1996; Burniston 2018). Single-sample GFR has the advantage of requiring only one blood sample, which makes it the least invasive of the three methods and requires the least time in the department for the patient. Furthermore, strong recent evidence supports its routine use (McMeekin et al. 2016 a; McMeekin et al. 2016b). The slope-only method has many proponents as it has various physiological and technical advantages over the other methods (Peters 1992). The methodology is fairly straightforward as preparation of a standard is not required, and it does not express GFR in terms of BSA. Height and weight measurement, which may be challenging in certain patients e.g. amputees, are also not required. (Peters et al. 2000).

In contrast, the slope-intercept method is the most complex technically, requiring many measurements. The patient is first weighed and his/her height is measured. After the radiopharmaceutical has been prepared, patient and standard doses are drawn up from the vial. These doses require accurate calibration by measuring their mass, activity or volume, both before and after administration. The technique used by departments to prepare the standard varies, but in our unit the tracer is added to a half-filled 100 ml volumetric flask, which is then filled with distilled water to the 100 ml mark. Two 1 ml aliquots are pipetted from this flask into a second half-filled volumetric flask, which is then filled to the 100 ml mark. After mixing, 2 x 1 ml samples are pipetted into counting tubes. The exact times of administration of the tracer to the patient and times of blood sampling are recorded. Once the blood samples have been centrifuged, duplicate plasma samples are pipetted into counting tubes. All samples (standard and plasma) are counted in a gamma well counter (Fleming et al. 2004). Errors in *any* of these measurements will affect the final GFR value.

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Studies that have attempted to quantify the effect of measurement errors on GFR are limited. In a simulation study, De Sadeleer et al (De Sadeleer et al. 2000) found that after introducing errors to the blood sampling times and activity measurements, errors in slope-only GFR were greater than slope-intercept GFR. A subsequent study by the same group found that these measurement errors had a smaller effect on single-sample GFR than slope-intercept GFR (De Sadeleer et al. 2001). However, these studies were criticized as they did not assess the impact of errors in other important measurements (Watson 2000; Peters 2001).

The previous BNMS GFR guideline recommended the slope-intercept method and described its methodology in detail (Fleming et al. 2004). Consequently, this became the preferred method in many departments around the world. More recently, literature has emerged showing the single-sample method to be as accurate as the slope-intercept method and the slope-intercept method to have no additional advantage in terms of quality assurance (McMeekin et al. 2016a; McMeekin et al. 2016b). For these reasons, combined with the convenience and comfort it confers to patients, the single-sample method has replaced the slope-intercept method in the current BNMS guideline (Burniston 2018). A more thorough comparison of the methods in terms of their sensitivity to measurement error will provide further evidence to inform this change in practice.

On completion of a GFR measurement study, a number of quality control (QC) checks are performed (Fleming et al. 2004; Burniston 2018). Comparing the volume of distribution (V_D) of the administered tracer and the half-time (T½) of its clearance to published reference data are two such checks that were considered important in the previous BNMS guideline (Fleming et al. 2004). An abnormality in either of these biological parameters alerts the nuclear physician to a potential problem with the GFR measurement. However, in addition to the vague origins of the reference data for these two parameters, a limitation to their widespread use is that the reference data was based on ^{51}Cr-EDTA clearance in predominantly White, British populations (Fleming et al. 2004; Fleming et al. 2009). It is not known whether the reference ranges are (i) applicable for ^{99m}Tc-DTPA and (ii) applicable in local populations who differ ethnically and genetically to British populations.

The value of QC parameters such as V_D has been challenged recently. In a systematic review, McMeekin et al (McMeekin et al. 2016b) classified errors that can occur during GFR measurement into 3 categories: model failure (type 1); errors that affect the whole clearance curve (type 2); and individual point measurement errors (type 3). They found most

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conventional QA parameters to be insensitive for detecting type 1 errors, a conclusion that was subsequently supported by Klein et al (Klein et al. 2019). However, conventional QA parameters are thought to have some utility in the detection of type 2 and type 3 errors, but the design of the systematic review did not enable it to assess these adequately.

In centres where GFR cannot be measured, or where the requirement for GFR measurement exceeds the department's capacity, clinicians are compelled to rely on a GFR estimate. In adults the Modification of Diet in Renal Disease (MDRD) (Levey et al. 2007) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (Levey et al. 2009) equations are most commonly used, with the CKD-EPI equation being recommended in international and local CKD guidelines (KDIGO 2013; SARS 2015). Both equations were developed in North American, predominantly White populations (Levey et al. 1999; Levey et al. 2009). Results of a more recent systematic review suggest that ethnicity has an effect on the performance of the equations, finding neither the MDRD nor CKD-EPI equation to perform as well in African or Asian populations as it did in North American and European populations (Earley et al. 2012). In African-American individuals an ethnicity factor needs to be applied to the equations as this population has higher average Scr levels due to genetic differences in creatinine generation (Worrall et al. 1990; Jones et al. 1998). A systematic overestimation of GFR by the MDRD equation in a Japanese population suggests that this population has lower Scr levels on average (Imai et al. 2007). There are a few studies that have evaluated the equations in Black and Indian South African adults (van Deventer et al. 2008; van Deventer et al. 2011; Stevens et al. 2011; Madala et al. 2011; Moodley et al. 2018). Their results varied substantially, but in general, the accuracy of the equations was poor. It is not known how the estimating equations will perform in individuals of mixed ancestry considering this population's genetic diversity (de Wit et al. 2010).

In children multiple GFR estimating equations have been described. The older equations were developed prior to routine standardization of Scr assays (Counahan et al. 1976; Schwartz et al. 1976; Morris et al. 1982; Léger et al. 2002; Mattman et al. 2006). A few are adult equations that have been adapted for paediatric use (Biörk et al. 2007). Others were developed in cancer populations (Cole et al. 2004; Brandt et al. 2006; Millisor et al. 2017), whereas the majority were developed in CKD populations (Schwartz et al. 2009; Pottel et al. 2010; Gao et al. 2012; Pottel et al. 2012; Schwartz et al. 2012; De Souza et al. 2012; Hoste et al. 2013; Pottel et al. 2016). The most well-known of these, the Schwartz formula (Schwartz et al. 2009), was used

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in the large Chronic Kidney Disease in Children (CKiD) study (Wong et al. 2012). The results of subsequent validation studies in CKD populations are discrepant, some showing excellent agreement with the measured GFR, others showing unacceptably low accuracy (Pottel et al. 2010; Staples et al. 2010; Bacchetta et al. 2011; Gao et al. 2012; Pottel et al. 2012; Selistre et al. 2012; De Souza et al. 2012; Blufpand et al. 2013; Hoste et al. 2013; Uemura et al. 2014; Deng et al. 2015; Pottel et al. 2016). Fewer studies exist that have tested the equations in children with cancer, but in general, estimated GFR has been found to be less accurate in this group (Blufpand et al. 2011; Bernhardt et al. 2015; Millisor et al. 2017; Llanos-Paez et al. 2018). The large number of equations that have been developed together with their varying performance in validation studies, suggests that the equations are largely population specific.

Due to the nephrotoxicity of certain chemotherapy drugs, e.g. the platinum-based agents, cancer patients form a large proportion of the patients requiring GFR measurement. The GFR is used in one of three main settings: (i) determining whether kidney function is adequate prior to starting nephrotoxic chemotherapy, (ii) serial monitoring of kidney function during treatment, and (iii) calculation of the dose of certain drugs e.g. carboplatin (Calvert et al. 1989; Launay-Vacher et al. 2008). CKD-based estimating equations have been found to be biased and imprecise in patients with cancer (Wright et al. 2001; Poole et al. 2002; Marx et al. 2004; Verhave et al. 2005; de Lemos et al. 2006; Barraclough et al. 2008; Ainsworth et al. 2011; Hartlev et al. 2012; Lauritsen et al. 2014), and the number of equations developed in cancer populations is limited (Jelliffe 1973; Martin et al. 1998; Wright et al. 2001; Janowitz et al. 2017). Those available were developed in European or American populations (Jelliffe 1973; Martin et al. 1998; Wright et al. 2001; Janowitz et al. 2017). It is thus required to test the CKD and cancer GFR estimating equations in a local cancer population in which differences in ethnicity, diet and pathology exist.

Brief paper overviews

Paper 1 (p. 1) is an original article that derived reference data for V_D and T½, two parameters that are frequently used for quality assurance of GFR studies. It also derived reference data for GFR using ^{99m}Tc-DTPA. This paper was published in *Nuclear Medicine Communications* (Holness et al. 2013).

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Paper 2 (p. 22) is a letter to the editor containing a mini meta-analysis, supporting the use of the plasma clearance of ^{99m}Tc-DTPA to measure GFR. It was published in *American Journal of Kidney Diseases* (Holness et al. 2015).

Paper 3 (p. 26) is an original article analyzing the effect of measurement errors on GFR. It compares their impact on the slope-intercept, single-sample and slope-only methods, and it performs a sensitivity analysis to the various sources of measurement error. It allows conclusions to be made about the clinical impact of the resultant error in GFR and provides recommendations for minimizing measurement error. This paper was published in *Nuclear Medicine Communications* (Holness et al. 2019).

Paper 4 (p. 48) is a prospective study evaluating the performance of the MDRD and CKD-EPI GFR estimating equations in a mixed ancestry, adult population. This paper has been accepted for publication in the *South African Medical Journal* (July 2019).

Paper 5 (p. 64) is a retrospective evaluation of GFR estimating equations in a largely non-White adult cancer population. The parameters of the equations are adapted, and the performance of the adapted equations is compared to that of the original equations. This manuscript is currently being finalised for submission.

Paper 6 (p. 83) is a combined prospective/retrospective study evaluating the performance of 11 GFR estimating equations in a Western Cape paediatric population. In order to improve their accuracy, it also examines the adaptation of the parameters of four of the equations and compares the performance with the original equations. This manuscript is currently being finalised for submission.

Candidate's role in the PhD

Retrospective studies (papers 1, 3 and 5)

Dr Holness developed the protocols for each of these studies. She reviewed the records of all participants. This entailed accessing the original GFR measurements from nuclear medicine records, obtaining additional clinical management from the Tygerberg Hospital Enterprise Content Management (ECM) system, and laboratory values from the National Health Laboratory Service (NHLS). Dr Holness was responsible for all data capture, and performed

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the data analysis with assistance from Prof James Warwick (papers 1, 3 and 5), Prof John Fleming (papers 1 and 3) and two biostatisticians, Justin Harvey and Maxwell Chirehwa (papers 1 and 3 respectively). Dr Holness was responsible for the writing of all three manuscripts, and all co-authors reviewed their respective manuscripts.

Prospective studies (papers 4 and 6)

Dr Holness developed the protocols for these studies. This included the development of the patient consent forms, participant check lists, and data capture forms. Screening and recruitment of participants was performed by Dr Karla Bezuidenhout in the Tygerberg Hospital Nephrology outpatient clinic (paper 4) and Dr Anita Brink in the Nuclear Medicine Division, Red Cross Children's Hospital (paper 6). The GFR measurements for paper 4 were performed by a research assistant who is experienced in GFR measurement. As the participants in paper 6 were all referred for GFR measurement as part of their clinical management, the actual measurements were performed by the radiography staff of the Nuclear Medicine Division, Red Cross Children's Hospital. The blood samples that were sent for creatinine measurement were taken by the research assistant/staff member performing the GFR study. Dr Holness was responsible for data capture. Data analysis was performed by Dr Holness with assistance from Prof James Warwick. Dr Holness wrote both manuscripts, and these were reviewed by all co-authors.

Letter to the editor (paper 2)

The data was analysed by Dr Holness with assistance from Prof James Warwick. Dr Holness wrote the letter and this was reviewed by the co-authors.

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1. ^{99m}Tc-DTPA volume of distribution, half-life and glomerular filtration rate in normal adults

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Abstract

Background and aim: Assessment of volume of distribution (V_D) and half-life ($T_{½}$) values during glomerular filtration rate (GFR) investigations is a useful quality control check. The aim of this study was to derive reference data for V_D and $T_{½}$ and also to provide reference data for GFR from studies performed using ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA).

Methods: This was a retrospective study of 126 healthy potential kidney donors (age range 18-59 years). GFR was evaluated from ^{99m}Tc-DTPA plasma clearance using the 2004 British Nuclear Medicine Society guidelines. The association between V_D and body surface area (BSA) was assessed. $T_{½}$ was correlated with age and with GFR. The correlation between Bröchner-Mortensen-corrected GFR ($BM\text{-}GFR_{Corr}$) and age was evaluated.

Results: Uncorrected V_D (L) was (10.1*BSA) ± 40.6% ($p<0.01$). Corrected V_D (L) was (8.19*BSA) ± 34.4% ($p<0.01$). In individuals under the age of 40 years mean $T_{½}$ was 95.0 min ± 36.2%. In individuals 40 years and older, $T_{½}$ increased at a rate of 0.49 min/year ($p=0.04$). $T_{½}$ (min) was [9480*(1/$BM\text{-}GFR_{Corr}$)] ± 35.1% ($p<0.01$). In individuals younger than 40 years the correlation of $BM\text{-}GFR_{Corr}$ and age was not statistically significant ($p=0.45$) and mean GFR was 108 $ml.min^{-1}.(1.73\ m^2)^{-1}$ ± 27.5%. In individuals 40 years and older $BM\text{-}GFR_{Corr}$ was [170 - (1.55*age)] $[ml.min^{-1}.(1.73\ m^2)^{-1}]$ ± 36.7% ($p<0.001$).

Conclusion: Well defined reference data for V_D and $T_{½}$ can be used as quality control checks in GFR investigations. In addition to these, reference data for GFR using ^{99m}Tc-DTPA have been defined. This will enhance the interpretation of adult ^{99m}Tc-DTPA GFR measurements.

Keywords: ^{99m}Tc-DTPA, volume of distribution, half-life, glomerular filtration rate, reference ranges

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Introduction

Glomerular filtration rate (GFR) is a standard measure of renal function. Although measuring plasma inulin clearance remains the gold standard for determining GFR, this technique is rarely used because it is time-consuming and difficult to perform (Blaufox et al. 1996; Fleming et al. 2004). An estimate of GFR can be obtained by measuring creatinine clearance; however, this technique is inaccurate, especially in cases of poor renal function (Bröchner-Mortensen and Rödbro 1976; Sawyer et al. 1982). Measurement of GFR using Nuclear Medicine techniques is considered a suitable alternative with clearance of ^{51}Cr-ethylenediaminetetraacetic acid (^{51}Cr-EDTA) having been shown to be similar to that of inulin (Garnett et al. 1967; Bröchner-Mortensen et al. 1969).

^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) is considered an acceptable alternative to ^{51}Cr-EDTA (Blaufox et al. 1996; Fleming et al. 2004). It has the advantages of being inexpensive, widely available and the radiation dose to the patient is low. It is also suitable for gamma camera imaging, allowing simultaneous acquisition of a renogram for calculation of differential renal function. Clearance of ^{99m}Tc-DTPA has been shown to correlate well with ^{51}Cr-EDTA clearance (Hilson et al. 1976).

In 2004 the British Nuclear Medicine Society (BNMS) published guidelines for the measurement of GFR (Fleming et al. 2004). The authors recommended measuring the plasma clearance of either ^{51}Cr-EDTA or ^{99m}Tc-DTPA using the slope-intercept method with Bröchner-Mortensen correction (Bröchner-Mortensen 1972; Fleming et al. 2004). In the clinical context this method provides a good compromise between accuracy and simplicity. Nevertheless, careful attention to technique is warranted since methodological errors can be introduced at a number of stages (Bird et al. 2007). These include, amongst others, errors in height or weight measurement, drawing up and injection of the patient dose, preparation or measurement of the standard, and preparation or measurement of the plasma samples.

The slope-intercept method does, however, offer a number of opportunities for quality control of the procedure (Fleming et al. 2004). Two parameters obtained during the calculation of GFR using the slope-intercept method are the volume of distribution (V_D) and the half-life ($T_{½}$) of the injected radiopharmaceutical (Bröchner-Mortensen 1972). While being of limited value for predicting the GFR in isolation, these values can be valuable to check for underlying methodological errors (Piepsz et al. 2001; Fleming et al. 2004; Piepsz et al. 2005). Using V_D

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and $T_{½}$ for this purpose requires clearly defined reference ranges for each of these parameters. The BNMS guidelines provide a reference range for the uncorrected V_D (L) as being linearly related to body surface area (BSA) (m^2) by the equation (Fleming et al. 2004):

$$V_{D\ (uncorrected)} = (8*BSA) \pm 25\%\ (2\ SD) \quad (1)$$

This range for V_D was obtained using ^{51}Cr-EDTA. It applies to an uncorrected value for V_D, calculated using the formula:

$$V_{D\ (uncorrected)} = A/C \quad (2)$$

where A is the administered activity and C the intercept at zero time obtained by back extrapolation of the terminal exponential of the curve of activity per unit volume versus time ((Waller et al. 1987), Personal communication: G. Blake, King's College London, UK).

The Medical Physics Department of University Hospital Southampton NHS Foundation Trust, UK, found the corrected V_D for ^{99m}Tc-DTPA to be related to BSA by the equation (Fleming et al. 2009):

$$V_{D\ (corrected)} = (6.61*BSA^{1.218}) \pm 32\%\ (2\ SD) \quad (3)$$

The values for V_D were calculated using the equation:

$$V_{D\ (corrected)} = BM\text{-}GFR / k \quad (4)$$

where BM-GFR is the Bröchner-Mortensen-corrected GFR (Bröchner-Mortensen 1972) and k is the slope of the terminal exponential.

Equation 2 leads to an overestimation of the volume of distribution as it takes into account only the terminal exponential of the plasma clearance curve after mixing has taken place between the vascular and extravascular compartments (Waller et al. 1987). The degree of overestimation is similar to that found when calculating GFR by the slope-intercept method without Bröchner-Mortensen correction. The corrected volume of distribution, $V_{D\ (corrected)}$ (equation 4), although still an approximation since it assumes k is the terminal exponential, tries to correct for the overestimation.

A technique of measuring extracellular fluid volume (ECV) using a combination of the slope-only and slope intercept methods has been described and validated (Peters 1992; Bird et al.

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2009). Using this technique, reference data for ECV have recently been described by Peters *et al* in a large multi-centre study in the UK (Peters et al. 2012b).

In the BNMS guidelines $T_{½}$ is described as being "typically in the range" of 100-120 min in adults (Fleming et al. 2004). However, to the authors' knowledge no data has been published supporting the use of the above or other reference ranges for V_D and $T_{½}$. Specifically, there is a lack of published data for normal values of V_D and $T_{½}$ that have been determined using ^{99m}Tc-DTPA (Fleming et al. 2006).

Reference data for GFR have been well-defined by Granerus (Granerus and Aurell 1981), Hamilton *et al* (Hamilton et al. 2000), Grewal and Blake (Grewal and Blake 2005), as well as by Peters *et a*l (Peters et al. 2012b). Although previous studies have shown only a small difference in GFR values obtained using ^{51}Cr-EDTA and ^{99m}Tc-DTPA (Fleming et al. 1991; Biggi et al. 1995), there are no published reference ranges for GFR using ^{99m}Tc-DTPA.

The aim of this study was to determine reference values for V_D and $T_{½}$ from GFR studies using ^{99m}Tc-DTPA in a healthy population. In addition, reference data for GFR using ^{99m}Tc-DTPA have been defined for the study population.

Methods

Patient population

This retrospective study included the GFR studies of all potential kidney donors referred to the Nuclear Medicine Department of Tygerberg Hospital, Cape Town, South Africa, between February 2007 and September 2012. In total 128 GFR studies were performed and 126 of these were included in the study (69 females, 57 males; age range 18-59 years). Two studies were excluded; one due to discrepancies with weight measurements and one as it was performed using ^{51}Cr-EDTA. In 113 subjects a renogram was performed in combination with the GFR study, while in 13 subjects the GFR study was performed on a different day to the renogram. All potential donors underwent a screening process by the hospital's Renal Unit. Subjects were excluded if they had chronic diseases that could potentially affect renal function or that placed them in a high-risk surgical category. Hypertension, diabetes mellitus and psychiatric illness were considered absolute contraindications to kidney donation. The initial blood tests included haematological and biochemical parameters (urea, creatinine, full blood count, liver function,

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sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, uric acid, glucose), as well as serology for HIV, syphilis, hepatitis A, hepatitis B, hepatitis C, and cytomegalovirus. If these tests were normal and the subject was considered a match based on ABO compatibility and T-cell cross-matching, more specific renal screening was performed. GFR was estimated from a plasma creatinine sample using either the Modification of Diet in Renal Disease (MDRD) (Levey et al. 2007) or Cockcroft-Gault formula (Cockcroft and Gault 1976). In addition, creatinine clearance was calculated from a 24-hour urine collection, 24-hour urinary protein excretion was determined, and a spot urine sample was collected to determine the protein-to-creatinine ratio. Only if the results of all tests were normal were subjects referred to the Nuclear Medicine department for a renogram and GFR study.

This work was approved by the Stellenbosch University Health Research Ethics Committee; study number N10/05/177.

Measurement of GFR, V_D and $T_{½}$

All GFR studies were performed based on the protocol described in the BNMS guidelines (Fleming et al. 2004). The subjects' heights and weights were recorded and the BSA calculated using the Haycock formula (Haycock et al. 1978). ^{99m}Tc-DTPA (TechneScan® DTPA, Covidien) was injected intravenously. Labelling efficiency was greater than 90% in all cases. The injection site was imaged to exclude extravasation. The dose was approximately 40 MBq when only the GFR study was performed on that visit, and about 400 MBq when the GFR study was combined with a renogram. The patient and standard doses were accurately calibrated by weighing the syringes pre- and post-injection on a Precisa 620 C balance, without flushing the syringes or removing the needles. Three 8 ml venous blood samples were drawn from the contralateral arm at 2, 3 and 4 hours respectively. The exact time of injection and the time of drawing each sample were recorded to the nearest minute. Samples were centrifuged immediately after being drawn. A standard was prepared by withdrawing a similar dose of ^{99m}Tc-DTPA from the same kit and adding it to a half-filled 100 ml flask, which was subsequently filled to the 100 ml mark with distilled water and mixed. Two millilitres of this solution was pipetted into a second 100 ml flask that was filled and mixed in a similar manner. The dilution volume of the standard was thus equivalent to 5 litres. Duplicate 1 ml aliquots of plasma samples and standard were pipetted into counting tubes. Background counts were recorded, followed by the counting of each sample in a Picker NaI(Tl) well counter. All

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samples were counted sequentially in one sitting. Linearity of the well counter was checked routinely and was acceptable, specifically at high count rates.

The GFR was calculated using the slope-intercept method as described in the 2004 BNMS guidelines (Fleming et al. 2004). The natural logarithm of the plasma ^{99m}Tc-DTPA concentrations were plotted against time. Linear regression analysis was used to determine the half-life ($T_{½}$) and $V_{D\ (uncorrected)}$. The slope-intercept GFR (SI-GFR) was calculated using the equation (Fleming et al. 2004):

$$\text{SI-GFR} = V_{D\ (\text{Uncorrected})} \times (0.693/T_{½}) \quad (5)$$

The SI-GFR was then corrected for body surface area:

$$\text{SI-GFR}_{\text{Corr}} = \text{SI-GFR} \times (1.73/\text{BSA } m^2) \quad (6)$$

Subsequently, the mean Bröchner-Mortensen (BM) equation was applied to correct for the missing area under the curve from the fast exponential (Fleming et al. 2004):

$$\text{BM-GFR}_{\text{Corr}} = 1.0004 \times \text{SI-GFR}_{\text{Corr}} - 0.00146 \times \text{SI-GFR}^2_{\text{Corr}} \quad (7)$$

The coefficients used in this equation are an average of those in the adult (Bröchner-Mortensen 1972) and paediatric equations (Bröchner-Mortensen et al. 1974).

The absolute GFR was calculated by reversing the BSA correction:

$$\text{BM-GFR} = \text{BM-GFR}_{\text{Corr}} \times (\text{BSA } m^2/1.73) \quad (8)$$

For each GFR study the uncorrected V_D was calculated using equation 2 and the corrected V_D was calculated using equation 4.

Using the methodology previously described (Peters 1992; Bird et al. 2009; Peters et al. 2012b), ECV-BSA (extracellular volume corrected to a BSA of 1.73 m^2) was calculated for each individual. Correction for BSA was reversed by multiplication of ECV-BSA with BSA/1.73 m^2 to give ECV (Peters et al. 2012b).

Defining reference ranges

Values for V_D, both uncorrected and corrected, were plotted against BSA. Using linear regression analysis the correlation was determined between V_D and BSA. Variability was

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defined by calculating the standard error of the estimate of the regression analysis. This gives the standard deviation of estimating V_D from BSA. In this report the 95% confidence limits, or two standard deviations, are expressed as a percentage relative to the mean V_D value. These results were compared to the accepted reference ranges described earlier (equations 1 and 3) (Fleming et al. 2004; Fleming et al. 2009) and to the ECV-BSA data described by Peters *et a*l (Peters et al. 2012b).

In order to define reference data for $T_{½}$, the correlations of $T_{½}$ and age as well as $T_{½}$ and 1/BM-GFR_{Corr} were determined. Similarly, the association between BM-GFR_{Corr} and age was investigated using linear regression. Variability for these parameters was also described by the relative two standard deviation, expressed as a percentage. The results of the BM-GFR_{Corr} vs. age correlation were compared to ^{51}Cr-EDTA reference ranges described by Granerus (Granerus and Aurell 1981), Hamilton (Hamilton et al. 2000) and Grewal and Blake (Grewal and Blake 2005) and to the mean values for GFR described by Peters *et a*l (Peters et al. 2012b).

Deviations from BNMS guidelines

The protocol used in this study deviated from the BNMS guidelines in two aspects and steps were taken to assess their impact on the calculated GFR and V_D. Firstly, in 70 of the 126 studies, low counts were recorded for some of the samples. The BNMS guidelines state that, where practical, a minimum of 10 000 counts should be obtained from each sample in order to reduce statistical error (Fleming et al. 2004). The effect of this factor on the accuracy of the GFR and V_D was assessed by introducing simulated random error into the counts that were obtained from all samples in all 126 studies. This was repeated 10 times and GFR and V_D were calculated in each instance. From this data, systematic, random and total error was estimated.

A second deviation from the BNMS guidelines was that no correction was performed for decay of ^{99m}Tc during the counting process. In order to quantify the error introduced by not correcting for radioactivity decay, an independent set of 26 GFR studies was evaluated. Counts obtained from all samples in these studies were higher than 10 000 and the exact time at which each sample was counted was recorded. GFR and V_D were then calculated for each study, with and without decay correction. From this set of data the systematic, random and combined errors were computed.

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Results

The scatter graphs of the uncorrected V_D (L) and corrected V_D (L) as a function of the BSA (m^2) are shown in Fig. 1.1 and Fig. 1.2 respectively with trendlines representing ± 2 SD.

The correlation between V_D (L), both uncorrected and corrected, and BSA (m^2) was significant ($p < 0.001$ for both correlations). Both were best described using linear functions:

$$V_{D\,(Uncorrected)} = (10.1 * BSA)\ L \pm 40.6\%\ (2\ SD) \quad (9)$$

(RMSE = 3.70 L; 95% CI for the coefficient: 9.79 to 10.5 L).

$$V_{D\,(Corrected)} = (8.19 * BSA)\ L \pm 34.4\%\ (2\ SD) \quad (10)$$

(RMSE = 2.53 L; 95% CI for the coefficient: 7.95 to 8.44 L).

In Fig. 1.1 the trendlines representing the upper and lower limits of the range described in the BNMS guidelines (equation 1) (Fleming et al. 2004) are displayed. Similarly, the trendlines representing the upper and lower limits of the range described by University Hospital Southampton NHS Foundation Trust, UK (Fleming et al. 2009) are displayed in Fig. 1.2.

Mean ECV normalised for BSA (ECV-BSA) was 12.7 ± 4.4 (2 SD) L/1.73 m^2. ECV-BSA in men was 13.5 ± 4.9 (2 SD) L/1.73 m^2 and in women 12.0 ± 3.5 (2 SD) L/1.73 m^2.

The association between $T_{½}$ (min) and age (years) was not statistically significant ($p = 0.16$), nor was the association between $T_{½}$ (min) and age (years) in subjects under the age of 40 years ($p = 0.65$). In this subgroup (< 40 years) the mean $T_{½}$ was 95.0 ± 36.2% (2 SD). In subjects 40 years and older the association between $T_{½}$ and age was statistically significant ($p = 0.046$). This bi-linear fit is illustrated in Fig. 1.3. Using linear regression the following equation describes the association in subjects 40 years and older:

$$T_{½} = [(0.49*age) + 75.9]\ min \pm 30\%\ (2\ SD) \quad (11)$$

The association between $T_{½}$ (min) and $1/BM\text{-}GFR_{Corr}$ ($min.(1.73\ m^2).ml^{-1}$) was statistically significant ($p < 0.001$) and it is illustrated in the scatter graph in Fig. 1.4. Using linear regression it was best described using the equation:

$$T_{½} = 9480*(1/BM\text{-}GFR_{Corr})\ min \pm 35.1\%\ (2\ SD) \quad (12)$$

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Fig. 1.5 is the scatter graph of BM-GFR_{Corr} [$ml.min^{-1}.(1.73\ m^2)^{-1}$] plotted as a function of age (years). In individuals younger than 40 years the correlation was not statistically significant ($p = 0.45$). The mean GFR in this group was 108 $ml.min^{-1}.(1.73\ m^2)^{-1}$ ± 27.5% (2 SD). In individuals 40 years and older the correlation between GFR and age was statistically significant ($p < 0.001$). The following equation describes this association:

$$BM\text{-}GFR_{Corr} = 170 - (1.55*age)\ [ml.min^{-1}.(1.73\ m^2)^{-1}] \pm 36.7\%\ (2\ SD) \quad (13)$$

Mean BM-GFR_{Corr} in men was 107 ± 29.8 (2 SD) [$ml.min^{-1}.(1.73\ m^2)^{-1}$] and in women 100.7 ± 35.8 (2 SD) [$ml.min^{-1}.(1.73\ m^2)^{-1}$]. This difference was statistically significant ($p = 0.04$), however, men were significantly younger than women, mean age 30.5 vs. 36.4 years ($p = 0.003$).

Considering all 126 studies, the systematic and random errors (1 SD) introduced to GFR data through statistical noise were -0.19% and 2.97% respectively, and for V_D, 0.64% and 10.19% respectively. In the prospective series of 26 studies the systematic and random errors (1 SD) introduced to GFR data through not correcting for radioactivity decay were -0.12% and 1.81% respectively, and for V_D, -0.22% and 2.37% respectively. In this series the counting of all samples was completed within 14 minutes (range 6 – 14 min, mean 9 min).

Discussion

In this study reference ranges for a South African adult population were determined for V_D, $T_{½}$ and GFR using ^{99m}Tc-DTPA and the slope-intercept method as described in the BNMS Guidelines (Fleming et al. 2004). The slope-intercept method remains prone to methodological errors (Garnett et al. 1967; Bröchner-Mortensen et al. 1969) and various quality control checks have been proposed: the fit of the counts to a single exponential can be assessed, either graphically or by checking that the correlation coefficient is greater than 0.985 (Fleming et al. 2004). Alternatively, slope-intercept GFR measurements can be checked using single-sample estimates (Fleming et al. 2005a; Fleming et al. 2005b) or using the slope-only technique (Peters 1992; Bird et al. 2007).

V_D and $T_{½}$ are two quantities that are obtained during calculation of GFR using the slope-intercept method. The BNMS guidelines recommend reviewing these quantities as an additional quality control check (Fleming et al. 2004). For this purpose it is necessary to

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compare values to normal values for V_D and $T_{½}$ defined for the patient population and for the radiopharmaceutical used.

In the present study a reference range for uncorrected V_D in litres was identified as (10.1 * BSA) ± 40.6% (2 SD). These values are systematically higher and show greater variability than those described in the BNMS guidelines (equation 1, Fig. 1.1) (Fleming et al. 2004). Although the values for V_D in the BNMS guidelines were derived from GFR measurements using ^{51}Cr-EDTA, previous studies demonstrated no significant difference in V_D between ^{51}Cr-EDTA and ^{99m}Tc-DTPA (Rehling et al. 1984; Fleming et al. 1991). Therefore, it is believed that it is unlikely that the radiopharmaceutical justifies for the differences between the BNMS range and the values in the current study.

In this study the reference range for corrected V_D in litres was found to be (8.19 * BSA) ± 34.4% (2 SD). The variability for corrected V_D (34.4%) is noted to be lower than for uncorrected V_D (40.6%). This is expected because uncorrected V_D is overestimated relative to the true value and the degree of overestimation depends on GFR. Thus a subject of a given size will have a higher value for uncorrected V_D if GFR is normal than if it is reduced. This GFR-related variability of V_D is reduced by applying a Bröchner-Mortensen correction.

In this study the values for corrected V_D are systematically higher than those reported by University Hospital Southampton NHS Foundation Trust (equation 3, Fig. 1.2) (Fleming et al. 2009). For example, for a BSA of 1.73 m^2, the corrected V_D using the Southampton equation would be 12.9 L whilst it would be 14.2 L using equation 11, leading to a 9% higher value. However, considering that the 2 SD error for the Southampton data is 32% and for the data in the current study it is 34.4%, the difference in variability between the two centres is within the estimated error on the V_D.

Radiopharmaceutical factors are even less likely to account for the differences in corrected V_D between this centre and Southampton University Hospitals NHS Trust. In fact, both centres used TechneScan® DTPA, Covidien. This specifically excludes differences in protein binding of different DTPA preparations as a cause for the higher values or greater variability seen in V_D. The study populations in the two centres differ. The Southampton data was obtained from a general clinical GFR population, which included normal and abnormal GFRs and both children and adults, while the data in this study was obtained from a carefully selected normal adult population. This will affect the uncorrected values of V_D. The overestimation of

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uncorrected V_D will be higher in the normal group compared to the mixed population as the GFR will on average be higher. In terms of environmental and ethnic factors, the population in this study is likely to be more diverse than a population originating from Southampton. The current study population is heterogeneous, with roughly equal numbers of subjects of Caucasian, African and mixed ancestry. It has been shown in previous studies that there are differences in muscle mass amongst different ethnic groups and this may translate to differences in V_D (Cohn et al. 1977; Harsha et al. 1978; Worrall et al. 1990).

An additional factor contributing to the variability in the results for V_D might have been experimental error due to low counts; however, this is thought to play a minor role and it will be discussed later in this section.

Using the technique described by Peters *et al* ECV-BSA was calculated for each subject (Peters 1992; Bird et al. 2009; Peters et al. 2012b). The mean ECV-BSA was 12.7 ± 4.4 (2 SD) L/1.73 m^2, whilst for males it was 13.5 ± 4.9 (2 SD) L/1.73 m^2 and females 12.0 ± 3.5 (2 SD) L/1.73 m^2. These GFR values were corrected for the one-pool assumption using the mean Bröchner-Mortensen correction as recommended in the BNMS guidelines (Fleming et al. 2004). When corrected using the adult Bröchner-Mortensen equation (Bröchner-Mortensen 1972), ECV/BSA in males was 13.9 ± 5.1 (2 SD) L/1.73 m^2 and in females 12.3 ± 3.7 (2 SD) L/1.73 m^2. These values for ECV-BSA agree reasonably well with those described by Peters *et al* in their recent multi-centre UK-based study (Peters et al. 2012b).

The mean value for ECV in this study is 27% lower than the mean value for uncorrected V_D. This is in close agreement with previous work in which a difference of 30% was described (Peters et al. 2000). The mean value for ECV-BSA in the current study is, however, also approximately 10% lower than the mean value for corrected V_D normalized for BSA. This is due to the approximation used in this study that the slope of the second exponential is equal to the clearance constant. The work of Bird *et al* (Bird et al. 2009) shows that the slope systematically underestimates the constant by about 10% leading to an overestimate in the volume of distribution.

Calculation of $T_{½}$ may be used as a quality control check by comparing it against the value expected for the subject's GFR. The association between $T_{½}$ (min) and 1/BM-GFR_{Corr} [min.(1.73 m^2).ml^{-1}] was statistically significant ($p < 0.001$) and $T_{½}$ (min) was found to be [9480*(1/BM-GFR_{Corr})] ± 35.1%.

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In the study by Grewal and Blake, the authors noted that it was apparent that there was a break in the age dependence of GFR at approximately 40 years (Grewal and Blake 2005). They found no statistically significant correlation between GFR and age for individuals under the age of 40 years, while there was a statistically significant decrease in GFR from the age of 40 years onward. In this study a cut-off of 40-years was used based on this work and it supports that conclusion: for individuals under the age of 40, the correlation between GFR and age was not statistically significant ($p = 0.45$), while it was significant ($p < 0.001$) in individuals 40 years and older. In individuals younger than 40 years the mean BM-GFR_{Corr} was 108 $ml.min^{-1}.(1.73\ m^2)^{-1}$. This is the same as the 108 $ml.min^{-1}.(1.73\ m^2)^{-1}$ reported by Hamilton *et al* (Hamilton et al. 2000), but higher than the 103 $ml.min^{-1}.(1.73\ m^2)^{-1}$ reported by Grewal and Blake (Grewal and Blake 2005) and the 105 $ml.min^{-1}.(1.73\ m^2)^{-1}$ reported by Granerus and Aurell (Granerus and Aurell 1981). The slightly higher GFR is expected for DTPA compared to EDTA (Fleming et al. 1991; Biggi et al. 1995). In individuals 40 years and older, BM-GFR_{Corr} was expressed by the linear relation $170 – (1.55*age)$ $[ml.min^{-1}.(1.73\ m^2)^{-1}] \pm 36.7\%$ (2 SD). In this study the reference curve predicts a mean GFR at age 50 years of 93 $ml.min^{-1}.(1.73\ m^2)^{-1}$. This is in good agreement with the mean of 94 $ml.min^{-1}.(1.73\ m^2)^{-1}$ found by Grewal and Blake (Grewal and Blake 2005), but lower than the 98 $ml.min^{-1}.(1.73\ m^2)^{-1}$ in the Granerus and Aurell study (Granerus and Aurell 1981). The data in the subgroup 40 years and older has to be interpreted with caution, however, as it comprised only 44 individuals and covered a relatively limited age range compared to the other studies.

The results of Granerus and Aurell (Granerus and Aurell 1981) and Hamilton *et al* (Hamilton et al. 2000) are not directly comparable due to small differences in methodology. On the other hand, the current study is based on the protocol described in the BNMS guidelines (Fleming et al. 2004), as was the study by Grewal and Blake (Grewal and Blake 2005), making it more appropriate for comparison.

It is accepted that GFR declines with age, although a cut-off age for the start of the decline is difficult to establish as recently shown by Peters *et al* (Peters et al. 2012b). No clear age cut-off could be identified in the current study, however a threshold of 40 years of age was chosen in accordance with the cut-off age used in a previous study (Grewal and Blake 2005). Due to a relatively small study sample, individuals were not divided into groups based on gender, however, mean BM-GFR_{Corr} was 107.0 ± 14.9 (1SD) $ml.min^{-1}.(1.73\ m^2)^{-1}$ in men vs. 100.7 ± 17.9 (1 SD) $ml.min^{-1}.(1.73\ m^2)^{-1}$ in women. The difference was statistically significant ($p =$

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0.04), however, the difference might be explained by the fact that the male cohort was significantly younger than the female cohort (mean age 30.5 versus 36.4 years, p = 0.002). These mean values for GFR in men and women are higher than those described in the multi-centre UK study (Peters et al. 2012b), but this can be explained by two factors: firstly, the majority of GFR measurements (1783 of 1878) in the multi-centre study were performed using ^{51}Cr-EDTA and secondly, the mean age of subjects in all the individual centres was higher than the mean age of subjects in the current study. Due to the relatively small study population, individuals in the current study could not be sub-divided into groups based on other factors such as obesity as was done in the multi-centre study (Peters et al. 2012b).

In another study by the same authors the coefficient of variation (CV) of ECV-BSA in normal subjects was found to be useful in assessing departmental performance as it reflects the 'technical robustness' with which the department performs the GFR measurements (Peters et al. 2012a). The authors suggest a range of 10-20% as acceptable. The CV for ECV-BSA in this study (using the adult Bröchner-Mortensen correction equation (Bröchner-Mortensen 1972)) was 15%.

The BNMS guidelines state that, where practical, a minimum of 10 000 counts should be obtained for each sample in order to reduce statistical errors (Fleming et al. 2004). In this study, 70 of the 126 GFR studies contained samples with fewer than 10 000 counts. The systematic error (1 SD) in GFR and V_D values due to counting error was found to be low (0.19% and 0.64% respectively). As expected, the random error (1 SD) was higher (GFR 2.97% and V_D 10.19%). Another deviation from the BNMS guidelines was that no correction was made for the decay of Tc-99m. The resultant systematic error (1 SD) was negligible in all cases (GFR -0.12% and V_D -0.22%) therefore it was ignored in further calculations. The random error (1 SD) was larger (GFR 1.81% and V_D 2.37%). By assuming that the error measured in the 26 cases represented the error introduced through not correcting for decay in all 126 studies, the combined random error of noise and lack of decay correction could be calculated. This resulted in 3.5% for GFR and 10.5% for V_D (1 SD).

The components of the relatively high combined random error in V_D were assessed further. In the correlation of uncorrected V_D against BSA, a 1 SD error expressed as a percentage of the mean V_D is 20.3% (equation 9). Part of this variation will be genuine variability of V_D with BSA, y%, and part due to experimental error. The two components add in quadrature:

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$$20.3^2 = y^2 + 10.5^2 \quad (14)$$

The real standard deviation variation of uncorrected V_D with BSA, y, is therefore 17.4%. Similarly, for corrected V_D, a 1SD error expressed as a percentage of the mean is 17.2% (equation 10) and the real standard deviation variation with BSA is 13.6%. These results show that, because the error in V_D is relatively large, the contribution of low counts and lack of decay correction to this variability is small.

It is worth mentioning that a cohort of 126 studies is relatively small, therefore further larger studies are recommended to better define reference data for GFR using ^{99m}Tc-DTPA. Secondly, the age of all individuals fell between 18 and 59 years, with few over the age of 50, as these were the subjects being considered as kidney donors. Ideally, a study of this nature should include subjects over a wider range of ages, including individuals over the age of 60 years as these are often the patients referred for GFR studies. Moreover, although the hospital's screening process for kidney donors is intensive, it may not have been rigorous enough to exclude all subjects with mild renal pathology.

Having defined the variation of volume of distribution with body surface area and its expected variation, this data may be used for quality control. Studies in which the value of V_D lies outside the expected limits for the subject's BSA may be deemed fail the quality control (QC) test. Considering the corrected V_D data shown in Fig. 1.2, two of the studies lie well away from the 2 SD limits and therefore may be considered to fail the QC requirements. Using 2 SD limits, 5% of the studies will lie outside the limits due to natural statistical variation, therefore in practical use wider limits might be used e.g. 2.5 or 3 SD. Several different methods of calculating volume of distribution exist and it is therefore important that in using this parameter in quality control values must be compared to the corresponding normal range for that particular estimation of the volume. A similar test may be applied to the measured $T_{½}$. This is compared to the expected limits of $T_{½}$ for the subject's normalized GFR and if it lies outside these, then the study is deemed to fail the QC test (Fig. 1.4). One limitation of the current data in this respect is that it only contains data from control subjects. To obtain a better fit for low GFR further data is required.

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Conclusion

This study has defined reference data for GFR, V_D and $T_{½}$ from GFR studies using ^{99m}Tc-DTPA in a healthy South African adult population. V_D and $T_{½}$ values can provide useful quality control checks for GFR studies performed using the slope-intercept method as described in the BNMS guidelines (Fleming et al. 2004). Reference data for GFR will enhance the interpretation of adult ^{99m}Tc-DTPA GFR measurements. The small difference in normal values for GFR in comparison to previous studies using ^{51}Cr-EDTA is in agreement with previous publications.

Acknowledgements

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(Centre for Statistical Consultation, Stellenbosch University)

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Manuscript review: all authors
Supervisor: JM Warwick

Conflicts of interest

There are no conflicts of interest.

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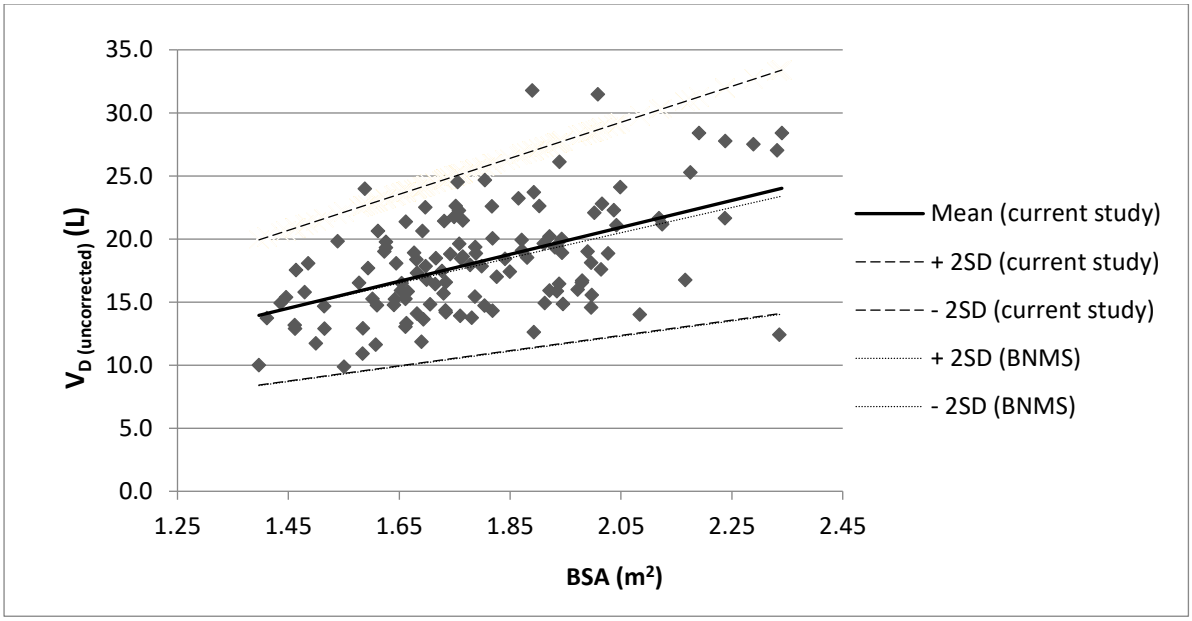
Tables and figures

Fig. 1.1



Scatter graph of the uncorrected values of volume of distribution [$V_{D\ (uncorrected)}$] in litres plotted as a function of body surface area. The central line represents equation 9, the upper and lower lines (dashes) represent ± 2 SD (± 40.6%). The faint dotted lines represent the upper and lower limits of the reference range described in the British Nuclear Medicine Society guidelines (8*BSA ± 25%) (2 SD) (Fleming et al. 2004). There is overlap of the two lines representing – 2 SD.

Abbreviations: **VD**, volume of distribution; **BSA**, body surface area; **SD**, standard deviation; **BNMS**, British Nuclear Medicine Society.

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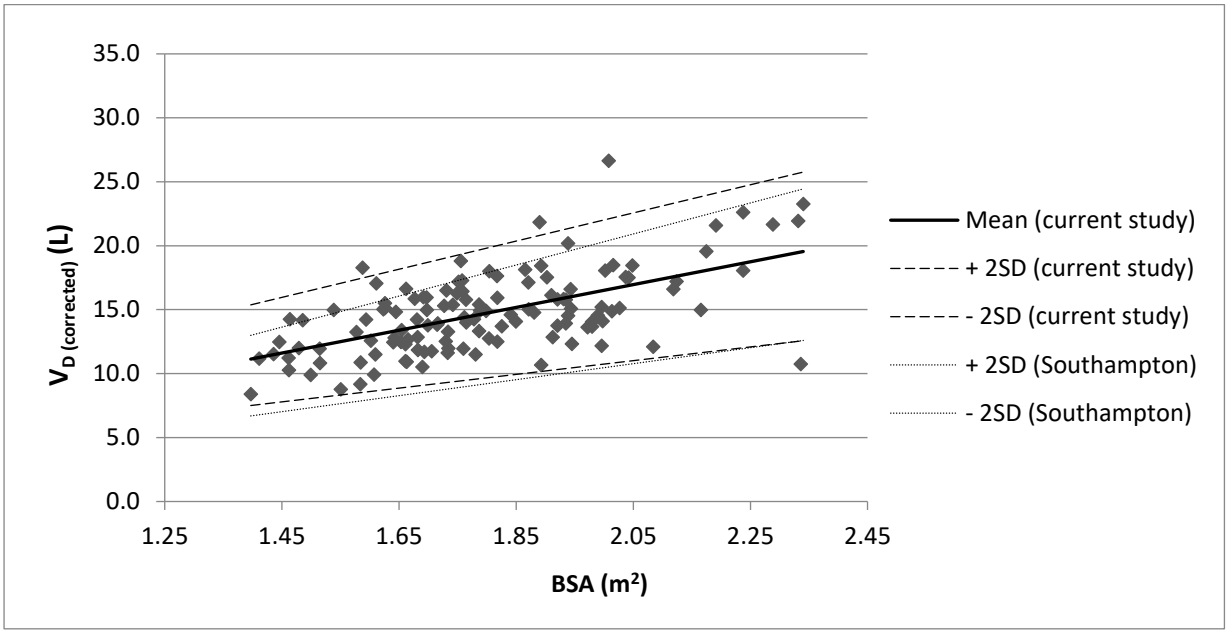
Fig. 1.2



Scatter graph of corrected values of volume of distribution [$V_{D\ (corrected)}$] in litres plotted as a function of body surface area. The central line represents equation 10, the upper and lower lines (dashes) are ± 2 SD (± 34.4%). The faint dotted lines represent the upper and lower limits of the reference range determined by University Hospital Southampton ($6.61*BSA^{1.218}$) ± 32% (2 SD) (Fleming et al. 2009).

Abbreviations: **VD**, volume of distribution; **BSA**, body surface area; **SD**, standard deviation.

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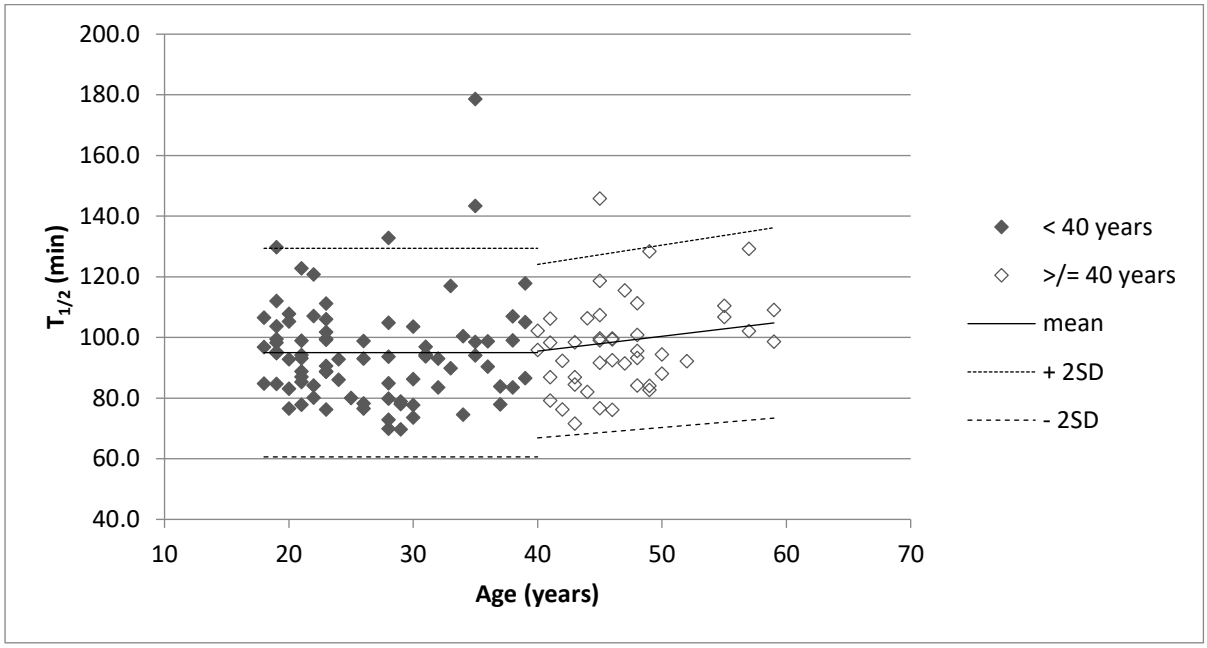
Fig. 1.3



Scatter graph of $T_{½}$ (min) plotted as a function of age (years). The central line represents the mean in individuals under the age of 40 years and the equation-predicted-mean in individuals 40 years and older (equation 11). The upper and lower lines are ± 2 SD (± 36.2% in individuals < 40 years and ± 36.7% in individuals ≥ 40 years).

Abbreviations: **SD**, standard deviation

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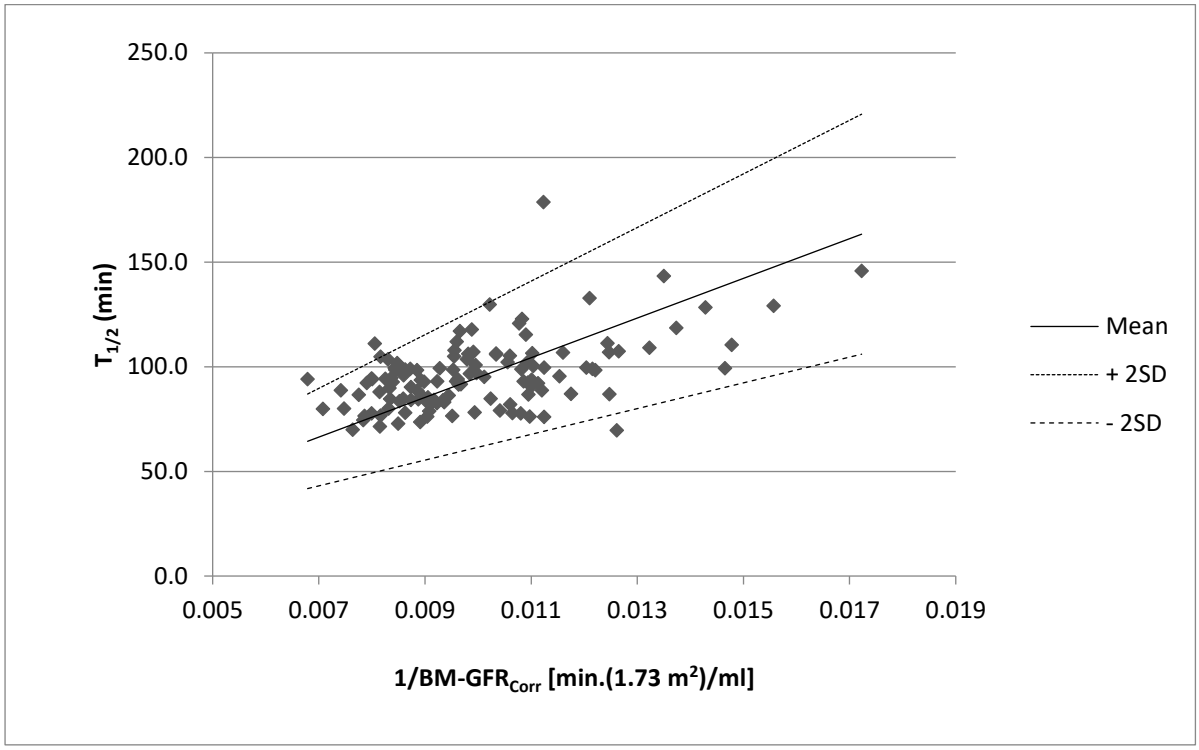
Fig. 1.4



Scatter graph of T½ (min) plotted as a function of (1/BM-GFR$_{Corr}$) [min.(1.73 m^2).ml^{-1})]. The central line represents T½ fitted to equation 12 and the upper and lower lines represent ± 2 SD (± 35.1%).

Abbreviations: **BM-GFR$_{Corr}$**, corrected Bröchner-Mortensen glomerular filtration rate; **SD**, standard deviation.

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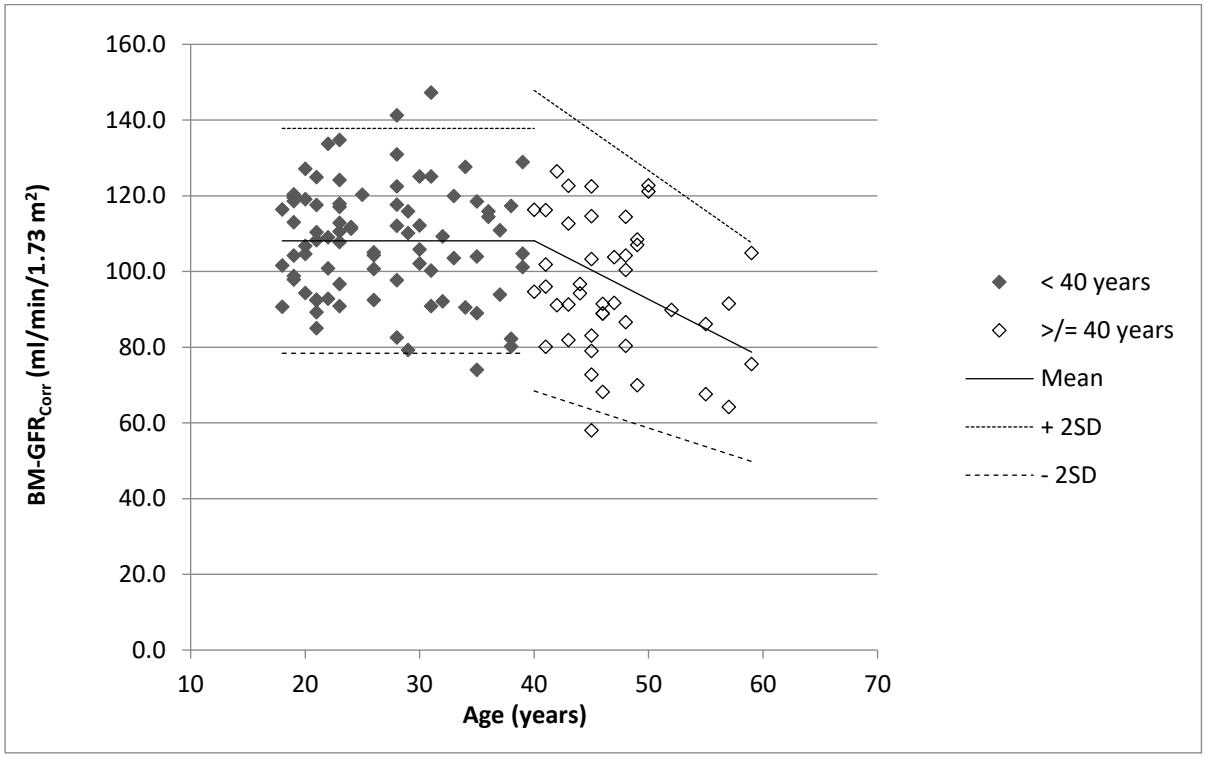
Fig. 1.5



Scatter graph of BM-GFR_{Corr} [$ml.min^{-1}.(1.73\ m^2)^{-}1$] as a function of age (18-59 years) in 126 potential kidney donors. GFR values were corrected for body surface area and using the mean Bröchner-Mortensen equation (Bröchner-Mortensen 1972; Fleming et al. 2004). The central line represents the mean GFR in individuals under the age of 40 years and the mean fitted to equation 13 in individuals 40 years and older. The upper and lower lines represent ± 2 SD (± 27.5% in individuals under the age of 40 years and 36.7% in individuals older than 40).

Abbreviations: **BM-GFR_{Corr}**, corrected Bröchner-Mortensen glomerular filtration rate; **GFR**, glomerular filtration rate; **SD**, standard deviation.

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2. Measuring GFR using the plasma clearance of ^{99m}Tc-DTPA

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To the editor:

A recent systematic review (Soveri et al. 2014) concluded that plasma clearance of ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) is inaccurate for measuring glomerular filtration rate (GFR). This was based on data from two studies with poor results (Shemesh et al. 1985; Dai et al. 2011), that are incompatible with a number of publications that demonstrate the suitability of DTPA plasma clearance. ^{51}Cr-ethylenediaminetetraacetic acid (^{51}Cr-EDTA) plasma clearance has been demonstrated to be comparable to renal inulin clearance (P_{30} value of 86%; bias 8%) (Soveri et al. 2014) and may thus be considered a secondary standard against which other techniques can be compared. Several publications compare plasma clearance of ^{99m}Tc-DTPA to ^{51}Cr-EDTA, and all demonstrate good overall precision and minimal bias (median bias <1%; P_{30}=93%; P_{10}=72%) (Tables and figures

Fig. 2.1) (Rehling et al. 1984; Fleming et al. 1991; Biggi et al. 1995).

In both included studies (Shemesh et al. 1985; Dai et al. 2011) the bias was large (14% and 24.8%), and in the paper by Shemesh et al (Shemesh et al. 1985), there was very poor correlation between plasma ^{99m}Tc-DTPA clearance and renal inulin clearance (r = 0.694). Given subsequently published data, the measurements obtained in these studies are likely to be fundamentally flawed. Likely reasons include inadequate correction of the slope-intercept GFR (Shemesh et al. 1985; Dai et al. 2011), unusual methodology to measure renal inulin clearance (Dai et al. 2011), and probable use of a poor quality DTPA preparation as was common in the earlier days of DTPA usage (Carlsen et al. 1980; Shemesh et al. 1985).

The recent review (Soveri et al. 2014) provides a valuable comparison of a number of techniques to measure GFR. However, we believe the authors have erred in advising against the use of ^{99m}Tc-DTPA plasma clearance based on data from two questionable studies. We are firmly of the view that there is sufficient evidence in the literature supporting its use.

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Contributors

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Letter review: JM Warwick and JS Fleming

Supervisor: JM Warwick

Conflicts of interest

There are no conflicts of interest.

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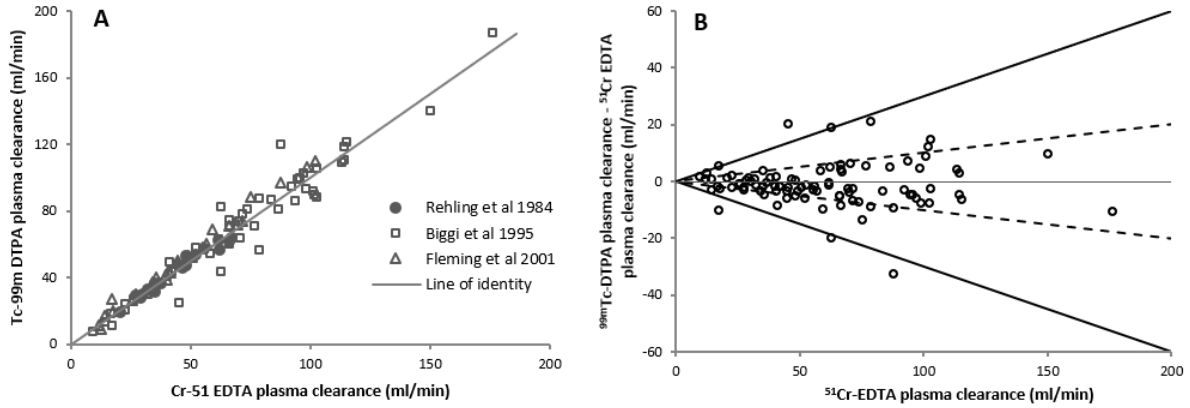
Tables and figures

Fig. 2.1



(A) Comparison of simultaneous plasma ^{99m}Tc-DTPA and ^{51}Cr-EDTA clearance; data derived from three studies (Rehling et al. 1984; Fleming et al. 1991; Biggi et al. 1995). (B) A Bland-Altman-like diagram of the difference between ^{99m}Tc-DTPA and ^{51}Cr-EDTA clearance plotted against ^{51}Cr-EDTA clearance. The solid lines show the proportion of ^{99m}Tc-DTPA measurements that were within 30% of the ^{51}Cr-EDTA values (P_{30}), and the dashed lines show the P_{10} limit.

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3. Propagation of measurement errors in glomerular filtration rate determination: a comparison of slope-intercept, single-sample and slope-only methods

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Publication status: published article (Holness et al. 2019)

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Abstract

Background: Measurement errors occurring during glomerular filtration rate (GFR) studies propagate to an error in the calculated GFR. Previous work has modelled measurement errors for slope-intercept (SI-GFR), single-sample (SS-GFR) and slope-only (SO-GFR) methods. In this study we have extended these models. The primary aims were to 1) compare measurement errors in 2-sample SI-GFR, 3-sample SI-GFR, SS-GFR and SO-GFR, and 2) determine the sensitivity of GFR to errors arising from different measurements.

Methods: This study expanded on previous models of GFR measurement error incorporating biological data from 786 patients, and realistic measurement errors. GFR median absolute error (MAE) and the coefficient of variation (CV) were calculated for each method. A sensitivity analysis was performed for individual measurement errors.

Results: MAE ranged between 1.2 and 2.3 ml/min/1.73 m^2, lowest for SS-GFR (4 h) and highest for SO-GFR. At higher rates of clearance, CV was < 5% for all methods. CV increased rapidly when GFR dropped below a threshold ranging between 34 and 56 ml/min/1.73 m^2, lowest for 3-point SI-GFR and highest for SO-GFR. SI-GFR and SS-GFR are most sensitive to injected activity quantification, but less sensitive to other measurement errors.

Conclusion: Measurement errors are probably insignificant relative to biological variation for GFR > 60 ml/min/1.73 m^2 but become significant regardless of biological variation at lower GFR, particularly in serial studies when GFR < 25 ml/min/1.73 m^2. Limits of precision recommended in the 2018 British Nuclear Medicine Society guideline are appropriate for once-off GFR measurement, whereas slightly more stringent limits are proposed for serial studies.

Keywords: glomerular filtration rate, adult, humans, radiopharmaceuticals, quality control

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Introduction

Using nuclear medicine techniques, glomerular filtration rate (GFR) is measured from the plasma clearance of ^{51}Cr-EDTA or ^{99m}Tc-DTPA with either the slope-intercept method (SI-GFR) (Chantler et al. 1969), a single sample technique (SS-GFR) (Fleming et al. 2004), or the slope-only method (SO-GFR) (Peters 1992; Bird et al. 2009). Regardless of the method, multiple measurements are required, and errors are introduced with every measurement. These errors propagate, leading to an error in the final GFR value. Previous work has shown that the magnitude of the resultant errors differs according to the method (De Sadeleer et al. 2000; De Sadeleer et al. 2001), but it can be argued that these analyses may not provide a complete and/or realistic reflection of the measurement errors likely to occur in a busy clinical department, particularly within a training hospital where staff are frequently inexperienced (Watson 2000; Peters 2001). In addition, while the magnitude of errors was well quantified in these analyses, their clinical significance was not addressed (Watson 2000). Patients are frequently referred for serial GFR measurements to detect deterioration in kidney function; however, GFR must change by at least 20% before it can be regarded as significant (Fleming et al. 2004). This is high because of the high coefficients of variation (CV) of repeat GFR measurements that have been found previously (Bröchner-Mortensen and Rödbro 1976; Wilkinson et al. 1990; Blake et al. 1997; Grewal and Blake 2005; Delanaye et al. 2008a; Bird et al. 2008). Measurement errors and biological fluctuations are the two sources of this variability (Blake et al. 1997), but the exact contribution of each is not known. This knowledge would be useful as many of the measurement errors can be controlled and minimised. Efforts to minimise measurement errors would be justified if this would appreciably reduce the variability of GFR measurements.

Many centres adopted SI-GFR after publication of the 2004 British Nuclear Medicine Society (BNMS) guideline (Fleming et al. 2004), and there are many proponents of SO-GFR. However, following a study that showed SS-GFR, using the technique described by Fleming et al (Fleming et al. 2005a), to have an accuracy comparable to that of SI-GFR, SS-GFR has become the recommended method in the 2018 updated BNMS guideline (McMeekin et al. 2016a; Burniston 2018). The current study therefore aims to model measurement error in adult GFR as accurately as possible by introducing realistic errors to as many measurements/sources as possible. The objectives were (i) to compare measurement errors in SI-GFR, SS-GFR, and SO-GFR; (ii) to estimate the magnitude of measurement errors relative to biological variation in GFR; (iii) to perform a sensitivity analysis of individual measurement errors; and (iv) to make

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practical recommendations to minimize errors in the context of a typical clinical unit.

Methods

Calculation of actual slope, intercept, and half-life

The demographic and original count data was extracted from the folders of all adults (≥ 18 years) who underwent GFR determination in the Tygerberg Hospital Nuclear Medicine Division between January 2008 and March 2016. The data was included only if 3 or more blood samples had been taken and if all information was complete.

GFR was measured using the slope-intercept method, with corrections for body surface area (BSA) and the missing first exponential, adhering to the methodology and calculations in the 2004 BNMS guideline (Fleming et al. 2004). After recording patient height and weight, ^{99m}Tc-DTPA (Technescan®DTPA, Mallinckrodt, Netherlands) was administered through an indwelling intravenous cannula. In the majority of patients the dose was 40 MBq, while in cases where simultaneous renography was performed, 400 MBq was administered. Both patient and standard doses were weighed before and after administration of the dose on a Precisa 620 C balance (Precisa Balances Ltd, Milton Keynes, United Kingdom), without flushing the syringe or changing needles or needle caps. Three venous blood samples were taken from the opposite arm at approximately 2, 3, and 4h. The exact times were recorded to the nearest minute. Blood samples were immediately centrifuged (1000 g for 10 min) and then duplicate 1 ml plasma samples were pipetted into counting tubes. A standard was prepared using a similar dose of ^{99m}Tc-DTPA obtained from the same vial. To avoid excessively high count rates, a double dilution of the standard was performed, resulting in an effective volume of 5 litres. The count rates of duplicate 1 ml standard samples, the plasma samples, and background samples were determined using a Picker NaI(Tl) well counter (Picker International Inc, Cleveland, Ohio, United States).

The natural logarithm of the plasma concentration of ^{99m}Tc-DTPA was plotted against time for each time point, and a linear curve fitted to the data points. From this curve, the slope (-k) and intercept ($\log_e P_0$) were obtained. The half-life ($T_{½}$) was calculated using the equation:

$$T_{½} = -0.693/k$$

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Modelling of measurement errors

To be as realistic as possible measurement errors were generated based on actual errors from our unit, and real patient data was used to reflect patient variation. The model is described in three steps described below:

1. Generation of idealised starting values

Based on the original patient data and assuming values for the count rate of the standard samples (counts/min/ml; CPM/ml), P_0 and k represented the "ground truth", idealised starting values were derived for the standard dose (CPM) and for plasma concentration (CPM/ml) at 2, 3 and 4 hours. Masses of the standard and patient dose syringes, pre- and post-administration, as well as the patients' heights and weights were also assumed to represent "ground truth" starting values. Background counts were initially set to a fixed value of 400 for all patients (typical background counts in our unit). Finally, in order to model minimum requirements of the 2004 BNMS guideline (Fleming et al. 2004), the average counts of the latest sample for each patient was scaled to a value of 10 000, with the same scaling factor being applied to all counts in that patient's study. Each step in the preparation of the standard was modelled. In our unit where a double dilution of the standard is performed, the following steps were modelled: (i) filling of flask 1 to 100 ml (after adding the dose), (ii) pipetting of 2 x 1 ml aliquots from flask 1 into flask 2, (iii) filling of flask 2 to 100 ml.

2. Calculation of reference GFR

Without the introduction of any measurement errors, the idealised starting values were used to calculate reference GFR values for the three methods.

SI-GFR was calculated using the equation (Fleming et al. 2004):

$$\text{SI-GFR} = V_D \times (0.693/T_{½}), \text{ where } V_D = 100/P_0$$

The SI-GFR value was corrected for BSA (m^2), which was calculated using the Haycock formula (Haycock et al. 1978):

$$\text{SI-GFR}_{Corr} = \text{SI-GFR} \times (1.73/\text{BSA})$$

Thereafter, the mean Bröchner-Mortensen equation (Bröchner-Mortensen 1972; Bröchner-Mortensen et al. 1974; Fleming et al. 2004) was applied to correct for the missing fast

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exponential:

$$\text{BM-GFR}_{Corr} = (1.0004 \times \text{SI-GFR}_{Corr}) - 0.00146 \times (\text{SI-GFR}_{Corr}^2)$$

All three plasma samples were used to generate a three-point SI-GFR [3pt SI-GFR (2, 3, 4 h)]. In addition, two-point SI-GFR values were calculated using the 2 and 3 h samples [2pt SI-GFR (2, 3 h)], the 3 and 4 h samples [2pt SI-GFR (3, 4 h)], and the 2 and 4 h samples [2pt SI-GFR (2, 4 h)]. While these create identical reference values, the SI-GFR error is expected to differ for each of the 4 methods.

SS-GFR was calculated from both 3 h [SS-GFR (3 h)] and 4 h [SS-GFR (4 h)] samples using the technique described by Fleming et al (Fleming et al. 2005a):

$$\text{SS-GFR}t = \{[-11297 - (4883 \times \text{BSA}) - 41.9t] + [5862 + (1282 \times \text{BSA})15.5t] + \ln[V\text{app}(t)]\}/t$$

where BSA is the body surface area in square metres, t is sampling time in minutes and Vapp is the apparent volume of distribution in litres/1.73 m^2.

$$V\text{app}\,(t) = [A \times 1.73]/[p(t) \times \text{BSA}]$$

where A is the total activity injected and $p(t)$ the plasma activity concentration at time t.

The units for SI-GFR and SS-GFR are ml/min/1.73 m^2.

SO-GFR was calculated using the equation described by Bird et al (Bird et al. 2009):

$$\text{SO-GFR} = \alpha_2 + [15.4 \times (\alpha_2^2)]$$

where α_2 is the rate constant of terminal exponential. SO-GFR is expressed in ml/min/L.

For purposes of comparison with SI-GFR and SS-GFR, all values for SO-GFR were multiplied by the extracellular fluid volume of the standard person (12.5 L/1.73 m^2) (Peters et al. 2000).

3. Introduction of error

Each method of GFR determination was modelled using realistic, normally distributed, random errors introduced to all measurements. The magnitude of error for each measurement was based on what has been observed within our institution. Table 3.1 lists the measurements, the one standard deviation (SD) error that was introduced to each, and the method used to determine the error. The calculated GFR values were compared to the reference values for each method.

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Modelling of real GFR measurements was performed using in-house software written in R (*The R-project for statistical computing*; version 3.4.3, The R foundation, Vienna, Austria, 2018). One thousand simulations were performed per patient.

Statistical analysis

The median absolute error was calculated for each patient. Subsequently, the median absolute error was calculated across all patients. This was performed for all 7 methods/variations: 3pt SI-GFR (2, 3, 4 h), 2pt SI-GFR (2, 3 h), 2pt SI-GFR (3, 4 h), 2pt SI-GFR (2, 4 h), SS-GFR (3 h), SS-GFR (4 h), and SO-GFR. CV was calculated for each patient and plotted against reference GFR. The 7 methods/variations were compared qualitatively as significance is expected due to the use of a large number of simulations.

Sensitivity analysis

In a second experiment, the sensitivity of each method to individual measurement errors was assessed. All measurement errors, other than the one being evaluated, were held at their realistic values. The standard deviation of the error in question was then varied between 0 and a value large enough to result in a mean error in GFR of 5 ml/min/1.73 m^2. Error in GFR was plotted against the SD of the evaluated measurement.

For counting errors, where the standard deviation of the error is a function of counts, the minimum counts were varied between 10 000 and a value small enough to result in a mean error in GFR of 5 ml/min/1.73 m^2. Error in GFR was plotted against the CV of counts. This was performed with background counts set to 400 and was repeated with background counts of 1500 and 3000, to reflect situations where lower count rates are expected (e.g. centres using ^{51}Cr-EDTA).

Ethical approval was obtained from the Stellenbosch University Health Research Ethics Committee (Protocol S14/08/166).

Results

GFR studies of 786 adults (512 female) were included in the error model. Mean (SD) GFR was 83.2 (26.7) ml/min/1.73 m^2 (range 10 to 158 ml/min/1.73 m^2). The diagnoses of the patients referred for GFR determination were cancer (536), chronic kidney disease (40) or urological

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problems (14), and a further 196 individuals were healthy potential kidney donors.

The resultant errors in GFR are summarised in **Table 3.2** and the box-and-whisker plots (Fig. 3.1). Median absolute error (MAE) ranged between 1.2 and 2.3 ml/min/1.73 m^2, lowest for SS-GFR (4 h) and highest for SO-GFR. The relative error ranged between 1.3% and 2.7%. The proportion with errors in GFR greater than 5% ranged between 0% for SS-GFR (4 h) and 6.2% for 2pt SI-GFR (2, 3 h). Figure 2 displays the CV as a function of GFR. At higher rates of clearance the CV was relatively low and independent of GFR for all methods, but as GFR decreased, the CV started increasing exponentially. The CV crossed a threshold of 5% at clearance values ranging between 34 and 56 ml/min/1.73 m^2, lowest for 3pt SI-GFR (2, 3, 4 h) and highest for SO-GFR (**Table 3.2**).

The sensitivity analyses of the individual measurements are displayed in Fig. 3.3. Only 3pt SI-GFR (2, 3, 4 h), SS-GFR (3 h) and SO-GFR are presented for the sake of brevity, however similar results were obtained for all methods. Using 3pt SI-GFR (2, 3, 4 h) and SS-GFR (3 h) an absolute error in GFR of 5 ml/min/1.73 m^2 was reached when the measurement errors exceeded the following values: weight 12 kg, height 40 cm, time 25 min, flask volume 6%, pipette volume 8%, balance 0.05 g, and CV of counts 4.3%. Using SO-GFR an absolute error in GFR of 5 ml/min/1.73 m^2 was induced when measurement errors exceeded the following values: time 6 min, pipette volume 6% and counts 3.4%.

Discussion

In this study we found that after introduction of realistic, random errors to all measurements, the resultant median absolute error (MAE) in GFR was low for all methods, ranging between 1.2 and 2.3 ml/min/1.73 m^2 (**Table 3.2**). Consistent with previous studies (De Sadeleer et al. 2000; Watson 2000; De Sadeleer et al. 2001), the error was lowest for SS-GFR (4 h) and highest for SO-GFR. SS-GFR (4 h) was also the most precise method (97.5th percentile 1.4 ml/min/1.73 m^2). Interestingly, 2pt SI-GFR (3, 4 h) was least precise (97.5th percentile 4.1 ml/min/1.73 m^2), while the precision of SO-GFR and the other SI-GFR variations was similar. A single median absolute value across all patients is useful as it is relatively independent of GFR, whereas expressing the error as a coefficient of variation (CV) allows for comparison with previous studies as well as estimation of the contribution of measurement error, as opposed to biological variation, to the variability of repeat GFR measurements. CV was highly dependent on GFR

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for all methods, rising sharply when the GFR fell below 60 ml/min/1.73 m^2 (Fig. 3.2). This is expected as the error remains stable in absolute terms even when the GFR becomes low. While the mean CV of SS-GFR was lower than the other methods at normal and higher rates of clearance, for GFR values < 50 ml/min/1.73 m^2, the mean CVs of 3pt SI-GFR (2, 3, 4 h) and 2pt SI-GFR (2, 4 h) were the lowest.

The differences in sensitivity of the methods to measurement errors can, in part, be explained by the methodology. SI-GFR, the method recommended in the 2004 British Nuclear Medicine Society (BNMS) guideline (Fleming et al. 2004), calculates GFR from the area under the second (slower) exponential of the plasma clearance curve, assuming a single compartment (Chantler et al. 1969). It requires at least two plasma samples taken after equilibration of the radiopharmaceutical with interstitial fluid. A disadvantage of SI-GFR is the procedure's relative complexity. However, SI-GFR is protected from measurement errors by an inherent robustness: errors that affect the slope have a reciprocal effect on the volume of distribution (V_D), and because SI-GFR is the product of the slope and V_D, resultant errors in GFR tend to be buffered (Blake et al. 1997; Peters 2001). SI-GFR is further stabilized by taking more than the minimum of two blood samples, as an increase in the number of samples leads to a better estimation of both slope and intercept, thus improving the precision of the GFR.

Single-sample methods use an empiric equation to estimate GFR from the apparent volume of distribution, calculated from the tracer concentration in the plasma sample at a specific time (Fleming et al. 2004). SS-GFR is more convenient and comfortable for patients, and, because it requires fewer measurements, the potential for measurement error may be reduced. SO-GFR calculates GFR purely from the slope of the second (slower) exponential, expressing GFR in terms of extracellular fluid volume (Peters 1992). Of the three methods, SO-GFR is the most straightforward to perform. It does not require preparation of a standard, and measurement of the doses, patient height and patient weight are not necessary. Although SO-GFR requires fewer measurements, it is generally more sensitive to these errors than SI-GFR, most likely because it lacks the buffering mechanism intrinsic to SI-GFR. Surprisingly, while the same disadvantage may be expected for SS-GFR, it was found to be generally more robust than SI-GFR. This may be due to the fact that the equation for the single sample is dependent on an estimate of the volume of distribution of tracer (Fleming et al. 2005a). This is proportional to the reciprocal of the intercept of the plasma clearance and so effectively provides an estimate of the intercept. This means that the single sample equation may contain some inherent

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buffering, which is sufficient to keep it robust against experimental errors.

In summary, for GFR values > 60 ml/min/1.73 m^2, the mean CV was found to be < 5% for all methods. At a GFR of 50 ml/min/1.73 m^2, the CV ranged between 3.7% and 5.8%, while at a GFR of 25 ml/min/1.73 m^2, the CV ranged between 6.2% and 9.3% (Fig. 3.2). These findings are consistent with the results reported by De Sadeleer et al (De Sadeleer et al. 2000; De Sadeleer et al. 2001) and Watson (Watson 2000). De Sadeleer et al used a Monte Carlo simulation to compare the magnitude of error in SI-GFR and SO-GFR after introducing normally distributed random errors to sampling time and activity measurement (De Sadeleer et al. 2000). From the data of this study, using minimum counts of 10 000, background counts of 400, pipetting error of 2%, and modelling determination of sample activity as the average of two samples, the activity measurement had a CV of 1.5 – 1.6%. Applying this and a timing error of 2 minutes to de Sadeleer's model resulted in an uncorrected GFR CV that was on average almost 2% lower than those generated by the model used in this study for GFR between 40 and 80 ml/min (data not shown), suggesting that additional errors from measurement of the standard dose, modelling the preparation of the standard, and counting of standard samples contribute significantly to GFR CV. This is consistent with the significant increases found by Watson et al (Watson 2000).

The model used in this study further incorporated errors in the measurement of weight and height to model error propagation in Bröchner-Mortensen corrected SI-GFR, and single sample techniques. It has built on existing models of measurement error as accurately as possible using errors that can be expected in a busy clinical unit. Furthermore, the model used in this study generated simulations based on ground truth characteristics derived from a large and diverse population of real patients. This approach is supported by plots of individual patient CV values, which still show significant inter-patient variation for a given GFR despite the use of 1000 simulations per patient (Fig. 3.2).

Patients with chronic kidney disease or those being treated with nephrotoxic drugs frequently require serial GFR measurements. For the nephrologist/oncologist, timely identification of a deterioration in kidney function is essential. The CV for repeat GFR measurements has been shown to be high, ranging between 7.5% and 12.2% (Bröchner-Mortensen and Rödbro 1976; Wilkinson et al. 1990; Blake et al. 1997; Grewal and Blake 2005; Delanaye et al. 2008a; Bird et al. 2008). Consequently, a large (> 20%) drop in GFR is required before the change can be considered significant (Fleming et al. 2004). It is useful to estimate the relative contribution of

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measurement error as these errors can, to a certain extent, be controlled. The CV of the biologic variation (CV_{BV}) and the CV of measurement errors (CV_{ME}), added in quadrature, will give the CV of repeat measurements (CV_{RM}):

$$(CV_{BV})^2 + (CV_{ME})^2 = (CV_{RM})^2$$

After matching the populations and methodology as closely as possible, the CV_{ME} for each of the previous studies was estimated and subsequently the CV_{BV} could be calculated. CV_{BV} was found to range between 2.8% and 11%. This wide range is likely due to heterogeneity of both study populations and study conditions. The population with the highest CV_{BV} had low mean GFR (45 ml/min) and the subjects were allowed free exercise during the study (Wilkinson et al. 1990), while the population with the lowest CV_{BV} had a higher mean GFR (79 ml/min) and were studied under conditions of strict bedrest (Bröchner-Mortensen and Rödbro 1976). It is difficult to postulate where within this range the CV_{BV} of the current study's population would lie, but assuming a value of 6.8% (median across the previous studies), in cases of normal clearance (GFR > 60 ml/min/1.73 m^2) where the CV_{ME} is consistently 3% - 4%, the CV_{RM} would be < 8%. In this scenario CV_{ME} is small in relation to CV_{BV} and reducing measurement errors further will have little impact on CV_{RM}. Interestingly data from the previous studies suggests that differences in patient preparation (i.e. exercise vs. bedrest and fasting vs. non-fasting) may have a greater impact on the CV_{RM} than measurement errors alone (Bröchner-Mortensen and Rödbro 1976; Wilkinson et al. 1990). At lower rates of clearance where CV_{ME} is large, its relationship relative to CV_{BV} is uncertain. There is little data on CV_{RM} at low GFR, but given the rapid rise in CV_{ME}, it is expected that CV_{RM} would increase similarly. The resultant high CV_{RM} is likely to render the investigation unusable. For example, even assuming a constant CV_{BV} of 6.8%, at a GFR of 20 ml/min/1.73 m^2, the CV_{ME} of 3pt SI-GFR (3, 4 h) is approximately 8.5% (Fig. 3.2) and therefore CV_{RM} will equal 13%. This means that GFR would have to drop by over 35% (2.8 x CV) before any change can be, with 95% confidence, regarded as significant (Fleming et al. 2004).

Error is inherent to every measurement and these errors propagate, resulting in an error in the final GFR value. The sources of the errors vary: counting error is statistical; some measurement errors arise due to finite equipment precision (e.g. patient scale, balance for weighing doses); others from user imprecision due to inexpert use of equipment (e.g. pipetting volume, recording of time). While equipment imprecision and lack of expertise are seldom problems in a research setting, they are more common in a busy clinical department, particularly in a training hospital

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where junior staff may be inexperienced. The second part of this paper has attempted to assess the sensitivity of GFR to individual measurement errors. SO-GFR is independent of many measurements (height, weight, flask volume and dose), yet it is more sensitive than SI-GFR and SS-GFR to errors in time and pipetting volume. Measurement errors had a similar effect on 3pt SI-GFR (2, 3, 4 h) and SS-GFR (3 h), both being relatively insensitive to errors in pipetting volume and flask volume, thus supporting the conclusions of De Sadeleer et al (De Sadeleer et al. 2001). Similarly, errors in height, weight and time measurement must be gross before the effect on GFR becomes clinically significant. Measurements performed based on the accuracy/precision requirements of the 2018 guideline (height 2 cm, weight 1 kg, time 1 minute) are expected to have minimal impact on GFR variability. Indeed even "suboptimal" measurements of these parameters e.g. using simple measuring devices without attention being given to whether shoes or coats are removed, or using unsynchronized clocks, are unlikely to have a major impact. This may be particularly relevant in patients who are difficult to measure due to being bedbound or wheelchair bound.

The measuring of dose syringes is however of greater importance. The precision of the balance in our unit is 0.005 g. After adding this error to the pre-administration syringe mass, and to the mass of the post-administration syringe, the error on the administered dose will be 0.007 g (i.e. $\sqrt{(0.005^2 + 0.005^2)}$, equivalent to 0.7% of the 1 g dose used in our department for both standard and patient doses. If the administered dose volume was halved (i.e. 0.5 g), the error would double (1.4%). It must also be noted that if the standard and patient dose volumes differ, their error will differ. The 2018 guideline (Burniston 2018) recommends an accuracy/precision of 2% on the difference of the pre- and post-administration syringe masses. Assuming balance precision of 0.005 g, the error on the administered dose exceeds the 2% threshold for doses < 0.35 g. If a less precise balance were to be used, the mass of the administered dose would need to be higher to achieve 2% accuracy e.g. for a balance with precision of 0.01 g, the minimum administered mass would need to be 0.7 g. The same principles apply to centres that measure their doses by volume or activity. It is suggested that individual centres determine the error on their full and empty syringe masses, for both patient and standard doses, based on the specific methodology and instruments used.

This study modelled the standard dilution protocol used in our institution. Different dilution protocols, both in terms of procedure and actual dilution, are expected to alter GFR error by altering the error of standard counts and altering the impact of flask volume error and pipetting

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errors. In our department we estimated an error of 0.5 ml on flask volume, which is 0.5% for the 100 ml flasks used, whereas a 0.5 ml error on a 1 L flask (i.e. 0.05%) would result in a smaller error in GFR. Similarly, the impact of a 2% pipetting error is greater for higher concentrations of activity i.e. when smaller flask volumes are used or when higher doses of activity are added to the standard. In our department a double dilution of the standard is performed resulting in an effective volume of 5 L. This will reduce the impact of pipetting error; however, this is likely negated by the additional steps required in preparation of the second dilution, together with the use of low-volume (100 ml) flasks.

An unexpected observation is the relative robustness of GFR to low counts. In the 2004 guideline (Fleming et al. 2004) it is stated that in order to minimize statistical counting error, a minimum of 10 000 counts is required for all samples. In this study it was found that total counts could reach surprisingly low levels before the error in GFR became significant (Fig. 3.3). The CV of counts is dependent on both sample and background counts. Obtaining sufficient counts is not a problem for centres using ^{99m}Tc-DTPA, as counts frequently exceed 10 000 after just a few minutes of counting with background counts that are low, resulting in a count CV of ~1%. However, this finding is reassuring to centres using ^{51}Cr-EDTA where lower doses of activity are administered. Here the prescribed minimum of 10 000 counts may be impossible to achieve despite much longer counting times, and this is compounded by background counts which are consequently higher. The results of this study suggest that a less strict minimum number of counts may be adhered to without significantly affecting GFR measurement precision. The 2018 guideline recommends precision better than 2% for sample counts (Burniston 2018). A simple approach to meet this would be for all count measurements to have counts >2500+background. In the 3 background scenarios modelled here count precision would vary from 1.3-1.9% with minimal impact on GFR variability.

How precise should individual measurements be? There is not a single, straightforward answer to this question as it depends on the indication for the study, the actual GFR, and the method used for GFR calculation. If using SO-GFR, precision is important. Time measurements should be kept accurate to within 1 minute and the error on pipetting volume should be < 1%. For a once-off GFR measurement using SS-GFR or SI-GFR extreme precision is not required and, as set out above, the equipment precision limits listed in the appendix of the 2018 BNMS guideline (Burniston 2018) are appropriate. Using these limits, median absolute errors would be < 2.3 ml/min/1.73 m^2 and 1.8 ml/min/1.73 m^2 for SI-GFR and SS-GFR respectively.

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However, while the impact is finite, the greatest care realistically possible is required when serial GFRs are required, particularly when GFR < 60 ml/min/1.73 m^2. In these cases, precision of every measurement is essential to keep CV_{ME} as low as possible. The problem is that one seldom knows at the outset which patients will require serial GFR measurement and which patients will have reduced kidney function. It is therefore reasonable to suggest that all measurements are kept as precise as possible. **Table 3.3** lists the measurements, suggested maximum errors for each, and methods to adhere to these limits. Using these slightly stricter limits, median absolute errors would be < 1.3 ml/min/1.73 m^2 and 1.0 ml/min/1.73 m^2 for SI-GFR and SS-GFR respectively. Values for minimum counts are based on the BNMS guideline recommendation of keeping counting error to < 2%, and this is appropriate for SI-GFR, SS-GFR and SO-GFR.

A recent meta-analysis of 1.7 million individuals with chronic kidney disease showed that a decline in estimated GFR of 30% was associated with a significantly increased risk of end stage renal disease (Coresh et al. 2014). In order to detect a 30% deterioration, CV_{ME} would have to be kept to < 8.3% (assuming a median CV_{BV} of 6.8%). Although the exact GFR at which CV_{ME} reaches 8.3% varies, it seems reasonable to suggest a single cut-off of 25 ml/min/1.73 m^2 for all methods, below which CV_{ME} becomes too high to meaningfully interpret serial GFR measurements. Should a referring clinician require detection of a 20% deterioration, CV_{ME} would have to be kept to approximately 2%, but this is only realistically achievable when GFR > 60 ml/min/1.73 m^2 and meticulous care is paid to precision.

The 2018 BNMS guideline (Burniston 2018) provides a list of quality control checks that should be routinely implemented when measuring GFR. One of the recommendations is that the difference in counts between duplicate samples should be less than expected levels when pipetting accuracy and counting error are both kept under 2%. It follows that the expected value would have to be determined for each centre as it is dependent on both minimum and background counts, which in turn, depends on the activity administered. In our unit, where 10 000 counts are obtained within a few minutes of counting with background counts of < 400 (due to higher doses of ^{99m}Tc-DTPA), the 95th percentile for the difference between duplicates is 3.5%. However, with background counts of 1500 and minimum counts are 4000, the 95th percentile for the difference between duplicates is 5.5%.

This study has several limitations. Only adult GFR data has been evaluated. However, it is expected that most measurements, and their errors will be similar in children. Importantly, this

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study did not specifically address the issue of gross human errors, e.g. inadvertent swapping of patients' samples or misinjections; mishaps that frequently lead to grossly erroneous GFR results. While the results of this study may provide further evidence to support a transition from SI-GFR to SS-GFR, in line with the updated BNMS guideline (Burniston 2018), there remains a concern that these not uncommon but potentially catastrophic errors/mishaps may not be detected using SS-GFR due to a lack of QC opportunities. Using SS-GFR, the current guideline recommends later blood sampling in cases of lower GFR (6 h for GFR 25 to 50 ml/min/1.73 m^2 and 24 h for GFR < 25 ml/min/1.73 m^2) (Burniston 2018). It is possible that CV_{ME} would be lower had the recommended time points been assessed, but this was not possible due to the lack of data at 6 h and 24 h. The approach taken for the sensitivity analysis of GFR to measurement errors represents a first order approximation as it focused on each error individually, thereby ignoring the possibility that errors may interact, either accentuating or attenuating each other. Radiopharmaceutical errors including small differences in protein binding between kits, or slight differences in the fraction of impurities ($^{99m}TcO_4^-$ and hydrolysed reduced ^{99m}Tc) (Rehling et al. 2001) were not included in our model, and their contribution is not known. It must also be noted that the intention of this study was to evaluate measurement errors only. Systematic errors arising from the use of simplified methods, as opposed to calculation of GFR from the area under the full plasma clearance curve, have been addressed elsewhere (Bird et al. 2007; McMeekin et al. 2016a).

In conclusion, measurement errors that occur on a day-to-day basis in a busy nuclear medicine department are unlikely to result in clinically significant errors in GFR when it is measured once-off, irrespective of the method. However, due to the considerable intra-individual variation in GFR, measurement errors must be kept to a minimum when serial GFR studies are being performed, particularly when GFR is below 60 ml/min/1.73 m^2. Serial measurements should not be performed when GFR < 25 ml/min/1.73 m^2, as detecting a significant decline in GFR becomes increasingly difficult.

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Conflicts of interest

None

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Tables and figures

Table 3.1 Summary of the measurement errors introduced

Measurement	SD error	Method to determine error
Patient height	5 cm	Departmental audit
Patient weight	2.5 kg	Estimation
Sample times	2 min	Estimation
Standard flask volume	0.5%	Departmental audit
Pipetting volume	2%	Departmental audit
Syringe masses	0.005 g	Precision of the balance
Counts	√counts	Poisson distribution

Abbreviations: **SD**, standard deviation

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Table 3.2 A comparison of methods after introduction of realistic errors to all measurements

Method	**Absolute error (ml/min/1.73 m^2)** **[median (2.5%; 97.5%)]**	**Relative error (%)** **[median (2.5%; 97.5%)]**	**Proportion with errors > 5%** **[% (95% CI)]**	**GFR at which CV exceeded 5%** **(ml/min/1.73 m^2)**
3pt SI-GFR (2, 3, 4 h)	1.7 (0.9; 2.5)	2.0 (1.6; 4.4)	1.7 (0.8; 7.8)	34
2pt SI-GFR (2, 3 h)	1.9 (1.4; 2.9)	2.2 (1.7; 8.3)	6.2 (4.5; 7.8)	52
2pt SI-GFR (2, 4 h)	1.8 (0.9; 2.6)	2.1 (1.7; 4.4)	1.5 (0.7; 2.4)	34
2pt SI-GFR (3, 4 h)	2.2 (1.1; 4.1)	2.6 (1.9; 6.9)	4.1 (2.7; 5.5)	42
SO-GFR	2.3 (1.6; 3.2)	2.7 (2.1; 5.9)	4.0 (2.6; 5.4)	56
SS-GFR (3 h)	1.6 (1.3; 1.8)	1.8 (1.2; 4.2)	0.8 (0.2; 1.4)	48
SS-GFR (4 h)	1.2 (1.0; 1.4)	1.3 (0.9; 3.4)	0.0 (0.0; 0.0)	40

Abbreviations: **GFR**, glomerular filtration rate; **CV**, coefficient of variation; **SI-GFR**, slope-intercept GFR; **SO-GFR**, slope-only GFR; **SS-GFR**, single-sample GFR

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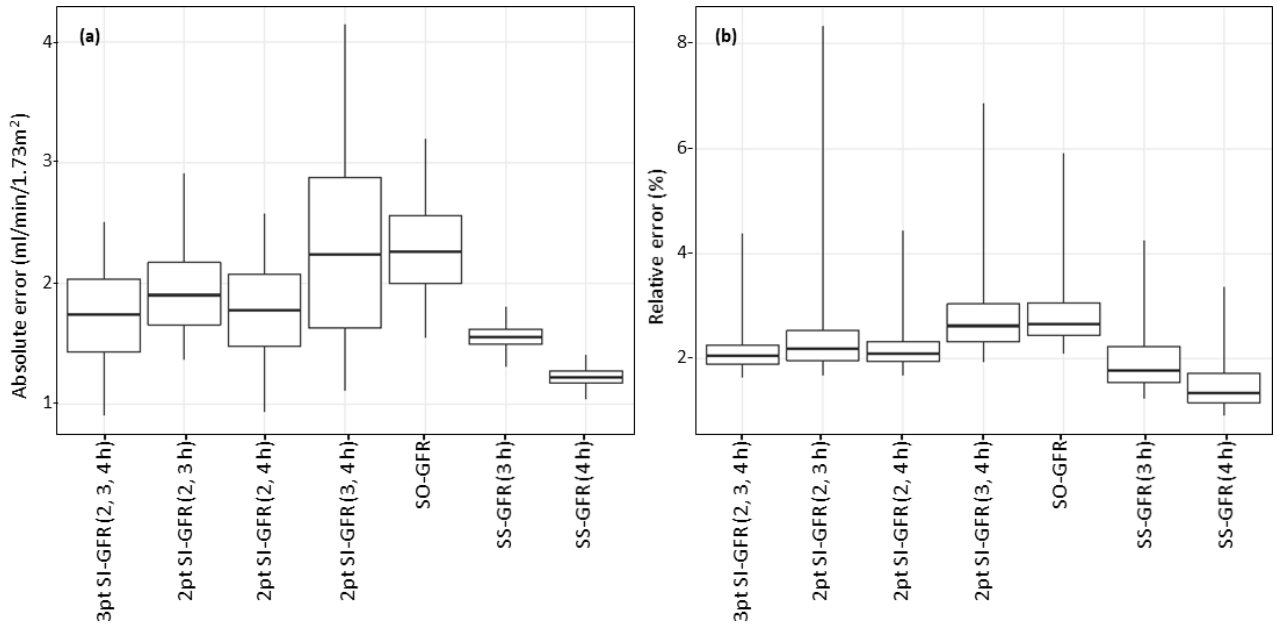
Fig. 3.1



Box-and-whisker plots comparing the seven methods of GFR calculation: (a) absolute error (ml/min/1.73 m^2) and (b) relative error (%). The solid lines represent the median values; the boxes represent the interquartile ranges; and the whiskers the 95% ranges.

Abbreviations: **GFR**, glomerular filtration rate; **SI-GFR**, slope-intercept GFR; **SO-GFR**, slope-only GFR; **SS-GFR**, single sample GFR.

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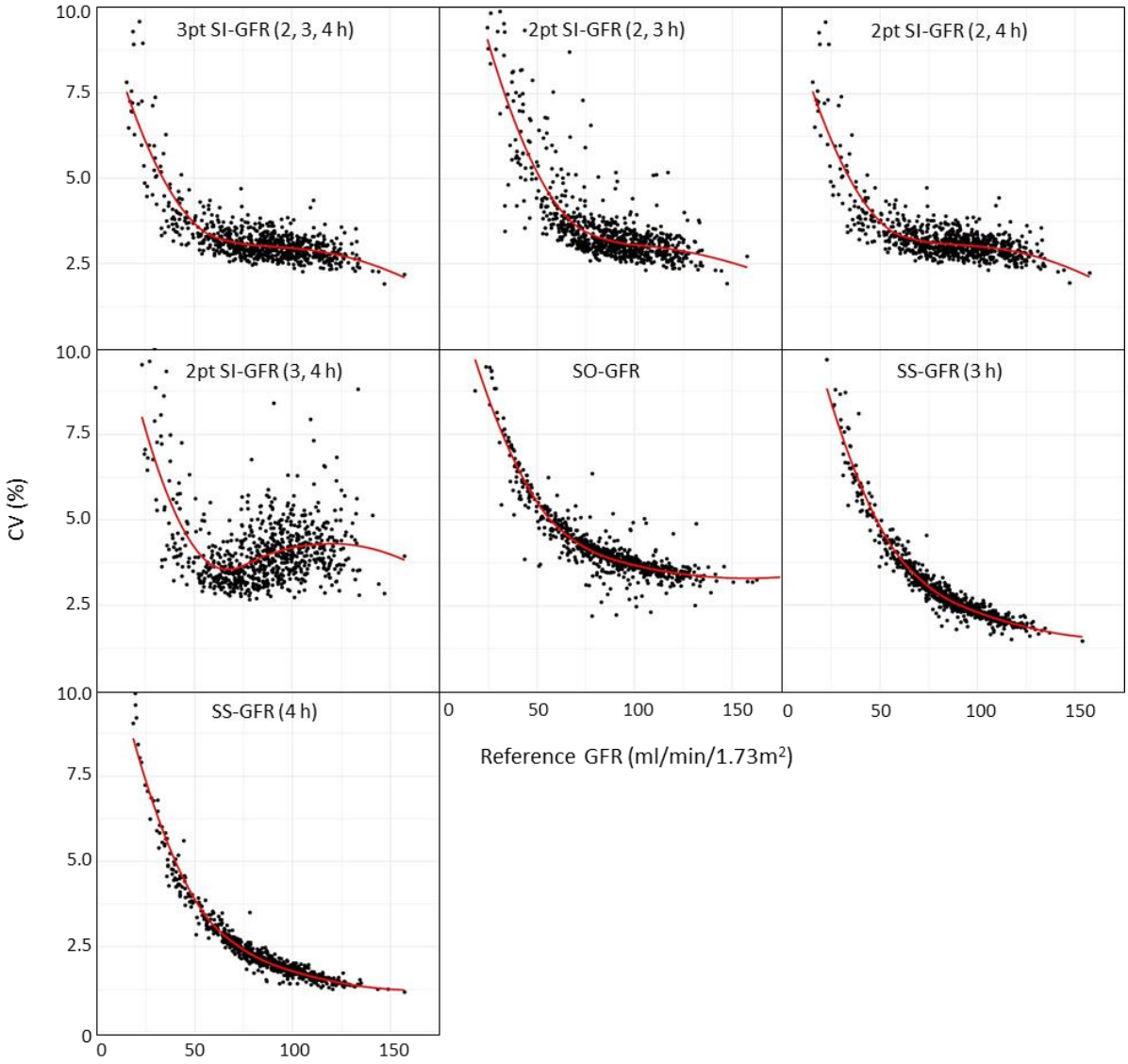
Fig. 3.2



Scatter plots of CV vs. GFR after introduction of realistic errors to all measurements for the 7 calculation methods/variations. The solid lines represent smoothed regression lines.

Abbreviations: **CV**, coefficient of variation; **GFR**, glomerular filtration rate; **SI-GFR**, slope-intercept GFR; **SO-GFR**, slope-only GFR; **SS-GFR**, single-sample GFR.

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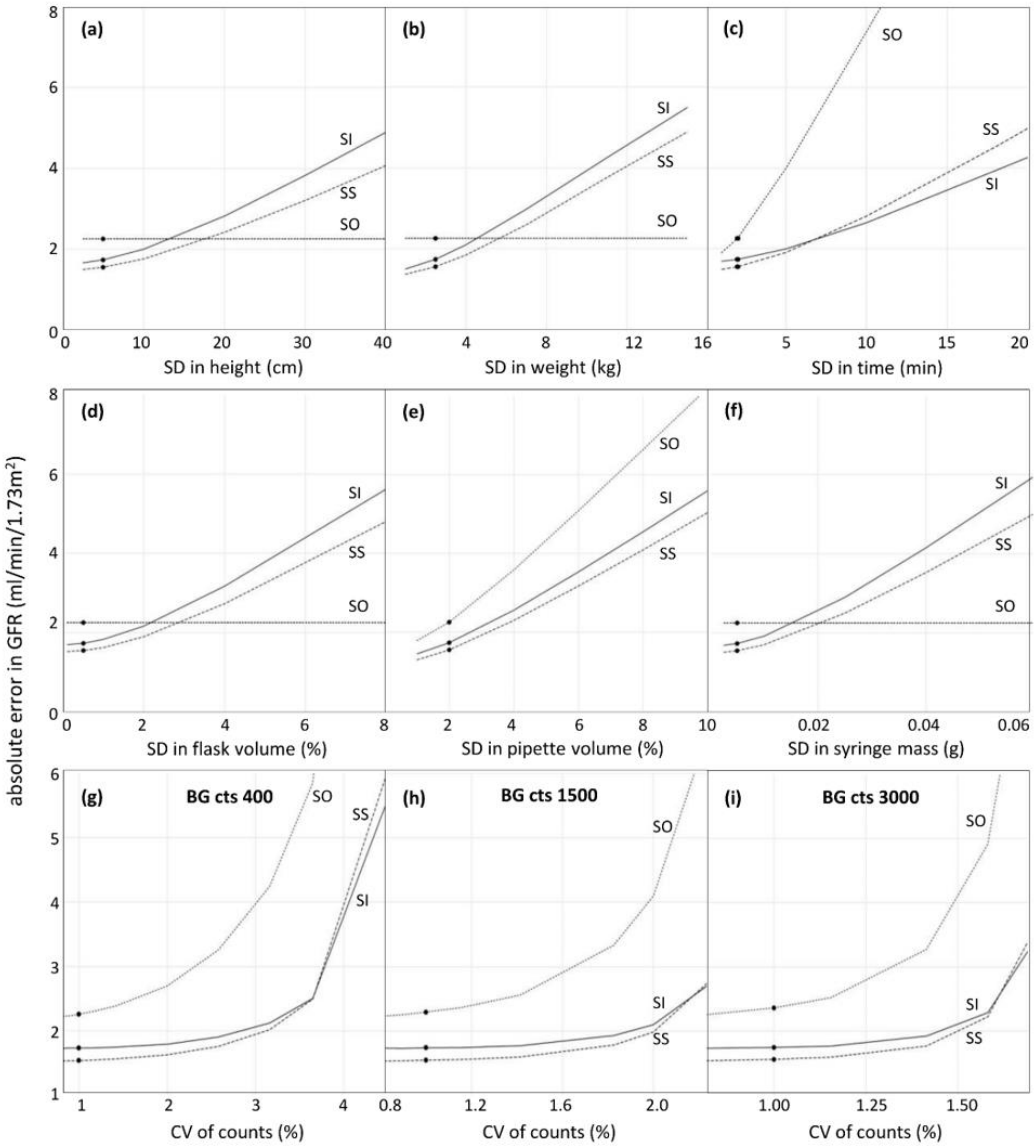
Fig. 3.3



Scatter plots of the error in GFR vs. a varying 1 SD error for each measurement (a-f), and error in GFR vs. the CV of the minimum counts obtained (g-i). ——— 3pt SI-GFR (2, 3, 4 h); ·····SO-GFR**;** - - - - - -SS-GFR (3 h). The solid dots represent realistic values. SO-GFR values are plotted on a, b, d, and f for purposes of comparison only.

Abbreviations: **GFR**, glomerular filtration rate; **SD**, standard deviation; **CV**, coefficient of variation; **BG cts**, background counts; **SI-GFR**, slope-intercept GFR; **SO-GFR**, slope-only GFR; **SS-GFR**, single-sample GFR.

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Table 3.3 Suggested maximum allowable errors for all measurements and methods to achieve this

Measurement	Suggested maximum error	Methods to minimize error or reduce its impact
Height	2 cm	Use of a single wall-mounted height rod Removal of shoes
Weight	1 kg	Use of a single scale Removal of bulky clothing, shoes and items in pockets
Time	1 min	Use of a single digital clock Synchronization of clocks if blood samples are taken outside the department (Important for SO-GFR)
Volumetric flask volume	0.5%	Use of a larger volume flask Adequate training of technologists;
Pipetting volume	1%	Adequate training of technologists
Administered doses	1%	Measurement by weight rather than activity or volume Measurement of pre- and post-administration syringe weights (rather than syringe weights before and after drawing up of doses) Use patient/standard dose weight ≥ 140 times balance precision (e.g. ≥ 0.7 g for a precision of 0.005 g)
Counts	If BG = 400, min cts > 2900 If BG = 1500, min cts > 4000 If BG = 3000, min cts > 5500	Administration of adequate doses Sufficient counting times

Abbreviations: **Min cts**, minimum counts; **BG**, background counts

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4. Validation of equations to estimate glomerular filtration rate in South Africans of mixed ancestry

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Abstract

Background: The Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations are two commonly used formulae to estimate glomerular filtration rate (GFR) in adults. The CKD-EPI equation is recommended in current international and local guidelines for the diagnosis and management of chronic kidney disease (CKD), unless an alternative equation has been shown to have superior accuracy. This requires validation and comparison of the equations in local populations. Previous studies have reported on the accuracy of these prediction equations in Black South Africans and those of Indian ancestry.

Objectives: The aim of this study was to evaluate the MDRD and CKD-EPI equations in mixed ancestry South African adults.

Methods: In all participants, GFR was measured from the plasma clearance of ^{99m}Tc-diethylenetetraaminepenta-acetic acid (^{99m}Tc-DTPA) using a standardised technique. Serum creatinine assays were isotope dilution mass spectrometry (IDMS) traceable. GFR was estimated (eGFR) using the MDRD and CKD-EPI equations, with and without the Black ethnicity factor. The agreement, bias, precision and accuracy of each equation was determined.

Results: Eighty adults were included (30 male, median age 39 years, median GFR 59 ml/min/1.73 m^2). Sixty had a diagnosis of CKD, 10 were potential kidney donors, and 2 were healthy volunteers. Both equations, without the Black ethnicity factor, had good agreement with measured GFR. The equations tended to overestimate GFR, with bias of 1.6 and 7.9 ml/min/1.73 m^2 for the MDRD and CKD-EPI, respectively. The interquartile ranges of the differences were 15.9 and 20.2 ml/min/1.73 m^2, and as a measure of accuracy, the P_{30} values were 80% and 72.5% (p=0.18). For identification of individuals with GFR <60 ml/min/1.73 m^2, the sensitivity of MDRD eGFR was 97.3% and CKD-EPI eGFR was 97.1%.

Conclusion: The MDRD and CKD-EPI equations have shown satisfactory and comparable performance in this South African mixed ancestry adult population, with the MDRD marginally less biased than the CKD-EPI equation.

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Introduction

Chronic kidney disease (CKD) is defined by the presence of abnormalities of kidney structure or function (such as a glomerular filtration rate (GFR) of less than 60 ml/min/1.73 m^2), present for > 3 months, with implications for health (KDIGO 2013). The Kidney Disease Improving Global Outcomes (KDIGO) guideline recommends using a creatinine-based equation to estimate GFR in the initial assessment of CKD and for monitoring disease progression (KDIGO 2013). The two most commonly used equations in adults are the Modification of Diet in Renal Disease (MDRD) equation (Levey et al. 1999; Levey et al. 2007), and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (Levey et al. 2009). Unless an alternative equation has been shown to have superior accuracy, use of the CKD-EPI equation is recommended (KDIGO 2013; SARS 2015).

A 2012 systematic review found that neither the MDRD nor the CKD-EPI equation performed well in populations outside North America, Europe or Australia and it concluded that neither equation could be used routinely across all populations and all GFR ranges (Earley et al. 2012). Studies conducted in various South African populations support this, and although the findings varied, the equations tended to have unacceptable bias, precision and/or accuracy (van Deventer et al. 2008; van Deventer et al. 2011; Stevens et al. 2011; Madala et al. 2011; Moodley et al. 2018). The poor performance of the equations in South African populations is concerning. South Africa is a developing country with limited centres that are able to measure GFR. Even where the service is available, the large number of CKD patients attending renal clinics precludes routine GFR measurement, compelling physicians to rely on estimated GFR. Furthermore, GFR estimates are essential in epidemiological studies, specifically to determine CKD prevalence.

The mixed ancestry (Coloured) population group is estimated to comprise 8.8% of South Africa's population (STATS SA 2018) and almost 49% of the Western Cape Province's population (STATS SA 2012). Its origins are Khoisan (32-43%), Bantu-speaking African (20-36%), European (21-28%), and Asian (9-11%) (de Wit et al. 2010). No previous studies have evaluated the GFR estimating equations in this group despite it having a high prevalence of CKD (Matsha et al. 2013; Adeniyi et al. 2017). Considering the population group's genetic diversity, it is hypothesized that the accuracy of the commonly used equations will be poor. The aim of this study was to evaluate the performance of the MDRD and CKD-EPI equations in mixed ancestry South African adults.

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Methods

Participants

Participants were recruited from the outpatient clinics of the Division of Nephrology at Tygerberg Hospital, Cape Town, South Africa between June 2015 and October 2018. Patients attending routine out-patient appointments or individuals being worked up for potential kidney donation were screened by a renal physician to exclude acute renal failure, concurrent illness, cancer, expanded extracellular fluid volumes (ascites, oedema, or pleural effusions), pregnancy or breastfeeding. After screening, adult patients (≥18 years) who self-classified as South Africans of mixed ancestry were invited to participate. All participants provided written, informed consent. The study was approved by the Stellenbosch University Health Ethics Research Committee (protocol S14/10/217).

GFR measurement

GFR was measured in the Nuclear Medicine Division of Tygerberg Hospital following our departmental protocol which is based on the methodology in the 2004 British Nuclear Medicine Society GFR guideline (Fleming et al. 2004). On arrival, the height and weight of the participant was recorded. Approximately 40 MBq of ^{99m}Tc-diethylenetetraaminepenta-acetic acid (^{99m}Tc-DTPA) was injected through an intravenous catheter. Extravasation was excluded by imaging the injection site using a gamma camera. A similar dose of ^{99m}Tc-DTPA was used for preparation of the standard. Patient and standard doses were calibrated accurately by weighing the syringes before and after administration of the doses on a Precisa 620C balance (precision = 0.005 g) (Precisa Balances Ltd, Milton Keynes, United Kingdom). In order to avoid excessively high count rates, a double dilution of the standard was performed, resulting in an effective volume of 5 L. Duplicate 1 ml samples of the standard were pipetted into counting tubes. Venous blood samples were taken from the contralateral arm at 2, 3 and 4 h after ^{99m}Tc-DTPA administration. After centrifugation at 1000 g for 10 min, duplicate 1 ml plasma samples were pipetted into counting tubes. The plasma and standard samples were counted simultaneously in a multichannel well counter (VIDEOGAMMA 4880, l'acn scientific laboratories, Italy) together with 2 empty counting tubes for background correction. GFR was calculated using the slope-intercept method (Chantler et al. 1969; Fleming et al. 2004). The clearance values obtained were corrected for body surface area (BSA) using the Haycock formula (Haycock et al. 1978), and to correct for the systematic underestimation of GFR that

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is inherent to the slope-intercept method, the mean Bröchner-Mortensen correction was applied (Bröchner-Mortensen 1972; Bröchner-Mortensen et al. 1974; Fleming et al. 2004). Routine quality control checks were performed on each GFR study. Measured GFR is denoted mGFR in this text.

GFR estimation

A venous blood sample was taken for creatinine measurement immediately prior to administration of the ^{99m}Tc-DTPA dose. Serum creatinine (Scr) was measured by the National Health Laboratory Service (NHLS) of Tygerberg Hospital. Initially a method based on the Jaffé reaction was used (Siemens ADVIA 1800, Siemens Healthineers, Munich, Germany). In September 2016, the analyser was replaced with a Roche Cobas 6000, c501 module (Roche, Basel, Switzerland) where an enzymatic method is applied. Both methods have been standardised against isotope dilution mass spectrometry (IDMS) (Myers et al. 2006). The analytical imprecision and total error respectively of the Siemens Advia 1800 was 2.0% and 2.4% at Scr=79 µmol/L; 2.1% and 7.2% at Scr=166 µmol/L; 1.8% and 6.7% at Scr=529 µmol/L. Values for the Roche Cobas 6000 analyser are 1.9% and 2.4% at Scr=90.5 µmol/L, and 1.3% and 4.1% at 327.9 µmol/L.

Estimated GFR, denoted eGFR, was calculated using the following equations, both of which are corrected for BSA:

1. The re-expressed, 4-variable MDRD equation (Levey et al. 2007):

$$eGFR\ (ml/min/1.73\ m^2) = 175 \times (Scr/88.4)^{-1.154} \times age^{-0.203} \times g \times e,$$

where Scr is serum creatinine in µmol/L, g is a gender factor (male=1, female=0.742), e is an ethnicity factor (African American=1.212, other ethnic groups=1).

2. The CKD-EPI equation (Levey et al. 2009):

$$eGFR = 141 \times \min(Scr/\kappa,1)^{\alpha} \times \max(Scr/\kappa,1)^{-1.209} \times 0.993^{age} \times g \times e,$$

where Scr is serum creatinine in µmol/L, κ is 61.9 for females and 79.6 for males, α is -0.329 for females and -0.411 for males, min(x,y) and max(x,y) respectively indicate the minimum and maximum of x and y, g is a gender factor (male=1, female=1.108), and e is an ethnicity factor (African American=1.159, other ethnic groups=1).

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Data analysis

Bias was calculated as the median difference between eGFR and mGFR. Precision was expressed as the interquartile range (IQR) of the differences, and as the root mean square error of the regression of eGFR vs. mGFR. As measures of accuracy, the RMSE (calculated as the square root of mean (log eGFR – log mGFR)^2), P_{30} and P_{20} were calculated. The P_{30} and P_{20} values are defined as the percentage of GFR estimates within 30% and 20% of measured GFR, respectively. Bland-Altman analyses (Bland and Altman 1986) were used to determine the agreement between eGFR and mGFR. The sensitivity, specificity and positive predictive value for identifying individuals with mGFR < 60 ml/min/1.73 m^2 were calculated for each equation. The Shapiro-Wilk test was used to test for normality. The McNemar test was used to compare proportions.

Results

In total, 80 participants were included (30 male; median age 39 years, age range 18 to 68 years) (**Fig. 4.1**). Sixty-eight had a diagnosis of CKD, 10 were potential kidney donors, and 2 were healthy volunteers. The median mGFR was 59 ml/min/1.73 m^2 (range 10 to 126 ml/min/1.73 m^2 (**Table 4.1**)**Error! Reference source not found.**.

In 46 individuals, serum creatinine (Scr) was measured using the Jaffé (alkaline picrate) method and in 34 the enzymatic method on the new analyser was used. There was no significant difference in bias or precision of the equations between the alkaline picrate and enzymatic groups (data not shown).

The performance of the GFR estimation equations is given in **Table 4.2**. The bias of the MDRD equation was 1.6 ml/min/1.73 m^2 whereas the CKD-EPI equation overestimated GFR by 7.9 ml/min/1.73 m^2 . The RMSE values of the regression of MDRD and CKD-EPI vs. mGFR were 15.1 and 13.5 ml/min/1.73 m^2 respectively, whereas the IQR of the differences was narrower for the MDRD equation than the CKD-EPI (15.9 vs. 20.2 ml/min/1.73 m^2). The P_{30} values did not differ significantly (MDRD 80.0%, CKD-EPI 72.5%; p=0.18). When the Black ethnicity factor was applied, bias, precision and accuracy of both equations deteriorated. The Bland-Altman analyses are shown in Fig. 4.2. Both equations had non-significantly higher P_{30} values in the subgroup of individuals with GFR ≥ 60 ml/min/1.73 m^2 (MDRD 84.6%, CKD-EPI 82.1%) than in the GFR < 60 ml/min/1.73 m^2 subgroup (MDRD 75.6%, CKD-EPI 63.4%). For

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identification of individuals in whom GFR was < 60 ml/min/1.73 m^2 the sensitivities of the MDRD and CKD-EPI equations were > 90% in the 3 subgroups analysed (**Table 4.3**).

Discussion

The performance of both equations was satisfactory in this mixed ancestry South African population. The MDRD equation was significantly less biased than the CKD-EPI equation, overestimating mGFR by 1.6 ml/min/1.73 m^2 compared to 7.9 ml/min/1.73 m^2 . The IQRs of the differences were 15.9 ml/min/1.73 m^2 for the MDRD equation and 20.2 ml/min/1.73 m^2 for the CKD-EPI equation, and the RMSE values were 16.0 and 16.4 ml/min/1.73 m^2 respectively. Accuracy was not significantly different with P_{30} values of 80% and 72.5% (p=0.18). In the original CKD-EPI study, the CKD-EPI equation was compared to the MDRD equation using a large validation set of 6646 individuals (Levey et al. 2009). Although the majority of participants were White and had CKD, the population was fairly diverse in terms of ethnicity (19% Black, ~4% Asian/Hispanic) and pathology (29% diabetes, 19% transplant recipients, 14% healthy kidney donors). Its mean GFR was 68 ml/min/1.73 m^2. The median difference, IQR, and P_{30} of the MDRD equation were -5.5 ml/min/1.73 m^2, 18.3 ml/min/1.73 m^2, and 80.6%. The CKD-EPI equation performed marginally better with bias of 2.5 ml/min/1.73 m^2, IQR of 16.6 ml/min/1.73 m^2, and P_{30} of 84.1%. In an earlier study, the MDRD equation was evaluated in a large population pooled from 10 studies (mean GFR 68 ml/min/1.73 m^2, 32% Black, 5% Asian/Native American, 29% diabetes, 5% transplant recipients) (Stevens et al. 2007a). Median bias was -2.7 ml/min/1.73 m^2, IQR 16.4 ml/min/1.73 m^2, and P_{30} 83%. The results in our study, specifically for the MDRD equation, are comparable to these and some of the other large validation studies from North American, European and Australian populations (Earley et al. 2012).

For identification of individuals with GFR < 60 ml/min/1.73 m^2, both equations performed well, with sensitivities of > 97%, and specificities and positive predictive values of > 80%. This is useful information for screening programmes or for researchers conducting epidemiological studies on the prevalence of CKD in local communities. Although the sensitivity decreased slightly when individuals with GFR < 30 ml/min/1.73 m^2 were excluded from the analysis, it remained > 90% for both equations. For the GFR subgroups analysed, the MDRD equation tended to perform better than the CKD-EPI, however, as expected from the small numbers in each group, the differences were not significant.

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A few investigators have evaluated the MDRD and CKD-EPI equations in other South African populations. One consistent finding across all the studies in Black South Africans was that incorporation of an African American ethnicity factor resulted in a substantial increase in bias and a decrease in precision and accuracy (van Deventer et al. 2008; Stevens et al. 2011; Madala et al. 2011; Moodley et al. 2018). It is therefore not surprising that similar results were found in this mixed ancestry population (**Table 4.2**). In a study of 100 Black South Africans with CKD the bias, precision and accuracy of the MDRD equation were similar to our values (van Deventer et al. 2008). In half of the same population (i.e. 50 Black South Africans) the CKD-EPI equation had a median bias of 4.9 ml/min/1.73m^2 and a P_{30} of 74%, values also very similar to ours (van Deventer et al. 2011). In a separate study of 91 Black South Africans with CKD, bias of the MDRD equation was negligible but the precision was poor as reflected by the wide 95% limits of agreement (LOA) in the Bland-Altman analyses (-37.9 to 40.0 ml/min/1.73m^2) (Madala et al. 2011). The P_{30} of the MDRD equation in this study ranged between 36% and 69% depending on the GFR. In a more recent study of 188 Black South Africans, the P_{30} value of the MDRD equation was 53%-54% and the CKD-EPI equation 53-54%, whereas the P_{30} values in 99 Indian South Africans were 49-66% and 54-66% for the MDRD and CKD-EPI equations respectively (Moodley et al. 2018). This population included individuals with malignancy (58%) and CKD (38%).

Possible reasons for the poorer performance of eGFR in previous South African studies include differences in ethnicity, mean GFR, pathology and/or methodology. Many other studies in populations outside the United States, Europe and Australia have shown similarly poor results, and in most cases this has been attributed to ethnic differences (Earley et al. 2012). Although South Africans of mixed ancestry are genetically diverse with Khoisan (32-43%), Bantu-speaking African (20-36%), European (21-28%) and Asian (9-11%) ancestral components (de Wit et al. 2010), this diversity did not significantly limit eGFR performance in this population. Furthermore, that some studies in Black Africans with CKD had similar results, suggests that ethnicity may be less important than other factors (van Deventer et al. 2008; van Deventer et al. 2011).

Possibly one of the most important determinants of the effectiveness of eGFR equations is patient pathology. The population in our study comprised CKD patients (85%) and healthy individuals (15%), similar in composition to the development populations of the MDRD and CKD-EPI equations. This is likely to have contributed towards the good performance of the

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equations in our study. In contrast, in the most recent of the South African studies, 58% of patients had cancer (Moodley et al. 2018). A lower average serum creatinine concentration in this population may explain the positive bias found for both MDRD and CKD-EPI equations as cancer, and other chronic illnesses, are known to reduce creatinine generation through muscle wasting (Stevens et al. 2006).

In the measurement of GFR, factors such as the use of a different filtration marker or method to calculate GFR will result in systematic differences in mGFR; however, provided mGFR is performed *correctly*, the impact should be small. This is because the error on eGFR-mGFR is equal to $\sqrt{[(\text{error on eGFR})^2 + (\text{error on mGFR})^2]}$, so if the error in mGFR << error in eGFR, then the latter would contribute little to the error on the difference. In this study, meticulous care was paid to methodology for GFR measurement. Our departmental guideline adheres strictly to the 2004 British Nuclear Medicine Society GFR guideline (Fleming et al. 2004) and we perform rigorous quality control checks on each GFR study. Furthermore, an analysis of measurement errors in our unit taking into account error in all aspects of the investigation (e.g. measuring patient height and weight, preparation of the standard, measuring of doses, recording of time, pipetting technique and counting of samples), revealed an error in mGFR of approximately 2 ml/min/1.73 m^2 (Holness et al. 2019).

All participants in this study were screened meticulously by a renal physician, a factor that may contribute to the relatively good performance of eGFR. Patients with expanded extracellular fluid volumes (ascites, oedema, or pleural effusions), acute renal failure, any acute concurrent illness, or any other reason to suspect unstable kidney function were not considered for inclusion. Furthermore, GFR and serum creatinine were measured on the same day in every patient. Although fluctuations in serum creatinine may, to a degree, mirror fluctuations in GFR, with coefficients of variation (CV) for repeat measurements of approximately 8-10% for GFR and 6% for serum creatinine (Fleming et al. 2004; Delanaye et al. 2017), measurement of these parameters on different days will limit the precision that is attainable. Use of a Jaffe assay rather than an enzymatic method for creatinine measurement will further limit the precision considering analytical CVs of approximately 5.5% and 2% respectively (Delanaye et al. 2017).

The main limitation of this study is the sample size; however, the study is sufficiently powered to draw conclusions about the performance of the equations in the population. The primary outcome variable is the P_{30} proportion. A sample size of 80 is sufficient to estimate the true population value of P_{30} using a 95% confidence interval to within 10% of the true value (i.e. a

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confidence interval width of 20%) given P_{30} is 70-80%. While a better estimation of P_{30} is ideal, an estimation within 10% can be regarded as acceptable. On the other hand, a sample size of ~ 150 would be required to detect a significant difference between the MDRD and CKD-EPI equations at P_{30} values of 80% and 72.5% respectively. It thus cannot be concluded that the MDRD equation out-performed the CKD-EPI equation in this study. Ideally, more patients with normal kidney function would have been included. This may have resulted in better performance of the CKD-EPI equation as (i) the MDRD equation has been shown to have poorer accuracy at higher GFR levels (Stevens et al. 2007a), and (ii) the CKD-EPI equation was found to be more accurate than the MDRD in most GFR subgroups (Stevens et al. 2010). Based mainly on a CKD population, our results are not necessarily generalisable to other populations e.g. patients with cancer. Four of the patients with lupus nephritis that were included used trimethoprim chronically. Trimethoprim is known to interfere with the tubular secretion of creatinine, thus raising serum creatinine levels (Perrone et al. 1992). However, the impact of this on the overall results of this study is thought to be minimal.

In conclusion, the MDRD and CKD-EPI equations have both shown satisfactory performance in this South African mixed ancestry adult population, with comparable accuracy. This information is reassuring to physicians treating patients with CKD and to researchers conducting epidemiological studies.

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Conflicts of interest

None

Author contributions

JL Holness: data analysis, manuscript preparation

K Bezuidenhout: participant recruitment, manuscript review

MR Davids: co-supervisor, manuscript review

JM Warwick: supervisor, manuscript review

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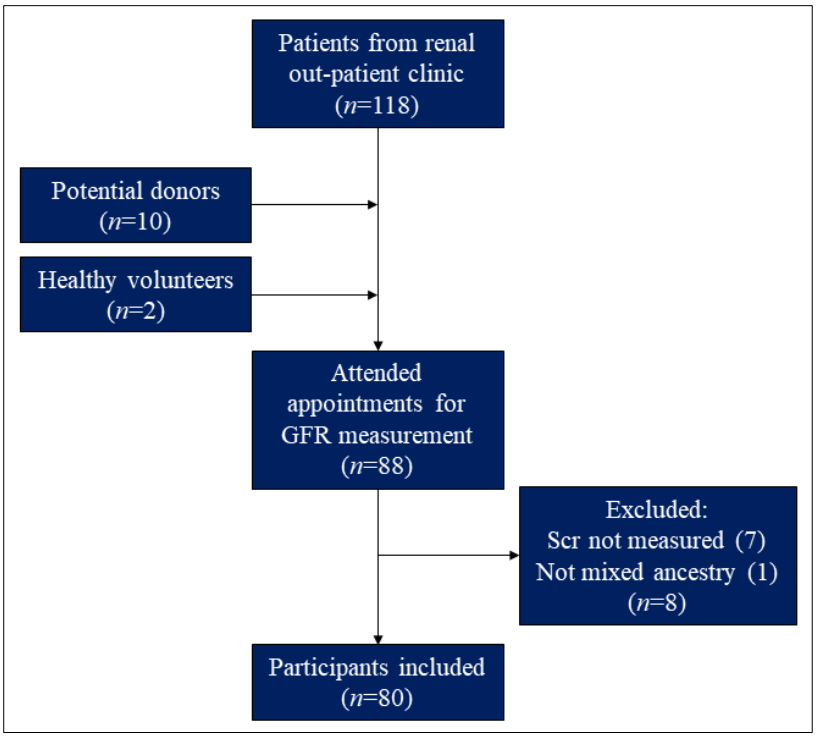
Tables and figures

Fig. 4.1 Study enrolment



Abbreviations: **GFR**, glomerular filtration rate. **Scr**, serum creatinine.

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Table 4.1 Participant characteristics (n = 80)

	Median (range)
Age (years)	39 (18 – 68)
Serum creatinine (µmol/L)	92 (66 – 191)
Serum creatinine (mg/dL)	1.0 (0.5 – 7.9)
Measured GFR (ml/min/1.73 m^2)	59 (10 – 126)
	***n* (%)**
Sex (male)	30 (37.5%)
GFR	
≥ 90 ml/min/1.73 m^2	27 (33.7%)
60 – 89 ml/min/1.73 m^2	12 (15.0%)
30 – 59 ml/min/1.73 m^2	19 (23.8%)
< 30 ml/min/1.73 m^2	22 (27.5%)
Diagnosis	
Glomerular disease	31 (51.3%)
Lupus nephritis	17 (21.3%)
Focal segmental glomerulosclerosis	7 (8.7%)
IgA nephropathy	4 (5.0%)
Membranous nephropathy	3 (3.8%)
Other glomerulonephritis	10 (12.5%)
CKD of unknown cause	8 (10%)
Autosomal dominant polycystic kidney disease	5 (6.2%)
Malignant hypertension	4 (5.0%)
Solitary kidney	3 (3.8%)
Obstructive nephropathy	2 (2.5%)
Other specified renal disease	5 (6.2%)
Potential kidney donor	10 (12.5%)
Healthy volunteer	2 (2.5%)
Chronic use of trimethoprim	4 (5.0%)

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

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Table 4.2 Bias, precision, accuracy and 95% limits of agreement of the MDRD and CKD-EPI equations

	MDRD	**CKD-EPI**	**MDRD with Black ethnicity factor**	**CKD-EPI with Black ethnicity factor**
Bias				
Median difference*† (95% CI)	1.6 (-0.3 to 7.5)	7.9 (5.4 to 11.5)	15.3 (11.1 to 20.3)	20.3 (14.6 to 24.0)
Precision				
IQR of the differences*†	15.9	20.2	25.9	28.1
RMSE*‡	15.1	13.5	18.2	15.7
Accuracy				
RMSE§	0.239	0.257	0.331	0.347
P_{30} % (95% CI)	80.0 (69.6 to 88.1)	72.5 (61.4 to 81.9)	51.3 (39.9 to 62.6)	47.5 (36.2 to 59.0)
P_{20} % (95% CI)	60.0 (48.4 to 70.8)	55.0 (43.5 to 66.2)	36.3 (25.8 to 47.8)	31.3 (21.4 to 42.6)
Agreement				
95% LOA*	-24.9; 34.4	-17.9; 35.7	-21.6; 60.0	-14.5; 55.2

*Units ml/min/1.73 m^2. †The differences are calculated as eGFR – mGFR. ‡RMSE of the regression of eGFR vs. mGFR. §RMSE calculated as the square root of mean (log eGFR – log mGFR)^2.

Abbreviations: **MDRD**, Modification of diet in Renal Disease; **CKD-EPI**, Chronic Kidney Disease Epidemiology Collaboration; **IQR**, interquartile range; **RMSE**, root mean square error; **95% CI**, 95% confidence interval; **LOA**, limits of agreement.

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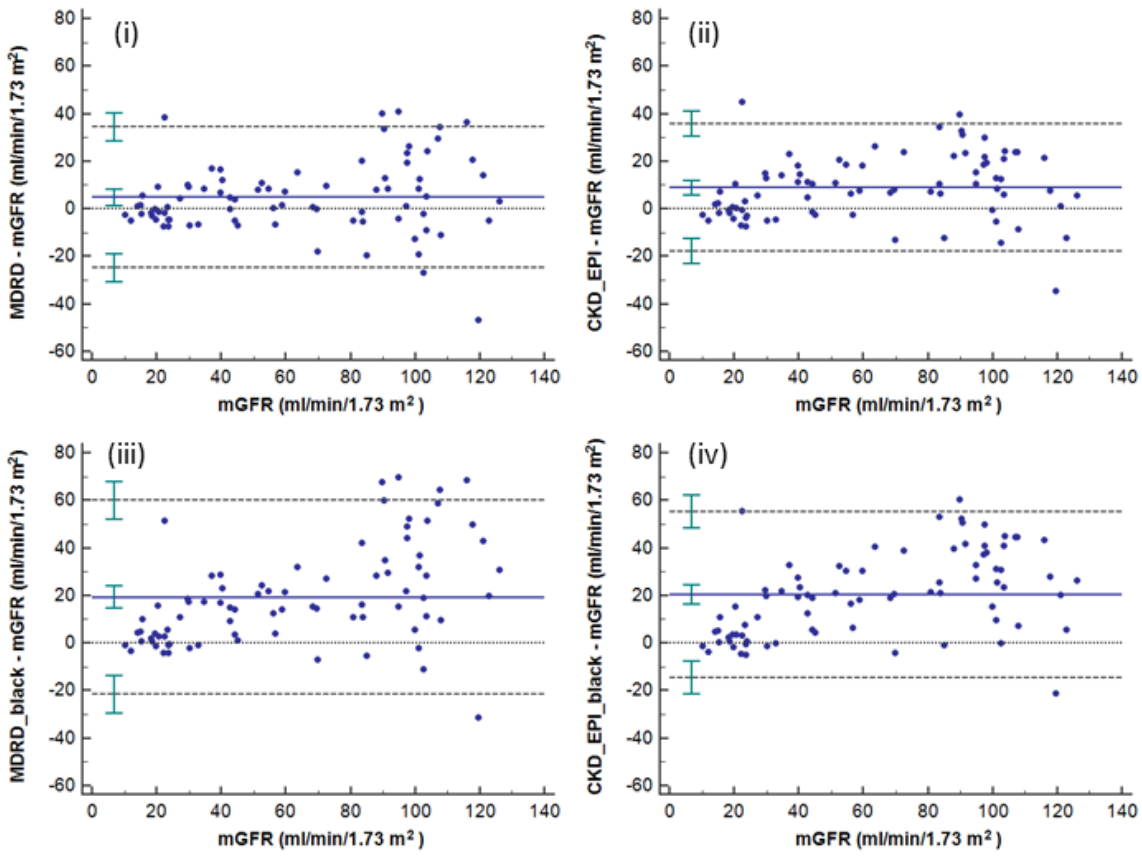
Fig. 4.2



Bland-Altman plots of the four equations (i) MDRD, (ii) CKD-EPI, (iii) MDRD with the Black ethnicity factor, and (iv) CKD-EPI with the Black ethnicity factor. The solid line represents the mean difference between eGFR and mGFR, and the dashed lines represent the upper and lower limits of agreement with 95% confidence intervals.

Abbreviations: **mGFR**, measured glomerular filtration rate; **eGFR**, estimated glomerular filtration rate; **MDRD**, Modification of Diet in Renal Disease, **CKD-EPI**, Chronic Kidney Disease Epidemiology Collaboration.

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Table 4.3 Diagnostic performance of the equations to detect patients with GFR < 60 ml/min/1.73 m^2

		Sensitivity % (95% CI)	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**
All patients (n = 80)	**MDRD**	97.3 (91.0 – 99.6)	88.4 (79.3 – 94.5)	87.8 (78.6 – 94.1)	97.4 (91.1 – 99.7)
	CKD-EPI	97.1 (90.7 – 99.6)	82.6 (72.7 – 90.3)	80.5 (70.1 – 88.5)	97.4 (91.1 – 99.7)
GFR 45-90 ml/min/1.73 m^2 (n = 31)	**MDRD**	93.8 (78.9 – 99.3)	73.3 (54.4 – 87.5)	78.9 (60.6 – 91.4)	91.7 (76.1 – 98.5)
	CKD-EPI	92.3 (76.9 – 98.8)	61.1 (42.0 – 78.0)	63.2 (44.1 – 79.7)	91.7 (76.1 – 98.5)
GFR > 30 ml/min/1.73 m^2 (n = 58)	**MDRD**	93.8 (84.2 – 98.4)	90.5 (79.7 – 96.6)	78.9 (66.2 – 88.5)	97.4 (89.4 – 99.8)
	CKD-EPI	92.3 (82.2 – 97.7)	84.4 (72.5 – 92.6)	63.2 (49.5 – 75.5)	97.4 (89.4 – 99.8)

Abbreviations: **GFR**, glomerular filtration rate; **MDRD**, Modification of Diet in Renal Disease, **CKD-EPI**, Chronic Kidney Disease Epidemiology Collaboration, **PPV**, positive predictive value; **NPV**, negative predictive value.

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5. Can glomerular filtration rate estimation be adapted for local oncology patients?

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Abstract

Purpose

Creatinine-based glomerular filtration rate (GFR) estimating equations that were developed for chronic kidney disease (CKD) populations tend to perform poorly in cancer patients. The Janowitz-Williams model is a new equation developed from a large population of White patients with cancer. Our study evaluates the performance of this equation and three commonly used CKD equations in a genetically and socioeconomically diverse cancer population. It also evaluates the utility of a simple tool to improve GFR estimation by adapting the equations' parameters for the patient population.

Methods

The records of consecutive adult cancer patients who had GFR measured as part of routine clinical management were reviewed. Using serum creatinine levels measured within a week of GFR measurement, GFR was estimated (eGFR) using the original Cockcroft-Gault (CG), Modification of Diet in Renal Disease (MDRD), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) and Janowitz-Williams (JW) equations. The population was then randomly divided into equal sized development and validation sets. A Microsoft® Excel add-in (Solver) was used to optimise the equations' parameters using the development set data. The bias, precision, accuracy and agreement of all original and adapted equations was determined using the validation set data. The impact of using eGFR on management decisions was also assessed.

Results

The data of 435 patients were included. Of the original equations, the JW equation was least biased and most precise. The original equations all overestimated GFR by 7.9 to 16.0 ml/min/1.73 m^2. Bias of the adapted equations was significantly lower (-2.2 to 3.4 ml/min/1.73 m^2, $p<0.0001$). The original equations had interquartile ranges (IQRs) of 21.8-30.4 ml/min/1.73 m^2 but adapting the equations reduced the IQRs to 21.2-24.0 ml/min/1.73 m^2. The accuracy, reflected by the P_{30} values, improved from 57.1%-73.7% to 78.3%-82.9% after adapting the equations ($p\leq0.0001$). The 95% confidence intervals (CI) were ± 42.3, 34.4, 32.9 and 33.3 ml/min/1.73 m^2 for the adapted CG, MDRD, CKD-EPI and JW equations respectively. Using eGFR with a CI allows for reliable exclusion of patients with impaired renal function, however eGFR remains equivocal in a significant proportion of patients.

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Conclusions

The JW equation provides the most accurate unadapted estimate. Adapting the equations for this population improved their performance. While reliable to exclude low GFR, when eGFR is normal to high, in the GFR range where most critical clinical decisions are made, GFR estimates are equivocal. In these cases, there is no alternative to GFR measurement.

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Introduction

Nephrotoxicity is a serious adverse effect of certain chemotherapeutic drugs e.g. the platinum-based agents, yet because of their efficacy, many of these drugs are widely used for the treatment and palliation of many types of cancer. Patients receiving potentially nephrotoxic chemotherapy require accurate determination of kidney function both prior to starting and during treatment (Launay-Vacher et al. 2008).

Glomerular filtration rate (GFR) is generally accepted as the best measure of kidney function but its measurement is associated with several real and perceived challenges including being inconvenient for the patient, comparatively expensive, and only available in specialized units. Consequently, many oncologists rely on an estimation of GFR. The most commonly used equations for this purpose are the Cockcroft-Gault (Cockcroft and Gault 1976), the Modification of Diet in Renal Disease (MDRD) (Levey et al. 1999), and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (Levey et al. 2009) equations. These equations were developed from the data of patients with chronic kidney disease (CKD) and healthy individuals, and while the formulae correct for various demographic and/or physiological characteristics (e.g. age, sex, ethnicity, weight), they do not take pathology into account.

Many studies have evaluated the performance of these GFR estimating equations in cancer patients, and although the results have varied, their accuracy was consistently poor with values for the mean/median absolute percentage error (APE) ranging between 10% and 18% (Wright et al. 2001; Poole et al. 2002; Marx et al. 2004; de Lemos et al. 2006; Barraclough et al. 2008a; Ainsworth et al. 2011; Hartlev et al. 2012; Lauritsen et al. 2014; Janowitz et al. 2017). One of the more recent studies found that carboplatin doses would have been acceptable in only one-fifth of patients (Lauritsen et al. 2014). However, CKD derived equations remain the most used in clinical oncology practice.

There are some GFR estimating equations that have been derived from cancer patient data. Three of these were developed prior to the era of routine standardization of serum creatinine assays (Jelliffe 1973; Martin et al. 1998; Wright et al. 2001). The recently described Janowitz-Williams equation was developed and validated in a White population from the United Kingdom (Janowitz et al. 2017). While it achieved improved results, the authors point out the need to validate its performance in oncology patients of different ethnicities. It is well known

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that in non-oncology populations outside Europe, North America and Australia, GFR estimating equations do not perform well, most likely due to genetic differences in muscle mass and diet (Stevens et al. 2006; Earley et al. 2012). It is thus hypothesized that similarly, the Janowitz-Williams equation will perform less well in a cancer population that is ethnically diverse with many patients coming from poor socioeconomic backgrounds.

The aims of this study were (i) to evaluate the performance of the Cockcroft-Gault, MDRD, CKD-EPI and Janowitz-Williams equations in this population, and (ii) to adapt the parameters of the equations using a simple Microsoft® Excel-based tool, and evaluate the performance of the adapted equations.

Methods

Study population

This was a retrospective case review of all adult (age ≥ 18 years) cancer patients who had GFR measured in the Nuclear Medicine Division of Tygerberg Hospital, Cape Town, between January 2011 and June 2016. GFR is measured prior to initiating therapy as part of routine clinical practice in all patients receiving platinum-based chemotherapy, and repeat measurements are performed in cases where there has been a significant increase in serum creatinine (Scr). Cases were included if Scr was measured within 7 days of the GFR study and no treatment was administered between the two measurements. Cases with missing information or methodological errors were excluded, as were cases of patients with documented ascites, pleural effusions, or any other source of expanded extracellular fluid volume. Ethical approval was granted by the institutional Health Research Ethics Committee (reference number S15/05/121).

GFR measurement

GFR was measured from the plasma clearance of ^{99m}Tc-diethylenetriaminepenta-acetic acid (^{99m}Tc-DTPA) following the methodology in the 2004 British Nuclear Medicine Society (BNMS) guideline (Fleming et al. 2004). This is an established and accepted reference method for GFR measurement in clinical practice. Three venous blood samples were taken at 2, 3 and 4 hours after administration of radiotracer. The clearance values obtained using the slope-intercept method (Chantler et al. 1969; Fleming et al. 2004) were corrected for body surface

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area (BSA) using the Haycock formula (Haycock et al. 1978). Thereafter, the mean Bröchner-Mortensen equation was used to correct for the systematic error arising from use of a mono-exponential clearance model (Bröchner-Mortensen 1972; Bröchner-Mortensen et al. 1974; Fleming et al. 2004). This is referred to in the text as measured GFR (mGFR). Various quality assurance checks, as recommended in the BNMS guideline, were performed for all GFR studies (Fleming et al. 2004).

GFR estimation

Serum creatinine was measured by the Tygerberg Hospital National Health Laboratory Service (NHLS) using a Jaffe kinetic alkaline picrate assay with blank rate correction (Siemens Advia 1800, Siemens Healthineers, Erlangen, Germany). The analytical imprecision was 2% at 79 µmol/L, 2.1% at 166 µmol/l and 1.8% at 529 µmol/l. Total error at these levels was 2.4%, 7.2% and 6.7%. This assay is traceable to the reference method, isotope dilution mass spectrometry (IDMS) (Myers et al. 2006). Estimated GFR, denoted eGFR, was estimated using the following equations (**Table 5.1**):

1. The Cockcroft-Gault equation (Cockcroft and Gault 1976)
2. The re-expressed, 4-variable MDRD equation (Levey et al. 2007)
3. The CKD-EPI equation (Levey et al. 2009)
4. The Janowitz-Williams equation (Janowitz et al. 2017)

The MDRD and CKD-EPI equations have additional correction factors that are applied for African American individuals. However, because previous studies in Black South Africans have concluded that the accuracy of the equations is better *without* this correction, and because the population in this study is ethnically diverse, these corrections were omitted (van Deventer et al. 2008; Stevens et al. 2011; Madala et al. 2011).

Data analysis

The bias, precision and accuracy of each equation was calculated based on previous recommendations (Stevens et al. 2007b; Earley et al. 2012). Bias was measured as the median difference between eGFR and mGFR (eGFR-mGFR), expressed on the raw scale and as a percentage of mGFR. Precision was measured using interquartile ranges (IQR) and the root mean square error (RMSE) of the regression of eGFR versus mGFR. Accuracy was expressed as the percentage of eGFR values that were within 30% of mGFR (P_{30}). The median absolute

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percentage error (APE) was calculated as an additional metric for purposes of comparison with previous studies. Bland-Altman plots were used to determine the agreement between mGFR and eGFR (Bland and Altman 1986). The Shapiro-Wilk test was used to test for normality. As the CKD-EPI equation is recommended in current, international, chronic kidney disease guidelines (KDIGO 2013), it was thus regarded as the reference method against which the other equations were compared. The McNemar test was used to compare proportions.

Adapting the equations

The population was then randomly divided into two equal groups, a development and a validation set. Using the development set data, an iterative non-linear least squares regression technique was used to adjust parameters of the equations to better fit the given data. This was performed using a readily available and simple tool (Microsoft® Excel Solver Add-in), which has been described in detail previously (Brown 2001). Using the validation set data, the bias, precision and accuracy of the adapted equations was calculated and compared to that of the original equations. Based on previous recommendations, relative reductions in bias of ≥ 50% or RMSE of ≥ 20% were regarded as significant improvements (Earley et al. 2012). The McNemar test to compare the P_{30} values of the original and adapted estimates.

Impact on patient management

The sensitivities and specificities of the original and adapted equations for identifying individuals with mGFR < 60 ml/min/1.73 m^2 were calculated using (i) point values, and (ii) the 95% confidence intervals for the adapted equations. Replicating the methodology in the Janowitz study (Janowitz et al. 2017), hypothetical carboplatin doses were calculated using the Calvert formula [dose (mg) = target AUC × (GFR + 25)] (Calvert et al. 1989) based on mGFR and the original and adapted equations. The APE in the dose was calculated for each patient. Dose deviations > 20% were regarded as significant.

Statistical analysis was performed using MedCalc Statistical Software for Windows v.18.10.3 (MedCalc Software bvba, Ostend, Belgium; https://www.*medcalc*.org; 2019).

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Results

A total of 435 patients (340 female) with a median age of 53 years (range 22-78 years) met the inclusion criteria. The patient characteristics are summarized in **Table 5.2**. The majority of patients (54%) had a diagnosis of cervical cancer. GFR was normally distributed with a mean (SD) of 81 (24) ml/min/1.73 m^2. Based on the Kidney Disease: Improving Global Outcomes (KDIGO) classification (KDIGO 2013), 155 patients (35.5%) had a normal GFR, in 203 (47%) GFR was mildly decreased, and in 77 (17.5%) it was moderately to severely decreased. The mean body mass index was 25 kg/m^2 with 45% classifying as overweight or obese (WHO 2018). HIV status and ethnicity were not consistently recorded.

The bias, precision, accuracy and agreement of the 4 original equations are summarized in **Table 5.3**. All equations overestimated GFR with median bias ranging between 7.7 ml/min/1.73 m^2 (Janowitz-Williams) and 16.5 ml/min/1.73 m^2 (Cockcroft-Gault). The biases of the CKD-EPI and MDRD equations were similar (12.6 and 13.1 ml/min/1.73 m^2 respectively). The Janowitz-Williams equation was the most precise with a RMSE value of 13.0 ml/min/1.73 m^2 and IQR of 21.4 ml/min/1.73 m^2. Accuracy of the Cockcroft-Gault equation was poor (< 60%), whereas for the other 3 equations it was moderate (60-80%). The P_{30} value of the Janowitz-Williams equation (77.0%) was significantly higher than all other equations ($p<0.0001$), and the P_{30} of the CKD-EPI equation (69.7%) was significantly higher than both Cockcroft-Gault (58.4%; $p=0.0008$) and MDRD equations (64.4%; $p=0.0165$). Median APE ranged from 15.3% (Janowitz-Williams) to 24.4% (Cockcroft-Gault).

The formulae for the adapted equations are listed in **Table 5.4** with the original equations alongside for comparison. In all four adapted equations bias was reduced by > 50%, with absolute values ranging between -2.2 and 3.4 ml/min/1.73 m^2. Reductions in RMSE of > 20% were found for the Cockcroft-Gault, MDRD and CKD-EPI equations. P_{30} values of the adapted equations ranged between 78.3% and 82.9%; the values all significantly higher than the original equations ($p=0.0001$ for Janowitz-Williams; $p<0.0001$ for the others) (**Table 5.4** and **Fig. 5.1**). There was no significant difference in the P_{30} values of the different adapted equations.

Using point values from eGFR equations the specificities for identifying patients with mGFR < 60 ml/min/1.73 m^2 were 94%-98%. However, the sensitivities ranged between 31% and 50%, lowest for the Janowitz-Williams equation, and highest for the MDRD. Adapting the equations did not improve the sensitivity to above 50% except in the case of the Cockcroft-Gault formula

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where it increased to 64%. The 95% predictive confidence intervals (CI) were ± 42.3, 34.4, 32.9 and 33.3 ml/min/1.73 m^2 for the adapted Cockcroft-Gault, MDRD, CKD-EPI and Janowitz-Williams equations respectively (**Fig. 5.2**). Only when eGFR exceeded approximately 90 ml/min/1.73 m^2 did the 95% confidence intervals not include the value of 60 ml/min/1.73 m^2. This was true for 41-61/217 validation set cases depending on the equation, with only 1-3 of these 41-61 cases having mGFR values below 60 ml/min/1.73 m^2. Overall, applying this threshold to the validation set for excluding patients with low GFR yielded sensitivities of 93%-98%, with specificities of 21%-27%. The APE in hypothetical carboplatin doses (AUC5) was > 20% in 60-99 (28-46%) for the original equations, lowest using the Janowitz-Williams equation, and in 45-57 (21-26%) for the adapted equations.

Discussion

In this population the CKD-based equations (Cockcroft-Gault, MDRD and CKD-EPI) all overestimated mGFR substantially with biases ranging between 12.6 and 16.5 ml/min/1.73 m^2. The precision was also poor with RMSE values of 16.4 to 26.8 ml/min/1.73 m^2 and IQRs of 23.5 to 31.8 ml/min/1.73 m^2. Despite being significantly more accurate than the Cockcroft-Gault and MDRD, the CKD-EPI equation had a modest P_{30} value of 69.7% meaning that almost one-third of patients had eGFR values that differed from the true GFR by more than 30%. The new Janowitz-Williams equation performed better with median bias of 7.7 ml/min/1.73 m^2, RMSE of 13.0 ml/min/1.73 m^2, IQR of 21.4 ml/min/1.73 m^2, and a significantly higher P_{30} value of 77.0%.

The systematic overestimation of GFR implies lower serum creatinine levels on average in this population, which is not surprising. Cancer and other chronic illnesses frequently result in muscle wasting leading to a decrease in the serum creatinine concentration (Stevens et al. 2006). Nausea or poor appetite, common symptoms in cancer patients, may perpetuate this by reducing a patient's dietary intake of protein. It is important to note that neither the MDRD nor the CKD-EPI development set included oncology patients and that these equations were never intended to be used in cancer patients. They were developed in predominantly chronic kidney disease (CKD) populations and perform satisfactorily in other populations with similar pathology. (Levey et al. 1999; Levey et al. 2009; Earley et al. 2012).

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The lower bias of the Janowitz-Williams equation is likely to be related to its derivation from oncology patient data, yet with a bias of 7.7 ml/min/1.73 m^2, it still overestimated GFR. Differences in genetics and nutrition are most likely to account for these systematic differences in GFR between the two populations. This is supported by the finding in the Janowitz study of negligible bias (< 1 ml/min/1.73 m^2 and < 1%) for the CKD-EPI equation (Janowitz et al. 2017). In that study the equations also showed greater precision. In their internal validation set the Janowitz-Williams equation had an IQR of 17.7 ml/min whereas in this study population it was 21.1 ml/min. Similarly, the CKD-EPI equation had an IQR of 19.3 ml/min/1.73 m^2 compared to 23.5 ml/min/1.73m^2 in this study. The poorer precision in our population is thought to be due to its greater diversity.

The poor performance of the unaltered Cockcroft-Gault equation is not unexpected. It was originally developed from data limited to men, used non-standardized serum creatinine measurements, and as an estimate of creatinine clearance, it systematically overestimates GFR due to tubular secretion of creatinine (Cockcroft and Gault 1976; Stevens et al. 2006). Yet it remains the most frequently used equation in our and many other oncology departments and was thus evaluated in this study. However, even after adapting its parameters, its accuracy was not significantly better than that of the unaltered Janowitz-Williams equation.

Many factors influence the performance of GFR estimating equations. In some populations the effect of ethnicity is well documented e.g. African Americans in whom eGFR without correction for ethnicity underestimates mGFR (Levey et al. 1999; Levey et al. 2017), and Japanese in whom eGFR gives an overestimation (Matsuo et al. 2009). In other populations, particularly those of mixed ancestry, the effect of ethnicity is largely unknown. The mean GFR of the population studied will also influence the performance of creatinine-based estimations, particularly the MDRD equation (Stevens et al. 2010), as may obesity (Verhave et al. 2005). Diet, whether due to cultural or socioeconomic factors, is known to influence eGFR through its effect on the serum creatinine concentration (Perrone et al. 1992; Stevens et al. 2006). In our population 78% were female, 62% of the total population had a gynecological cancer, 45% were either overweight or obese by WHO definitions (WHO 2018), the mean GFR was 81 ml/min/1.73 m^2, and the majority fell into a low-income group. The patients were ethnically diverse comprising Black African, White and mixed ancestry individuals. Given the numerous factors potentially affecting GFR estimating equations, these should ideally be adapted for the populations in which they will be used.

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The Microsoft® Excel Solver add-in is a simple, readily available tool that uses an iterative, non-linear, least squares regression technique to adapt parameters to better fit multiple variables to development data (Brown 2001). Provided a centre can measure both GFR and serum creatinine, it can adapt equations for its own patient population in-house relatively easily with the assistance of a medical physicist. Testing the adapted equations (**Table 5.4**) using independent internal validation set data showed significant improvements in bias and accuracy of all the equations, and significant improvements in precision in 3/4. Bias was close to zero for all equations, RMSE values ranged from 12.2 to 20.0 ml/min/1.73 m^2, and the P_{30} values were 78.3% to 82.9%. Various mathematical models (functions) exist to predict GFR from Scr, with most showing similar performance once the parameters are adapted to a specific patient population.

When using point values for directing management decisions, none of the equations performed well. For detecting patients with mGFR < 60 ml/min/1.73 m^2, while specificity was good, the sensitivity of all the equations was < 50%. Unfortunately, this is the GFR level at or around which critical treatment decisions are made. Using point values will therefore fail to detect most patients with reduced renal function, creating a false security for clinicians. Thus, using eGFR point values to avoid cisplatin therapy in patients with poor renal function is expected to result in inappropriate therapy in more than half of these patients. Extrapolating from these data, using the Calvert formula (AUC5) (Calvert et al. 1989) with eGFR point values can be predicted to result in inappropriate carboplatin doses in 46-57/217 (21-26%) of patients.

Using 95% confidence intervals with eGFR yields some improvement in clinical value. The main utility would be in cases of normal/high eGFR (eGFR > ~90 ml/min/1.73 m^2) where a low GFR (false positive) can be reliably excluded when the confidence intervals do not include the value of 60 ml/min/1.73 m^2. Using this approach, a clinical decision to proceed with cisplatin therapy based on adequate GFR can be safely made in about 1/3 of patients in this study population using eGFR alone. However, when the confidence interval includes a clinical threshold e.g. 60 ml/min/1.73 m^2, reliable determination of GFR requires measurement. The lack of universal availability of reliable GFR measurement, even in the developed world, is a challenge that should be addressed by the oncology community globally, as these measurements are not overly sophisticated, especially with the adoption of new simpler single sample techniques (Burniston 2018).

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A limitation of this study is that it was based on a specific local cancer population, and it cannot be assumed that its findings can be generalized to cancer populations elsewhere in the world. The intention of this study is however to illustrate that estimation equations can be adapted locally without high levels of expertise. Furthermore, the number of patients with mGFR < 60 ml/min/1.73 m^2 was relatively small. While this accurately reflects the distribution of GFR in our cancer population, it is at this level that critical therapeutic decisions are made. Further studies focusing on eGFR in patients with mild to moderately decreased GFR (GFR 45-90 ml/min/1.73 m^2) are required. As a retrospective study there were time lapses between serum creatinine and mGFR measurements, which will have reduced the precision of the equations, but this probably reflects normal practice where serum creatinine is measured before chemotherapy is due to start. However, it is possible that there were a few patients who received nephrotoxic chemotherapy between serum creatinine and GFR measurements without this being detected during the record review.

The authors elected not to evaluate other equations developed for oncology patients, specifically the Jelliffe (Jelliffe 1973), Martin (Martin et al. 1998) and Wright (Wright et al. 2001) equations as they are used infrequently having been developed prior to routine standardization of serum creatinine assays. In addition, many previous studies that have evaluated existing GFR estimating equations in cancer patients exist (Wright et al. 2001; Poole et al. 2002; Marx et al. 2004; de Lemos et al. 2006; Barraclough et al. 2008; Ainsworth et al. 2011; Hartlev et al. 2012; Lauritsen et al. 2014). However, due to significant differences in methodology to measure GFR, compounded by non-standardization of creatinine assays in the majority, these studies were not compared. Of the numerous equations that have been developed for CKD populations, only the MDRD and CKD-EPI were evaluated: the MDRD equation as our hospital laboratory service routinely provides an MDRD estimate alongside every serum creatinine result, and the CKD-EPI equation as it is recommended in local and international CKD guidelines (KDIGO 2013; SARS 2015).

In conclusion, most GFR estimation equations that were developed in other patient populations are suboptimal for use in cancer patients. Adapting these equations for use in a specific population of oncology patients is practical and preferable as it improves their performance. For centers unable to adapt published equations, the new Janowitz-Williams equation with a confidence interval is preferred in oncology patients. However, to ensure correct treatment, in a significant proportion of patients GFR measurement is unavoidable.

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Conflicts of interest

None

Author contributions

JL Holness: data analysis, manuscript preparation

HM Simonds: manuscript review

P Barnardt: manuscript review

JM Warwick: supervisor, manuscript review

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Tables and figures

Table 5.1 Equations evaluated in this study

Cockcroft-Gault (Cockcroft and Gault 1976)	eGFR (ml/min) = (140 – age) x wt/72 x Scr x g where Scr is serum creatinine in mg/dL, age is in years, wt is weight in kilograms, and g is a gender factor (male=1, female=0.85). The value obtained was corrected for BSA by multiplying by 1.73/BSA
MDRD equation (Levey et al. 2007)	eGFR (ml/min/1.73 m^2) = 175 x $Scr^{-1.154}$ x $age^{-0.203}$ x g where Scr is serum creatinine in mg/dL, g is a gender factor (male=1, female=0.742)
CKD-EPI (Levey et al. 2009)	eGFR (ml/min/1.73 m^2) = 141 x $\min(Scr/\kappa,1)^{\alpha}$ x $\max(Scr/\kappa,1)^{-1.209}$ x 0.993^{age} x g where Scr is serum creatinine in mg/dL, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min(x,y) and max(x,y) respectively indicate the minimum and maximum of x and y, and g is a gender factor (male=1, female=1.108)
Janowitz-Williams (Janowitz et al. 2017)	$\sqrt{}$GFR (ml/min) = $\beta_0 + \beta_1 age + \beta_2 BSA + \beta_3 \ln(Scr) + \beta_4 \ln(Scr)^2 + \beta_5 \ln(Scr)^3 + [(\beta_6 + \beta_7) \times g] + (\beta_8 age \times BSA)$ where Scr is serum creatinine in mg/dL, BSA is body surface area, age is in years, β_0 = 1.8140, β_1 = 0.0191, β_2 = 4.7328, β_3 = -3.7162, β_4 = -0.9142, β_5 = 1.0628, β_6 =0.0202, β_7 = 0.0125, β_8 = -0.0297, g is a gender factor (male=1, female=0). The value obtained was squared and then corrected for BSA by multiplying by 1.73/BSA

Abbreviations: **eGFR**, estimated glomerular filtration rate; **BSA**, body surface area

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Table 5.2 Patient characteristics (n=435)

Characteristic	**Category**	***n* (%)**
Gender	Male	95 (22)
	Female	340 (78)
Cancer diagnosis	Gynaecological	269 (62)
	Lung	53 (12)
	Gastrointestinal	48 (11)
	Head and neck	21 (5)
	Urological	18 (4)
	Neuroendocrine	17 (4)
	Other	9 (2)
GFR category	> 90 ml/min/1.73m^2	155 (35.5)
	60-89 ml/min/1.73m^2	203 (47)
	30-59 ml/min/1.73m^2	70 (16)
	15-29 ml/min/1.73m^2	7 (1.5)
	< 15 ml/min/1.73m^2	0

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Table 5.3 Bias, precision, accuracy, and agreement of the equations

	Cockcroft-Gault	**MDRD**	**CKD-EPI**	**Janowitz-Williams**
Bias				
Median difference* (95% CI) (ml/min/1.73 m^2)	16.5 (13.6 to 19.4)	13.1 (11.1 to 16.2)	12.6 (10.0 to 15.0)	7.7 (6.3 to 9.7)
MPE* (95% CI)	20.0 (17.7 to 25.1)	17.4 (14.5 to 19.8)	16.9 (13.0 to 20.0)	10.8 (7.8 to 13.5)
Precision				
IQR of the differences* (ml/min/1.73 m^2)	31.8	28.3	23.5	21.4
RMSE† (ml/min/1.73 m^2)	26.8	27.0	16.4	13.0
Accuracy				
P_{30} % (95% CI)	58.4 (53.6 to 63.1) ‡	64.4 (59.7 to 68.9) §	69.7 (65.1 to 74.0)	77.0 (72.8 to 80.9) ‡
MAPE (%) (95% CI)	24.4 (19.7 to 26.2)	20.7 (19.2 to 23.6)	18.6 (16.6 to 21.6)	15.3 (13.8 to 17.2)
Agreement				
95% LOA* (ml/min/1.73 m^2)	-32.8; -72.2	-36.6; -69.1	-22.5; 48.1	-26.0; 40.5

*The errors are calculated as eGFR – mGFR. †RMSE of the regression of eGFR vs. mGFR. ‡P_{30} value significantly different to the P_{30} of the CKD-EPI equation.

Abbreviations: **MDRD**, Modification of diet in Renal Disease; **CKD-EPI**, Chronic Kidney Disease Epidemiology Collaboration; **MPE**, median percentage error; **IQR**, interquartile range; **RMSE**, root mean square error; **95% CI**, 95% confidence interval; **LOA**, limits of agreement.

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Table 5.4 Comparison of the original and adapted equations (n=217)

	Equation	Median difference (95% CI) ml/min/1.73 m^2	Δ bias	RMSE ml/min/1.73 m^2	Δ RMSE	IQR of differences* ml/min/1.73 m^2	P_{30}	*p*
Original Cockcroft-Gault	eGFR = (140-age) x wt/(72 x Scr) x g x 1.73/BSA where g=1 (male) or 0.85 (female)	16.0 (12.5 to 21.2)	-86%	27.1	-26.2%	30.4	57.1%	<0.0001
Adapted Cockcroft-Gault	eGFR = (188-age) x wt/(129 x Scr) x g x 1.73/BSA where g=1 (male) or 0.75 (female)	-2.2 (-5.1 to 0.9)		20.0		23.5	78.3%	
Original MDRD	eGFR = 175 x $Scr^{-1.154}$ x $age^{-0.203}$ x g where g=1 (male) or 0.742 (female)	14.7 (11.1 to 18.0)	-77%	26.7	-54.3%	28.2	62.7%	<0.0001
Adapted MDRD	eGFR = 274 x $Scr^{-0.533}$ x $age^{-0.33}$ x g where g=1 (male) or 0.89 (female)	3.4 (1.3 to 5.6)		12.2		24.0	80.6%	
Original CKD-EPI	eGFR = 141 x $\min(Scr/\kappa,1)^{\alpha}$ x $\max(Scr/\kappa,1)^{-1.209}$ x 0.993^{age} x g where g, κ, α = 1, 0.9, -0.411 (male) or 1.108, 0.7, -0.329 (female)	13.3 (10.3 to 16.8)	-84%	17.2	-20.3%	22.9	67.3%	<0.0001
Adapted CKD-EPI	eGFR = 108 x $\min(Scr/\kappa,1)^{\alpha}$ x $\max(Scr/\kappa,1)^{-1.365}$ x 0.993^{age} x g where g, κ, α = 1, 1, -0.607 (male) or 1.106, 0.8, -0.318 (female)	2.1 (0.3 to 4.5)		13.7		21.9	80.6%	
Original Janowitz-Williams	eGFR = $\{\beta_0 + \beta_1 age + \beta_2 BSA + \beta_3 \ln(Scr) + \beta_4 \ln(Scr)^2 + \beta_5 \ln(Scr)^3 + [(\beta_6 + \beta_7) \times g] + (\beta_8 age \times BSA)\}^2 \times 1.73/BSA$ where β_0 = 1.8140, β_1 = 0.0191, β_2 = 4.7328, β_3 = -3.7162 β_4 = -0.9142, β_5 = 1.0628, β_6 =0.0202, β_7 = 0.0125, β_8 = -0.0297, g =1 (male) or 0 (female)	7.9 (6.3 to 10.4)	-77%	13.8	- 9.4%	21.8	73.7%	0.0001
Adapted Janowitz-Williams	eGFR = $\{\beta_0 + \beta_1 age + \beta_2 BSA + \beta_3 \ln(Scr) + \beta_4 \ln(Scr)^2 + \beta_5 \ln(Scr)^3 + [(\beta_6 + \beta_7) \times g] + (\beta_8 age \times BSA)\}^2 \times 1.73/BSA$ where β_0 = 3.8309, β_1 = 0.0027, β_2 = 3.1063, β_3 = -3.3135 β_4 = -0.7315, β_5 = 0.8270, β_6 =0.9616, β_7 = -0.0157, β_8 = -0.0142, g =1 (male) or 0 (female)	1.8 (-1.7 to 4.7)		12.5		21.2	82.9%	

Scr is serum creatinine in mg/dL. The differences are calculated as eGFR – mGFR. RMSE is calculated from the regression of eGFR vs. mGFR.

Abbreviations: **BSA**, body surface area; **eGFR**, estimated glomerular filtration rate; **mGFR**, measured glomerular filtration rate; **MDRD**, Modification of Diet in Renal Disease; **CKD-EPI**, Chronic Kidney Disease Epidemiology Collaboration; **CI**, confidence interval; **RMSE**, root mean square error; **IQR**, interquartile range.

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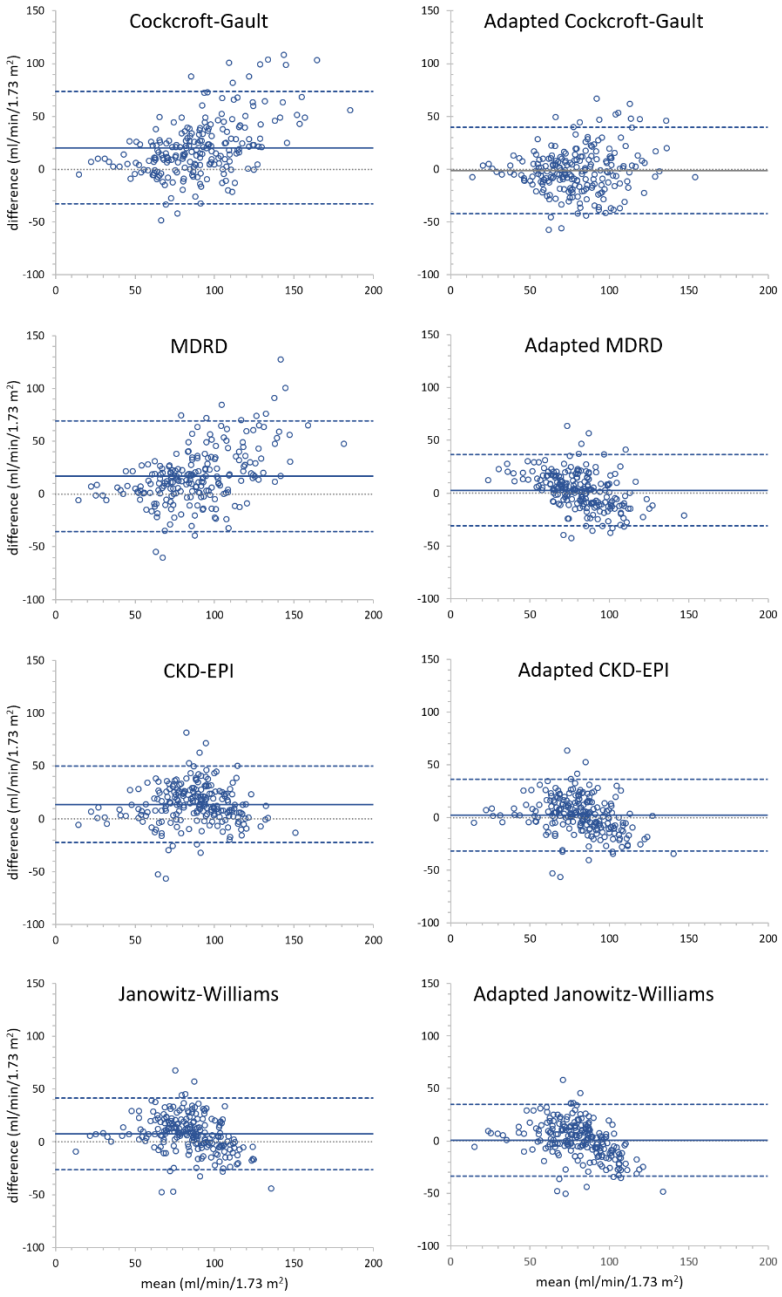
Fig. 5.1



Bland-Altman plots of the original (1st column) and adapted (2nd column) equations. The differences are calculated as eGFR-mGFR, and the mean is the average of eGFR and mGFR. The solid line represents the mean difference, the dashed lines represent the 95% upper and lower limits of agreement, and the dotted grey line is the zero line.

Abbreviations: **eGFR**, estimated glomerular filtration rate; **mGFR**, measured glomerular filtration rate; **MDRD**, Modification of diet in Renal Disease; **CKD-EPI**, Chronic Kidney Disease Epidemiology Collaboration.

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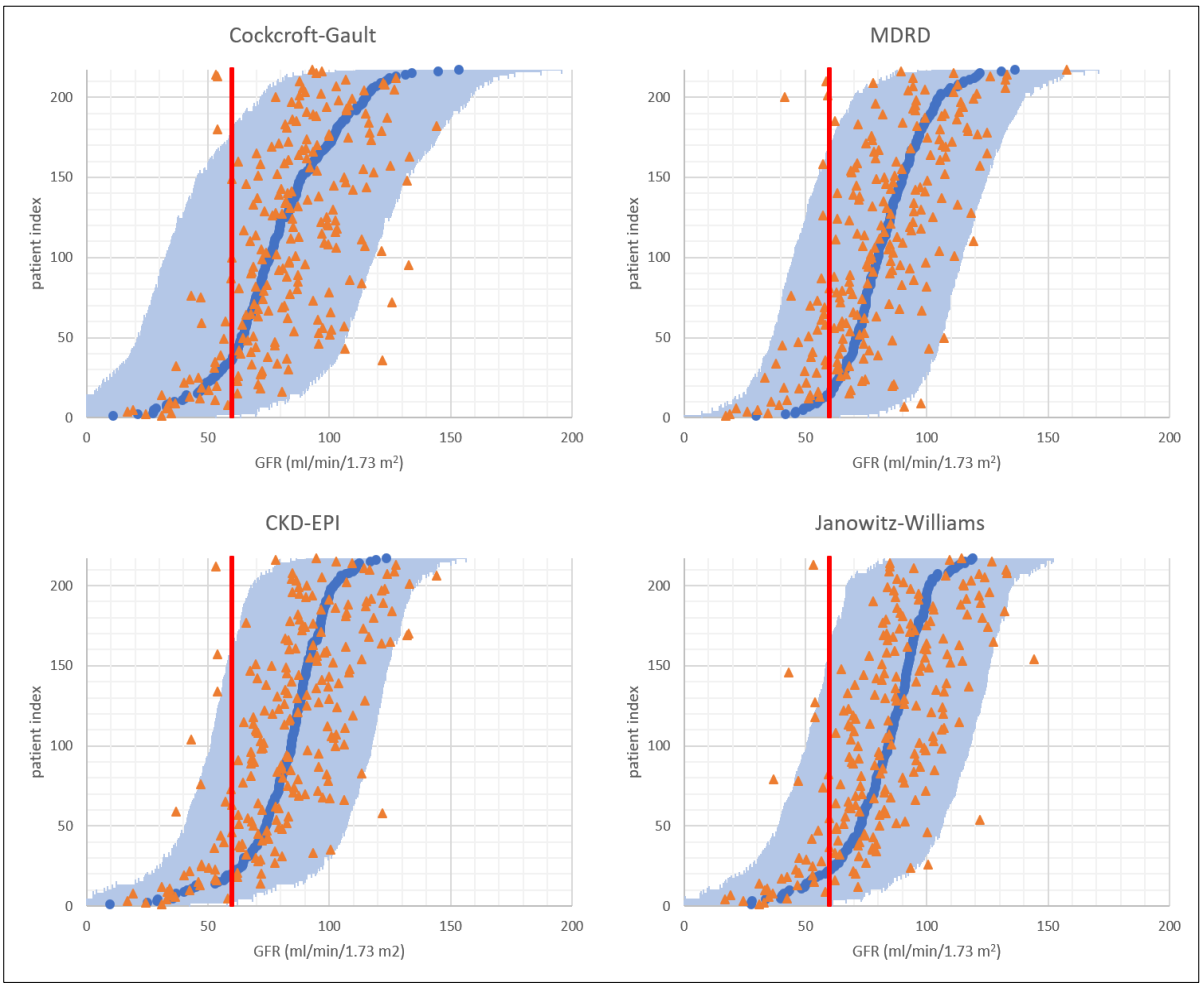
Fig. 5.2



Predictive confidence intervals for the four adapted equations for each patient in the validation set. The patients in the validation set are ordered by eGFR. The overlapping blue points represent eGFR and the orange triangles mGFR. The light blue horizontal lines represent the 95% confidence interval for eGFR for each patient. The solid red vertical lines represent the threshold of 60 ml/min/1.73 m^2, the level at or around which critical treatment decisions are made.

Abbreviations: **GFR**, glomerular filtration rate; **eGFR**, estimated glomerular filtration rate; **mGFR**, measured glomerular filtration rate; **MDRD**, Modification of diet in Renal Disease; **CKD-EPI**, Chronic Kidney Disease Epidemiology Collaboration.

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6. Estimated glomerular filtration rate in children: evaluating and adapting existing equations for a specific population

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Abstract

Background

Numerous creatinine-based equations have been developed for estimating glomerular filtration rate (GFR) in children. However, these equations frequently do not perform as well in other populations that differ in terms of mean GFR, age, pathology, ethnicity and diet. After first evaluating the performance of existing GFR estimating equations in non-cancer and cancer populations, the aim of this study was to demonstrate the utility of an in-house modification of the equations to better fit a specific population.

Methods

A total of 256 GFR measurements (mGFR) were performed in 160 children using the plasma clearance of ^{51}Cr-ethylenediaminetetra-acetic acid (^{51}Cr-EDTA). Same-day GFR estimations (eGFR) were performed using 11 creatinine-based equations. The agreement of eGFR and mGFR, and the bias, precision and accuracy of each equation was determined for non-cancer and cancer groups independently. The groups were then divided into development and validation sets. Using the development set data, the Microsoft® Excel SOLVER add-in was used to modify the parameters of 4 equations to better fit the data. Using the validation set data, the performance of the original and modified equations was compared.

Results

In the non-cancer group, bias was 3.4-22.6 ml/min/1.73 m^2. The root mean square error (RMSE) was 14.1-30.5 ml/min/1.73 m^2, and the P_{30} values were 48.4-76.1%. In the cancer group, bias was 16.8-46.0 ml/min/1.73 m^2, RMSE was 13.4-39.5 ml/min/1.73 m^2, and the P_{30} values were 20.6-67.0%. The Chronic Kidney Disease in Children Study (CKiD) univariate equation was the most accurate equation in both groups. Modifying the equations parameters resulted in significant improvements in bias and good accuracy (P_{30} > 80%) in 3/4 of the non-cancer group. All 4 of the adapted cancer equations demonstrated significant improvements in bias and RMSE, with good accuracy in 3/4.

Conclusions

Existing paediatric GFR estimating equations performed poorly in both non-cancer and cancer populations. However, modifying the equations' parameters using a simple Excel-based tool significantly improved their accuracy.

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Introduction

In centres where glomerular filtration rate (GFR) cannot be routinely measured, whether due to non-availability, limited capacity or cost, it is frequently estimated from the serum creatinine concentration. Numerous creatinine-based equations have been developed for use in children. The majority were developed in populations with chronic kidney disease (CKD), with just a few developed specifically for cancer populations. However, the performance of the equations in subsequent validation studies has been shown to vary widely (Counahan et al. 1976; Léger et al. 2002; Mattman et al. 2006; Biörk et al. 2007; Schwartz et al. 2009; Pottel et al. 2010; Staples et al. 2010; Bacchetta et al. 2011; Gao et al. 2012; Pottel et al. 2012; Schwartz et al. 2012; Selistre et al. 2012; De Souza et al. 2012; Blufpand et al. 2013; Hoste et al. 2013; Uemura et al. 2014; Deng et al. 2015; Pottel et al. 2016). For example, in CKD populations, values for bias of the new bedside Schwartz formula (Schwartz et al. 2009) have ranged between -15 ml/min/1.73 m^2 (underestimation) and 22 ml/min/1.73 m^2 (overestimation), and accuracy, reflected by P_{30} values (percentage of estimates within 30% of measured GFR), has ranged from good (~90%) to poor (~50%) (Pottel et al. 2010; Staples et al. 2010; Bacchetta et al. 2011; Gao et al. 2012; Lee et al. 2012; Pottel et al. 2012; Selistre et al. 2012; De Souza et al. 2012; Blufpand et al. 2013; Hoste et al. 2013; Uemura et al. 2014; Pottel et al. 2016). There is less data on the performance of estimated GFR (eGFR) in children with cancer, but in general, the accuracy has been lower (Millisor et al. 2017; Jeong et al. 2018; Llanos-Paez et al. 2018).

The large discrepancies in estimating equation performance are likely to be due to systematic differences in age, kidney function, pathology, genetics, and nutrition between the validation population and the population in which the equation was originally developed. The accuracy of eGFR in children has been shown to be dependent on both age and GFR (Blufpand et al. 2013; Hoste et al. 2013). Serum creatinine levels are known to be lower in individuals with low-meat or vegetarian diets (Perrone et al. 1992). Ethnicity may also result in differences in serum creatinine levels as this has been shown in certain adult populations (Stevens et al. 2006; Levey et al. 2009). Ideally, before using estimated GFR in clinical practice, the equations should be validated in the population in which they will be used. If no equation with acceptable accuracy exists, the only remaining alternatives are to measure GFR in *all* cases or to develop a new equation for that population.

The development of new estimating equations typically involves the analysis of large data series in extensive and costly projects, which is impractical for most paediatric populations,

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especially outside of the developed world. However, in centres with even limited access to GFR measurement, it may be possible to *adapt* existing equation parameters for use in a specific population. This may be possible using a relatively simple and widely available tool that requires limited expertise.

The aims of this study were (i) to evaluate existing GFR estimation equations in diverse, non-cancer and cancer paediatric populations, and (ii) to evaluate the adaptation of equations using a simple spreadsheet-based tool.

Methods

Participants

Children were recruited both prospectively and retrospectively. In the prospective group (September 2015 to May 2018), children (<18 years) meeting the following inclusion criteria were invited to participate: (i) GFR measured in the Nuclear Medicine Department of the Red Cross War Memorial Children's Hospital, Cape Town, South Africa, (ii) assessed by the attending Nuclear Physician to be clinically stable without expanded third spaces (e.g. oedema, ascites), and (iii) informed consent signed by a parent or guardian. Prospectively recruited cases were excluded if insufficient blood was obtained for serum creatinine measurement. In the retrospective group (January 2011 to August 2015), all consecutive studies in children who had GFR and serum creatinine measured on the same day were included. In both groups additional cases were excluded if the child or his/her parents were not South African, if information in the child's records was missing or inconsistent, or if the GFR measurement failed routine quality assurance checks.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Health Research Ethics Committee of Stellenbosch University (reference S14/10/217), the Human Research Ethics Committee of the University of Cape Town (reference 184/2015), and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from the parents or guardians of all individual participants included in the study's prospective group. In addition, assent was obtained from children of appropriate age and understanding. For the group of children recruited retrospectively, formal consent was not required.

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GFR and serum creatinine measurement

GFR was measured following departmental protocol that is based on the 2001 European Association of Nuclear Medicine (EANM) guideline for GFR determination in children (Piepsz et al. 2001). There was no change in protocol during the study period. The children's heights and weights were measured, and body surface area (BSA) was calculated using the DuBois and DuBois formula (Du Bois and Du Bois 1989). The dose of ^{51}Cr-ethylenediaminetetraacetic acid (^{51}Cr-EDTA), calculated as 2.8 x BSA (MBq), was administered intravenously. Venous blood samples were taken from the contralateral arm at 2 h and 4 h after administration. The plasma clearance was calculated using the slope-intercept method (Chantler et al. 1969). This value was adjusted for BSA, and the Jodal Bröchner-Mortensen (Jodal and Bröchner-Mortensen 2009) correction was applied. The measured GFR value is referred to in the text as mGFR. Serum creatinine (Scr) was measured using an enzymatic method (Beckman Coulter AU480 analyzer) with calibration that is isotope dilution mass spectrometry (IDMS) traceable (Myers et al. 2006).

Evaluation of existing equations

GFR was estimated using 11 paediatric, creatinine-based equations (**Table 6.1**). Equations that were developed prior to IDMS-standardization of serum creatinine assays were not evaluated. The estimated GFR value is referred to in the text as eGFR. Previous studies point to the need to develop separate estimation equations for patients with cancer (Millisor et al. 2017; Llanos-Paez et al. 2018). As all evaluated equations were developed almost exclusively from chronic kidney disease (CKD) populations, the children in the current study were divided into cancer and non-cancer groups. Bland-Altman analyses were used to determine the agreement between eGFR and mGFR. The performance of the equations in each group was quantified in terms of bias, precision and accuracy, calculated based on previous recommendations (Stevens et al. 2007b; Earley et al. 2012). Bias was measured as the median difference between eGFR and mGFR (eGFR – mGFR), expressed on the raw scale (ml/min/1.73 m^2). As a measure of precision, the root mean square error (RMSE) of the regression of eGFR vs. mGFR was calculated. Accuracy was expressed as the percentage of eGFR values that fell within 30% of mGFR (P_{30}).

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Modification of the equations

In the second part of this study, the cancer and non-cancer populations were randomly divided into equally sized development and validation sets. A spreadsheet-based tool applying an iterative least squares technique (Excel Solver add-in, Microsoft® Corporation, Albuquerque, New Mexico, United States) was used to adjust parameters of three of the equations to optimize the fit of the development set data, following methodology described previously (Brown 2001). Six of the equations were not adapted as these require normalization of the children's serum creatinine concentrations to the median or average, age-matched, serum creatinine concentrations in their populations. These values are denoted *Q-values* in the simple-height independent (SHI) (Pottel et al. 2012), Q (age), Q (height) (Hoste et al. 2013), FAS (age) and FAS (height) (Pottel et al. 2016) equations, and *reference serum creatinine* in the Japanese equation (Uemura et al. 2014). These values are population-specific and are not available for our patient population. The Schwartz-Lyon variation of the Schwartz equation (De Souza et al. 2012) was also not assessed due to the paucity of males ≥ 13 years.

The performance of each modified equation was evaluated and compared to the original using the validation set data. Cancer and non-cancer groups were analyzed separately. The changes in bias (Δbias) and RMSE (ΔRMSE) were calculated, and Bland-Altman analyses were performed to compare the agreement of the original and calibrated equations.

Statistical analysis was performed using MedCalc Statistical Software for Windows v.19 (MedCalc Software bvba, Ostend, Belgium; https://www.*medcalc*.org; 2019). Normality was tested using the Shapiro-Wilk test. The McNemar and chi-squared tests were used to compare proportions within groups and between groups respectively.

Results

A total of 256 GFR measurements in 160 children were included (median age 8.9 years; age range 0.1-17.9 years). The diagnosis was cancer in 97 (37.9%) and non-cancer conditions in the remaining 159: CKD in 63 (24.6%), vasculitis (Takayasu's arteritis or polyarteritis nodosa) in 52 (20.3%), and liver transplant in 44 (17.2%). Mean (SD) GFR was 88.9 (29.9) ml/min/1.73 m^2. The characteristics of the children are summarized in **Table 6.2**. One hundred and fifty-five (61%) children were recruited prospectively.

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In the non-cancer group, median bias ranged between 3.4 ml/min/1.73 m^2 for the Chronic Kidney Disease in Children Study univariate equation (CKiD) and 22.6 ml/min/1.73 m^2 for the Q(age) equation (Table 6.3). RMSE ranged between 14.1 (Gao's quadratic formula (QF)) and 30.5 (Q(age) equation) ml/min/1.73 m^2. The P_{30} values ranged between 76.1% (CKiD) and 48.4% (Q(age) equation) (**Fig. 6.1**). The P_{30} of the CKiD equation was not significantly different to the Japanese equation P_{30} (p=0.21) but was significantly higher than all the other equations. The 95% limits of the Bland-Altman analyses were narrowest for Gao's QF and widest using the Q(age) equation.

In the cancer group, bias of every equation was systematically greater. Median bias ranged between 16.8 ml/min/1.73 m^2 (CKiD) and 46.0 ml/min/1.73 m^2 (Schwartz) (Table 6.3). Similarly, RMSE increased in the cancer group for all equations with the single exception of Gao's QF. With a RMSE of 13.4 ml/min/1.73 m^2, Gao's QF was most precise, and the Q(height) the least precise (RMSE = 39.5 ml/min/1.73 m^2). Only the CKiD equation and Gao's QF had P_{30} values > 50% (67.0% and 62.9% respectively) (**Fig. 6.1**). There was no significant difference between the two (p=0.5), but the CKiD was significantly more accurate than the other equations. The P_{30} values of the CKiD and Gao's QF equations were also not different for cancer patients compared to non-cancer patients (p=0.1138 and p=0.7590 respectively). In the remaining 9/11 equations the P_{30} values were significantly lower in the cancer group compared to the non-cancer group (p-values <0.0001 to 0.0008).

For modification of the equations, the development and validation sets respectively included 79 and 80 cases (non-cancer), and 48 and 49 cases (cancer). The optimized equations are listed in Table 6.4. On testing with validation set data, in both non-cancer and cancer groups there was a reduction in bias of > 50% in all cases, with the exception of the CKiD in non-cancer patients which showed a reduction of only 5% (Table 6.5). None of the non-cancer group showed reductions in RMSE that exceeded 20%. However, the RMSE decreased by >20% in all four cancer-adapted equations, with the greatest reduction in the refitted CKiD equation (38%). The P_{30} values of the refitted non-cancer equations ranged from 70.0% to 83.8%, in comparison to 53.8% to 71.3% for the original equations, significantly improving for the Schwartz formula (p<0.0001), Flanders metadata equation (p=0.002) and Gao's QF (p=0.019). The P_{30} values of the refitted cancer equations increased from 14.3%-67.3% to 77.6%-89.8%, significantly improving for the Schwartz formula (p<0.0001), Flanders metadata equation (p<00001), and Gao's QF (p=0.003) (**Fig. 6.2**). Bland-Altman plots

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illustrate the difference between the original and adapted versions of the 4 equations (**Fig. 6.3**). The 95% confidence intervals (CI) in ml/min/1.73 m^2 for the adapted non-cancer equations were: Schwartz ± 44.7, Flanders metadata ± 42.4, CKiD ± 41.7 and Gao's QF ± 36.3. For the adapted cancer equations, the 95% CIs were Schwartz ± 57.8, Flanders metadata ± 57.8, CKiD ± 41.5 and Gao's QF ± 41.5.

The adapted Flanders metadata equation had the highest P_{30} in the non-cancer group (83.8%) and the adapted Schwartz formula had the highest P_{30} in the cancer group (89.8%). When compared to the original CKiD equation, the best performing original equation in both groups, the P_{30} of the adapted equations was significantly improved (non-cancer p=0.013; cancer p=0.001).

Discussion

In this study, paediatric GFR estimating equations generally performed poorly in non-cancer patients. GFR was consistently overestimated with biases ranging between 3.4 and 22.6 ml/min/1.73 m^2. Only Gao's QF, the CKiD and Japanese equations had RMSE values < 20 ml/min/1.73 m^2. The accuracy, reflected by the P_{30} values, was moderate (60-80%) for 7 of the equations and low (< 60%) for the remaining 4 equations.

Previous validation studies have shown variable results although, in general, the accuracy of all the equations was lower in our non-cancer population. Using the Schwartz formula, some studies found good accuracy (> 80%), while in others it was moderate to poor, with P_{30} values similar to the 58% found in this study (Pottel et al. 2010; Bacchetta et al. 2011; Pottel et al. 2012; Selistre et al. 2012; De Souza et al. 2012; Uemura et al. 2014; Deng et al. 2015; Pottel et al. 2016). The Schwartz-Lyon equation was found to have P_{30} values consistently > 77% in comparison to our 68% (Bacchetta et al. 2011; Selistre et al. 2012; Hoste et al. 2013), and the SHI equation had P_{30} values of 64% and 77% in two separate studies compared to our 54% (Blufpand et al. 2013; Deng et al. 2015). In a study that evaluated the Flanders metadata equation, results were presented in age and GFR categories. However, in 4/6 groups the P_{30} values were > 80% compared to the 67% in our study (Hoste et al. 2013). Having been developed relatively recently, Gao's QF and the FAS equation have not been evaluated in many independent validation studies, although one found Gao's QF to have a P_{30} of 72%, marginally higher than the 65% in this study (Deng et al. 2015).

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The consistent overestimation of GFR in our non-cancer population reflects systematically lower serum creatinine levels in comparison to the populations in which the equations were developed and validated. Lower dietary protein intake, which is likely in this population due to poor socio-economic conditions, possibly combined with ethnic/genetic differences are likely causative factors (Stevens et al. 2006). The poor precision of eGFR is unsurprising considering the population's diversity. In the non-cancer group, the children fell into one of three main ethnic groups. The kidney function and ages of the children also varied widely, with 25% of children having a GFR of < 60 ml/min/1.73 m^2, 45% > 90 ml/min/1.73 m^2, half under 10 years in age, approximately one-third between 10 and 14 years, and the remaining 15% > 14 years. Such diversity places a limit on the precision that can be achieved with any GFR estimating equation.

In the cancer group, there was consistently poorer performance in terms of bias, precision and accuracy. Bias ranged between 16.8 ml/min/1.73 m^2 (CKiD) and 46.0 ml/min/1.73 m^2 (Schwartz formula), and 9 of the 11 evaluated equations had RMSEs of > 30 ml/min/1.73 m^2. Interestingly, Gao's QF had a RMSE of 13.4 ml/min/1.73 m^2, which is similar to the value in the non-cancer group. No equations demonstrated good accuracy, and at 63% and 67%, Gao's QF and the CKiD were the only equations with P_{30} values > 50%.

Although the cancer and non-cancer groups differed significantly in terms of age (median 5.9 vs. 9.7 years) and mGFR (mean 95 vs. 85 ml/min/1.73 m^2), the poorer performance of eGFR in the cancer group likely mostly reflects differences in pathology. The greater overestimation of GFR in this group is not unexpected as chronic illnesses such as cancer are known to decrease the serum creatinine concentration (Stevens et al. 2006). This is likely compounded by a reduced dietary protein intake due to the symptoms of cancer or its treatment, further reducing the serum creatinine concentration (Stevens et al. 2006). The poor precision of eGFR may in part reflect a diversity of cancer types, stages of disease and timing of the GFR determination in relation to treatment.

In comparison to other studies in paediatric cancer populations, the accuracy in our population was lower. In 124 GFR measurements in 73 children with cancer, Llanos-Paez et al (Llanos-Paez et al. 2018) evaluated 22 equations, 8 of which were also evaluated in our study. In 7/8 the accuracy was substantially lower in our study with differences in P_{30} values of 14.5-48.9% between the two studies. Millisor et al (Millisor et al. 2017) found a P_{30} value of 55% for the Schwartz formula. Although this is considered low, it was more than double the value in our

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study. Jeong et al (Jeong et al. 2018) found values of 64% and 66% for the Schwartz formula and FAS(age) equations respectively, again much higher than our values of 21% and 33%. Similar to the non-cancer population, systematically lower Scr levels may be attributable to differences in diet and ethnicity. It is also possible that our population included patients who presented later with more advanced disease. The population's greater diversity may again account for the poorer precision.

Ideally, GFR should be measured in all children, but as mGFR remains unavailable in many centres, clinicians may be compelled to rely on eGFR. However, eGFR should not be used without initial confirmation of its validity in the patient population. This is particularly relevant for children with cancer. Furthermore, within the same hospital, separate equations are likely to be required for children with and without cancer. When performance of published equations is unsatisfactory, adapting the equations provides a practical option without the need for large expensive studies. This still requires at least limited availability of mGFR, but it then allows for population-specific adaptation of the equations to be performed. A simple tool, the Microsoft® Excel Solver add-in, is readily available for this purpose, with a step-by-step guide for its use (Brown 2001).

It must be noted that while the Schwartz formula could be adapted using a simple linear regression, Solver allows fitting of non-linear functions with multiple variables, allowing for numerical adaptations of all 4 estimating equations. When adapting equations, there must be an internal validation set with independent data, to avoid overfitting. For an adapted eGFR equation to be valid it is essential for the adaptation to be derived from a similar patient population with renal function over the same range. The minimum number of cases required to successfully adapt equations is expected to depend on the number of equation parameters and the diversity of the population being modelled. If the population were to have a wide range of heights, GFRs, or ages, larger numbers would be required, whereas in a more homogeneous population fewer would suffice. In order to get an indication of the minimum number of cases required for a satisfactory adaptation we studied the effect of development set size on bias, precision and P_{30} for an independent validation set. Bias and P_{30} changed little after surprisingly few cases (~40), while precision continued to improve after a large number of cases (data not shown). Based on this we used development sets of at least 40-50 cases, but we would use as many cases as possible when these were available.

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Before being put into clinical practice, a new equation needs to be shown to sufficiently improve the accuracy of GFR estimates compared to existing equations (Earley et al. 2012; KDIGO 2013). Relative reductions in bias of > 50% or RMSE of > 20% have been proposed (Earley et al. 2012). In 3/4 adapted non-cancer equations in this study, there were significant improvements in bias. Based on the P_{30} values, accuracy of 3/4 was good (> 80%) and 1/4 was moderate. All 4 of the adapted cancer equations demonstrated significant improvements in both bias and RMSE, with good accuracy (> 80%) in 3/4 and moderate accuracy in 1/4.

As the GFR estimating equations were modified to fit specific non-cancer and cancer populations, the adapted versions cannot be assumed to work well in other populations that differ in terms of age or GFR range, pathology, genetics and nutrition. Rather, the intention of this study was to demonstrate the *utility* of modifying the equations. Another limitation of this study is that the performance of the modified equations should ideally have been assessed in different age and GFR categories; however, the small numbers in each group precluded this. Specifically, there were very few children in the cancer group with mGFR < 60 ml/min/1.73 m^2. A development dataset should ideally include a representative range of GFRs to ensure its validity for the full range of renal function (KDIGO 2013).

A limitation of eGFR in general is that the decision to use it in the clinic should always be made with a good understanding of the inherent uncertainty associated with these estimates. For example, for an eGFR of 100 ml/min/1.73 m^2 the P_{30} represents values between 70 and 130 ml/min/1.73 m^2. This in itself is a wide range, but for a P_{30} of 80%, which is often considered acceptable, 1 in 5 children will have a true GFR that is beyond even this range. If a specific clinical context permits a certain amount of uncertainty in the GFR value obtained, then it may be acceptable to use an eGFR with a P_{30} of 80%. In support of it, the Chronic Kidney Disease Improving Global Outcomes (KDIGO) guideline recommends use of eGFR for diagnosing and monitoring CKD in adults and children (KDIGO 2013). However, if the clinical context requires a high degree of certainty, eGFR should probably only be used to place the value within a 95% confidence interval, making it useful in only a limited proportion of patients, typically those with high eGFR values. For example, in oncology where GFR must be above a specific value (typically 60 ml/min/1.73 m^2) for a child to receive chemotherapy, only a very high eGFR result is useful to reliably exclude a reduced GFR, with eGFR expected to misclassify a large proportion of cases.

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Inclusion of children < 2 years of age in this study (10%) may have contributed to the original equations' poor overall performance as under 2-year-olds were excluded from many of the development datasets; however, the impact of this on the overall results is likely to be small. Six of the equations require normalization the child's Scr to the population's mean or median Scr (Pottel et al. 2012; Hoste et al. 2013; Uemura et al. 2014; Pottel et al. 2016). Had this data been available for our local population, it is expected that the accuracy of the relevant equations would have been better.

In conclusion, existing paediatric GFR estimating equations performed poorly in this specific population that differed from the development populations in terms of age, GFR, pathology, ethnicity and diet. Modifying the equations' parameters using a simple Excel-based tool is achievable in any department that has at least some access to GFR measurement, and significantly improved their accuracy. However, in specific clinical settings that require high degrees of certainty with GFR values, GFR measurement is essential.

Conflicts of interest

None

Author contributions

JL Holness: data analysis, manuscript preparation
A Brink: participant recruitment, manuscript review
MR Davids: co-supervisor, manuscript review
JM Warwick: supervisor, manuscript review

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Tables and figures

Table 6.1 The GFR estimating equations evaluated in this study

Equation name	Formula (ml/min/1.73m^2)	Units
New bedside Schwartz formula (Schwartz et al. 2009)	eGFR = k(ht/Scr) k = 0.413	Scr: mg/dL ht: cm
Flanders Metadata formula (Pottel et al. 2010)	eGFR = k(ht/Scr) k = [0.0414 x ln(age) + 0.3018]	Scr: mg/dL ht: cm
Schwartz-Lyon formula (De Souza et al. 2012)	eGFR = k(ht/Scr) k = 32.5 all females and males < 13y k = 36.5 males ≥ 13y	Scr: µmol/L ht: cm
Simple height-independent equation (Pottel et al. 2012)	eGFR = 107.3/(Scr/Q) Q = (0.0270 x age) + 0.2329	Scr: mg/dL age: years
Q(age) equation (Hoste et al. 2013)	eGFR = 107.3/(Scr/Q) Boys: Q = 0.21 + (0.057 x age) – (0.0075 x age^2) + (0.00064 x age^3) – 0.000016 x age^4) Girls: Q = 0.23 + (0.034 x age) – (0.0018 x age^2) + (0.00017 x age^3) – (0.0000051 x age^4)	Scr: mg/dL age: years
Q(height) equation (Hoste et al. 2013)	eGFR = 107.3/(Scr/Q) Q = 3.94 – (13.4 x L) + (17.6 x L^2) – (9.84 x L^3) + (2.04 x L^4) for boys and girls	Scr: mg/dL L: cm
CKiD univariate equation (Schwartz et al. 2012)	eGFR = a(ht/Scr)^b a = 42.3 b = 0.780	Scr: mg/dL ht: m
Gao's quadratic formula (Gao et al. 2012)	Boys: eGFR = 0.68 x (Ht/Scr) – 0.0008 x (Ht/Scr)^2 + (0.48 x age) – 21.53 Girls: eGFR = 0.68 x (Ht/Scr) – 0.0008 x (Ht/Scr)^2 + (0.48 x age) – 25.68	Scr: mg/dL ht: cm
Japanese equation (Uemura et al. 2014)	eGFR = 110.2 x (ref Scr/pt Scr) + 2.93 Boys: ref Scr = (-1.259 x L^5) + (7.815 x L^4) – (18.57 x L^3) + (21.39 x L^2) – (11.71 x L) + 2.628 Girls: ref Scr = (-4.536 x L^5) + (27.16 x L^4) – (63.47 x L^3) + (72.43 x L^2) – (40.06 x L) + 8.778	Scr: mg/dL L: m
Full age spectrum (FAS) equation (age) (Pottel et al. 2016)	eGFR = 107.3/(Scr/Q) See original papers for Q value table (Hoste et al. 2013; Pottel et al. 2016)	Scr: *
Full age spectrum (FAS) equation (height) (Pottel et al. 2016)	eGFR = 107.3/(Scr/Q) See original papers for Q value table (Hoste et al. 2013; Pottel et al. 2016)	Scr: *

*Scr units must correspond to the units of the Q value

Abbreviations: **CKiD**, Chronic Kidney Disease in Children study; **eGFR**, estimated glomerular filtration rate; **ht**, height; **Scr**, serum creatinine; **L**, length; **ref**, reference; **pt**, patient

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Table 6.2 Patient characteristics (n = 256)

Age (years)		8.9 (4.3 to 12.6)
Sex	Male	117 (46%)
GFR (ml/min/1.73 m²)		90.6 (69.9 to 108.6)
Weight (kg)		23.9 (14.2 to 37.7)
Height (cm)		120.8 (99.6 to 145.0)
Scr (mg/dL)		0.43 (0.32 to 0.57)
Scr (µmol/L)		38 (28 to 51)
Height/Scr	Non-cancer	220
	Cancer	328
GFR categories	≥ 90 ml/min/1.73m²	131 (51.2%)
	60 – 89 ml/min/1.73m²	81 (31.6%)
	45 – 59 ml/min/1.73m²	24 (9.4%)
	< 45 ml/min/1.73m²	20 (7.8%)
Diagnoses	**Non-cancer**	159 (62.1%)
	Vasculitis	52 (32.7%)
	Liver transplant drug monitoring	44 (27.7%)
	Neurogenic bladder	12 (7.5%)
	Posterior urethral valves	6 (3.8%)
	Uteropelvic junction obstruction	6 (3.8%)
	Solitary kidney	6 (3.8%)
	Fibromuscular dysplasia	5 (3.1%)
	Cystic diseases	4 (2.5%)
	Glomerular disease	3 (1.9%)
	Other causes of CKD (congenital and medical)	21 (13.2%)
	Cancer	97 (37.9%)
	Neuroblastoma	33 (34.0%)
	Osteosarcoma	17 (17.5%)
	Lymphoma	11 (11.3%)
	Wilms tumor	9 (9.3%)
	CNS tumors	8 (8.3%)
	Other	19 (19.6%)
Ethnicity	Mixed ancestry	148 (57.8%)
	Black African	74 (28.9%)
	Caucasian	34 (13.3%)

Values are either median (IQR) or *n* (%).

Abbreviations: **GFR**, glomerular filtration rate; **Scr**, serum creatinine; **CKD**, chronic kidney disease; **CNS**, central nervous system.

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Table 6.3 Summary statistics for the 11 original estimating equations

	Non-cancer (n=159)					Cancer (n=97)				
	Median GFR*	**Median difference (95% CI)***	**RMSE***	**P_{30} (%) (95% CI)**	**Mean difference (95% LOA)***	**Median GFR***	**Median difference (95% CI)***	**RMSE***	**P_{30} (%) (95% CI)**	**Mean difference (95% LOA)***
mGFR (^{51}Cr-EDTA plasma clearance)	85.5	NA	NA	NA	NA	95.1	NA	NA	NA	NA
New bedside Schwartz formula	103.5	18.4 (15.7 to 21.4)	23.4	57.9 (49.8 to 65.7)	19.4 (-27.3; 66.1)	141.1	46.0 (37.3 to 58.2)	35.8	20.6 (13.1 to 30.3)	51.7 (-18.1; 121.5)
Flanders metadata formula	99.5	12.8 (9.9 to 16.0)	21.6	67.3 (59.4 to 74.5)	13.5 (-29.3; 56.3)	123.4	31.1 (24.5 to 37.9)	33.9	42.3 (32.3 to 52.8)	35.2 (-30.8; 101.3)
Schwartz-Lyon formula	92.4	9.6 (6.7 to 12.7)	21.0	67.9 (60.0 to 75.1)	9.2 (-33.9; 52.3)	125.7	35.0 (25.5 to 42.2)	31.6	34.0 (24.7 to 44.3)	36.6 (-25.1; 98.3)
Simple height-independent equation	105.5	17.8 (15.0 to 25.3)	29.6	54.1 (46.0 to 62.0)	23.3 (-34.6; 81.1)	133.7	39.5 (31.0 to 44.0)	33.1	27.8 (19.2 to 37.8)	41.9 (-23.0; 106.7)
Q(age) equation	107.6	22.6 (17.1 to 28.1)	30.5	48.4 (40.4 to 56.4)	26.8 (-33.0; 86.5)	137.7	42.3 (34.5 to 48.7)	33.3	23.7 (15.7 to 33.4)	45.5 (-19.5; 110.5)
Q(height) equation	98.0	13.8 (8.7 to 17.2)	21.2	64.2 (56.2 to 71.6)	13.3 (-29.4; 56.0)	134.9	42.8 (32.4 to 48.8)	39.5	25.8 (17.4 to 35.7)	47.9 (-30.1; 125.9)
CKiD univariate equation	86.6	3.4 (0.5 to 7.2)	15.0	76.1 (68.7 to 82.5)	1.3 (-39.1 to 41.7)	110.3	16.8 (14.1 to 20.5)	21.3	67.0 (56.7 to 76.2)	17.7 (-27.4; 62.8)
Gao's quadratic formula	101.8	9.9 (6.1 to 17.1)	14.1	64.8 (56.8 to 72.2)	10.1 (-27.4; 47.6)	116.5	19.3 (16.2 to 22.9)	13.4	62.9 (52.5 to 72.5)	16.8 (-24.0; 57.7)
Japanese equation	90.2	5.4 (1.8 to 10.3)	18.6	71.7 (64.0 to 78.6)	5.2 (-34.0; 44.5)	121.3	28.2 (22.4 to 34.1)	30.3	49.5 (39.2 to 59.8)	31.6 (-27.8; 91.1)
FAS (age) equation	105.1	17.8 (14.3 to 23.2)	28.6	54.7 (46.6 to 62.6)	22.5 (-33.4; 78.5)	131.8	37.0 (29.3 to 42.2)	32.3	33.0 (23.8 to 43.3)	39.8 (-23.7; 103.3)
FAS (height) equation	96.6	12.9 (7.3 to 15.1)	20.9	63.5 (55.5 to 71.0)	11.6 (-30.8; 54.1)	129.9	36.3 (28.7 to 44.4)	33.9	32.0 (22.9 to 42.2)	40.9 (-25.5; 107.2)

*Units are ml/min/1.73 m^2. Median and mean differences are calculated as eGFR – mGFR. RMSE is calculated from the regression of eGFR vs. mGFR.

Abbreviations: **GFR**, glomerular filtration rate; **mGFR**, measured glomerular filtration rate; **eGFR**, estimated glomerular filtration rate; **LOA**, limits of agreement; **CI**, confidence interval; **RMSE**, root mean square error; **NA**, not applicable; **CKiD**, Chronic Kidney Disease in Children study; **FAS**, full age spectrum.

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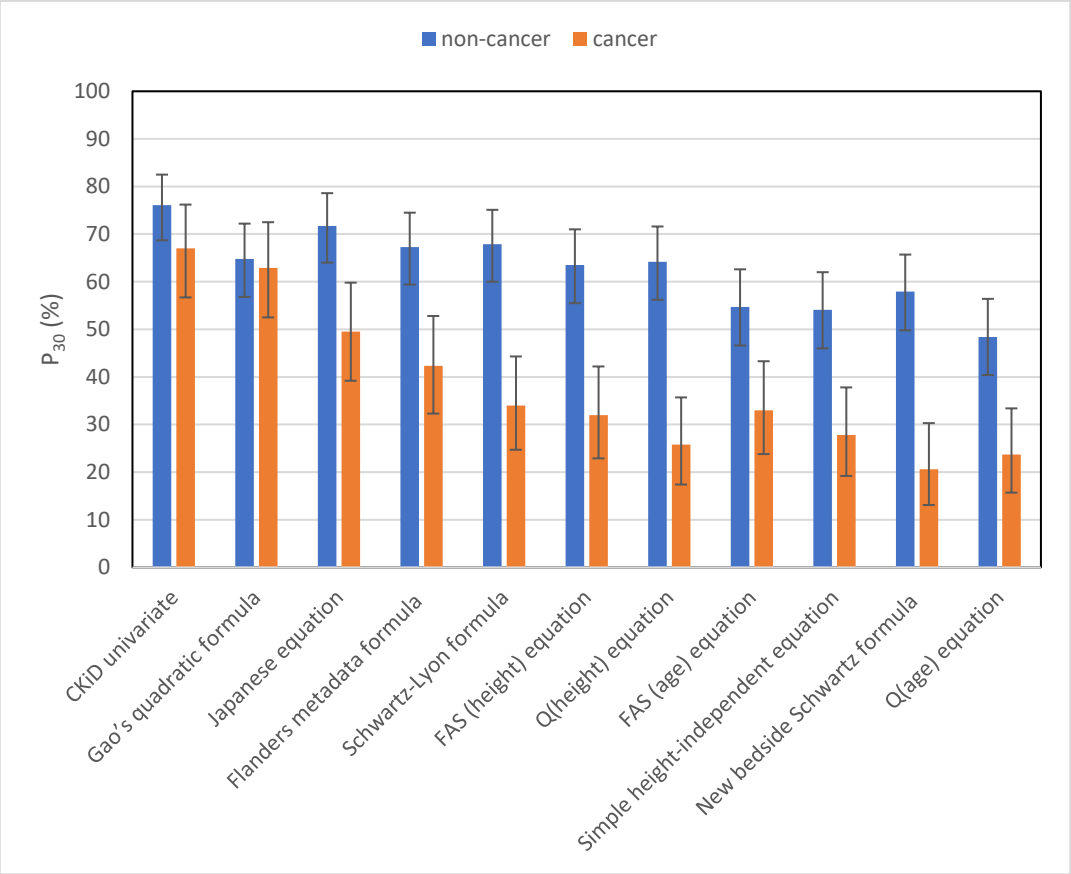
Fig. 6.1



Bar chart of the P_{30} values of the non-cancer (blue) and cancer (orange) groups for each equation. The equations have been arranged from left to right in descending order of average P_{30}. The error bars represent the 95% confidence intervals.

Abbreviations: **CKiD**, Chronic Kidney Disease in Children study; **FAS**, full age spectrum.

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Table 6.4 Original and adapted equations

Schwartz formula	Original	eGFR = 0.413(ht/Scr)
	Adapted non-cancer	eGFR = 0.333(ht/Scr)
	Adapted cancer	eGFR = 0.254(ht/Scr)
Flanders metadata equation	Original	eGFR = k(ht/Scr) k = [0.0414 x ln(age) + 0.3018]
	Adapted non-cancer	eGFR = k(ht/Scr) k = [0.0518 x ln(age) + 0.2198]
	Adapted cancer	eGFR = k(ht/Scr) k = [0.0117 x ln(age) + 0.2366]
CKiD univariate equation	Original	$eGFR = a(ht/Scr)^b$ a = 42.3; b = 0.780
	Adapted non-cancer	$eGFR = a(ht/Scr)^b$ a = 45.0; b = 0.728
	Adapted cancer	$eGFR = a(ht/Scr)^b$ a = 69.3; b = 0.280
Gao's quadratic formula	Original	Boys: $eGFR = 0.68(Ht/Scr) - 0.0008(Ht/Scr)^2 + (0.48 \times age) - 21.53$ Girls: $eGFR = 0.68(Ht/Scr) - 0.0008(Ht/Scr)^2 + (0.48 \times age) - 25.68$
	Adapted non-cancer	Boys: $eGFR = 0.61(Ht/Scr) - 0.0006(Ht/Scr)^2 + (1.68 \times age) - 37.03$ Girls: $eGFR = 0.61(Ht/Scr) - 0.0006(Ht/Scr)^2 + (1.68 \times age) - 43.29$
	Adapted cancer	Boys: $eGFR = 0.12(Ht/Scr) - 0.00004(Ht/Scr)^2 + (0.64 \times age) + 58.92$ Girls: $eGFR = 0.12(Ht/Scr) - 0.00004(Ht/Scr)^2 + (0.64 \times age) + 51.69$

Height in units m for the CKiD equation, and cm for the others. Scr in units mg/dL for all equations.

Abbreviations: **CKiD**, Chronic Kidney Disease in Children study; **eGFR**, estimated glomerular filtration rate; **ht**, height; **Scr**, serum creatinine.

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Table 6.5 Evaluation of the adapted non-cancer and cancer equations

	Non cancer (n=80)						Cancer (n=49)					
	Median difference (95% CI)*	**Δ bias (%)**	**RMSE***	**Δ RMSE**	**P_{30} (%) (95% CI)**	***p*-value**	**Median difference (95% CI)***	**Δ bias (%)**	**RMSE***	**Δ RMSE**	**P_{30} (%) (95% CI)**	***p*-value**
Original Schwartz	18.3 (12.2 to 24.3)	-93%	22.0	-19%	53.8 (42.3 to 65.0)	<0.0001	46.0 (36.8 to 62.3)	-88%	28.6	-38%	14.3 (6.0 to 27.3)	<0.0001
Adapted Schwartz	1.3 (-3.9 to 8.2)		17.8		80.0 (69.6 to 88.1)		-5.3 (-8.9 to -0.8)		17.6		89.8 (77.8 to 96.6)	
Original Flanders	11.5 (7.3 to 16.3)	-82%	20.2	-14%	67.5 (56.1 to 77.6)	0.0023	31.1 (24.5 to 50.5)	-82%	28.1	-36%	34.7 (21.7 to 49.6)	<0.0001
Adapted Flanders	-2.1 (-5.6 to 1.6)		17.4		83.8 (73.9 to 91.1)		-5.5 (-7.9 to 1.3)		17.9		87.8 (75.3 to 95.4)	
Original CKiD univariate	5.7 (0.7 to 10.2)	-5%	14.3	-6%	71.3 (60.1 to 80.9)	1.0000	16.4 (13.3 to 21.9)	-63%	17.2	-67%	67.3% (52.4 to 80.0)	0.2668
Adapted CKiD univariate	5.4 (0.7 to 11.7)		13.5		70.0 (58.7 to 79.7)		6.0 (-1.7 to 14.1)		5.6		77.6% (63.4 to 88.3)	
Original Gao	11.3 (5.7 to 18.8)	-85%	14.6	5%	58.8 (47.2 to 69.7)	0.0192	21.1 (16.6 to 26.3)	-79%	12.0	-44%	61.2% (46.2 to 74.8)	0.0034
Adapted Gao	1.7 (-3.1 to 6.6)		15.3		72.5 (61.4 to 79.7)		4.4 (-1.8 to 10.0)		6.7		83.7% (70.4 to 92.7)	

*Units are ml/min/1.73 m^2. Median and mean differences are calculated as eGFR – mGFR. RMSE is calculated from the regression of eGFR vs. mGFR.

Abbreviations: **CKiD**, Chronic Kidney Disease in Children study; **CI**, confidence interval; **RMSE**, root mean square error.

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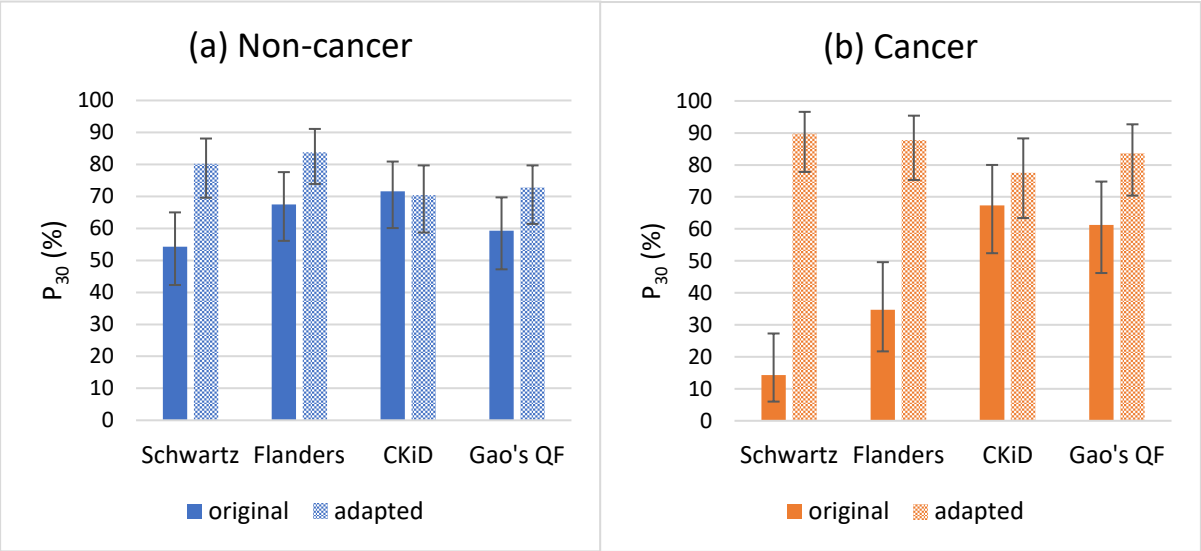
Fig. 6.2



Bar graphs showing the P_{30} values of the original (solid) and adapted (shaded) equations for the (a) non-cancer and (b) cancer groups. The error bars represent the 95% confidence intervals.

Abbreviations: **CKiD**, Chronic Kidney Disease in Children study; **Gao's QF**, Gao's quadratic formula

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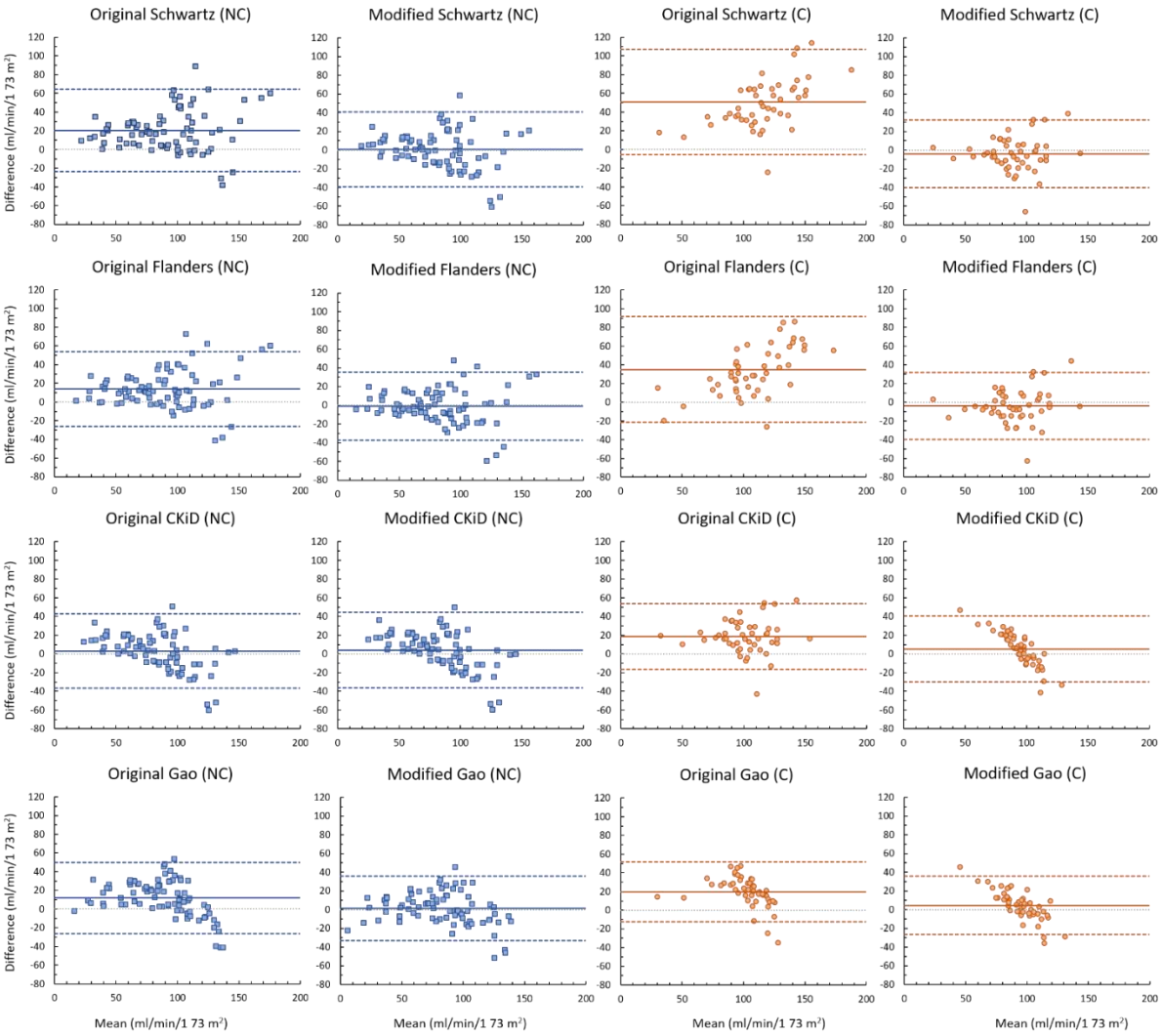
Fig. 6.3



Bland-Altman plots of the original and modified equations for non-cancer (blue; NC) and cancer (orange; C) groups.

Abbreviations: **CKiD**, Chronic Kidney Disease in Children study.

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Discussion

Optimising mGFR methodology

In paper 1, a post-hoc evaluation of the contribution of two deviations from the 2004 BNMS guideline (low counts and lack of decay correction) to the overall variability in V_D and GFR was performed. The resultant combined errors in GFR and V_D were small relative to the physiological variation of these parameters.

In paper 3, a more exhaustive evaluation of the effect of measurement errors on GFR was performed. We compared 2-point SI-GFR, 3-point SI-GFR, SS-GFR and SO-GFR. After introducing realistic, random errors to all measurements, the median absolute error for all methods/variations was low. The error in GFR, expressed as the CV, increased sharply for all methods when GFR decreased to below 60 ml/min/1.73 m^2. For normal or high GFR values, the mean CV of SS-GFR was the lowest, whereas for GFR values < 50 ml/min/1.73 m^2 the CVs for 3-point SI-GFR and 2-point SI-GFR (2 and 4 h) were the lowest.

Based on data from repeat GFR measurements, in cases of normal clearance, the CV of measurement errors was found to be small in relation to the CV of biological variation. However, for lower rates of clearance the CV of measurement errors approaches or even exceeds the CV of biological variation and would therefore contribute significantly to repeat GFR measurement variability.

In a sensitivity analysis of GFR to individual measurement errors, the effect of measurement errors on three-point SI-GFR and SS-GFR was found to be similar. Both methods were relatively insensitive to errors in pipetting volume, flask volume, height, weight and time measurements. On the other hand, errors in the administered dose measurements were found to be most significant. Of the measurements made, this therefore requires the most attention to avoid error.

Supporting the results of paper 1, GFR was found to be surprisingly robust with low counts. A minimum of 2500 counts for each sample (background-corrected) was shown to be adequate to avoid a significant error in GFR.

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The first conclusion drawn from paper 3 is that in most patients the measurement errors that occur on a day-to-day basis in an ordinary nuclear medicine department are unlikely to result in significant errors in GFR. In fact, it is very unlikely that the resultant error in GFR will be > 5 ml/min/1.73 m^2, which in turn means that patient management is unlikely to be affected. This is useful information for individuals undergoing once-off GFR measurement e.g. for potential kidney donors, or for previously healthy patients prior to receiving nephrotoxic chemotherapy for cancer.

However, frequently GFR is measured in patients with known or suspected poor kidney function, or is required to detect a deterioration in kidney function. In these cases, the repercussions of measurement errors are far more significant. Below a value of 25 ml/min/1.73 m^2 the effect of measurement errors becomes too great to meaningfully interpret changes in GFR over serial measurements. However, this conclusion applies to SI-GFR, SO-GFR and SS-GFR using the methodologies described here, and in the case of SS-GFR the equation developed by Fleming et al (Fleming et al. 2005a), whereas the current BNMS guideline recommends an alternative SS-GFR equation when GFR is < 25 ml/min/1.73 m^2 (Gref and Karp 2009; Burniston 2018). As the study for paper 3 was developed prior to the 2018 guideline, it did not evaluate whether measurement errors would have the same effect on GFR measurements using this method. Perhaps more importantly, it did not assess the effect of measurement errors on SI-GFR, SO-GFR and SS-GFR based on later sampling times and/ or prolonged sampling intervals, as are recommended in the latest BNMS guidelines. This would be an area for further research.

Some of the recommended measurement error limits listed in paper 3 are more strict than those in the 2018 BNMS guideline (Burniston 2018; Holness et al. 2019). For the majority of indications for GFR measurement, these stricter limits are not necessary. However, as stated in the paper, one may not know at the outset whether patients will have reduced kidney function. It is in these cases that a high level of precision of every measurement is ideal.

The 2004 BNMS guideline stated that a minimum of 10 000 counts should be obtained for all samples to avoid statistical counting error (Fleming et al. 2004). The effect of counts was explored further in paper 3 and all methods were found to be surprisingly robust to low counts (Holness et al. 2019). Accordingly, a lower value for minimum counts could be recommended. This is consistent with the 2018 BNMS guidelines' recommendation of a precision of less than

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2% for background corrected counts. This is particularly relevant to departments that use ^{51}Cr-EDTA as lower doses of activity are administered.

When using the 2-point SI-GFR, paper 3 provided clear evidence that taking the blood samples at 2 h and 4 h is optimal (Holness et al. 2019). Ideally, if the first sample is delayed for whatever reason, the second sample should be delayed by the same amount of time.

As discussed, based on two studies by McMeekin et al (McMeekin et al. 2016a; McMeekin et al. 2016b), SS-GFR became the recommended technique in the updated BNMS guideline (Burniston 2018). The findings of paper 3 support this conclusion as SS-GFR was found to be the least susceptible to measurement errors in the majority of patients. A change to SS-GFR is likely to be welcomed by nuclear medicine departments. For the patient, it is less invasive and generally requires less time in the department, and for the technologist the procedure is simplified, which may translate into an increase in capacity.

QC checks for mGFR

In paper 1, as a primary aim, we defined reference data for ^{99m}Tc-DTPA T½ and V_D in a healthy adult population. The ranges represent 2 SD of the mean. For T½, this was derived from the regression of T½ vs. the inverse of BM-GFR$_{corr}$. No published data with similar methodology was available for comparison.

For V_D, the reference data was derived from the regression of V_D vs. BSA. For uncorrected and corrected V_D, the values obtained in our study were systematically higher than the reference data that was available at the time (Fleming et al. 2004; Fleming et al. 2009). *Uncorrected* V_D will always overestimate true V_D, but the degree of overestimation is dependent on GFR. Being a healthy population of potential kidney donors, the mean GFR in our population is expected to be higher than the BNMS guideline population, thus providing a plausible explanation for the higher uncorrected V_D values. However, our values for *corrected* V_D fell within the 2 SD error limits of the published reference data, and vice versa (Fleming et al. 2009). The apparent systematic differences therefore may not be significant. Supporting this was the finding of very similar values for ECV to those found in a study of 1878 healthy kidney donors from the United Kingdom (Peters et al. 2012). The values for V_D in our study also showed greater variability than the published reference data. This was thought to be largely

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due to the greater ethnic/genetic diversity in our population, with a smaller contribution from experimental error.

During GFR measurement, ensuring that values obtained for T½ and V_D are within defined reference ranges was regarded as an important QC check in the original (2004) BNMS guideline (Fleming et al. 2004). Subsequent to the publication of paper 1, questions have been raised about the utility of GFR QC parameters. McMeekin et al. evaluated the effectiveness of various commonly used QC checks, including V_D (McMeekin et al. 2016b). In this study measurement errors were classified into 3 categories: (1) model failure errors (failure of the simplified, single-compartment method e.g. in a patient with ascites); (2) whole curve errors (measurement errors that affect the whole curve in the same way e.g. due to an error in the standard or inaccuracy in measuring the administered activity); and (3) individual point errors (errors in the plasma samples e.g. due to inaccurate recording of time or inaccurate pipetting). The conclusion was that *all* conventional QC checks have poor sensitivity and positive predictive value for detecting clinically significant errors in SI-GFR and SS-GFR (McMeekin et al. 2016b). However, the authors point out that due to its design, it was unable to detect type 2 (whole curve) errors, and only 1 out of 412 GFR tests had a type 3 (individual point) error.

In 2018, an updated GFR guideline was released by the BNMS (Burniston 2018). Based on the findings of the McMeekin paper (McMeekin et al. 2016b) and another paper published by the same authors in parallel (McMeekin et al. 2016a), SS-GFR is recommended as the preferred method in the new guideline. Thus, QC checks such as V_D would fall away in the majority of GFR measurements. In 2019 Klein et al (Klein et al. 2019) provided a SI-GFR spreadsheet as a useful tool with many embedded QC checks, however, V_D was not included.

While there is evidence that as a QC measure V_D can be both non-specific and insensitive (McMeekin et al. 2016b), we are of the opinion that in specific scenarios and if interpreted optimally, V_D may still contribute to QC of SI-GFR. We are unaware of work addressing gross human errors, e.g. mixing up of patient samples or misinjections. These mishaps, or ‘blunders’, frequently result in grossly erroneous GFR results that may be difficult to detect using SS-GFR. Our experience in a busy clinical department where GFR measurements are performed by one of several generically functioning radiographers or by trainees under supervision is that despite attempts to eliminate them, infrequent but clinically potentially catastrophic type 2 and 3 errors still occur. When this happens, the causes are typically related to significant procedural

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or patient identification errors that are often large and clearly detected using the battery of QC checks that are in place, including V_D.

The reference ranges that were defined for T½ and V_D were based on the mean ± 2 SD (Holness et al. 2013). As for any reference range, the use of 2 SD error limits will result in 5% of *normal* studies falling outside of the range. One in twenty GFR measurements would thus fail this QC test and would need to be repeated, an unacceptably high proportion. The specificity can be increased by increasing the error limits to 2.5 SD or 3 SD, but this would come at the expense of sensitivity, with the risk of providing a false sense of security to the nuclear medicine physician. Further research would be useful to define an optimal threshold.

A further potential criticism of the reference ranges defined in paper 1 (Holness et al. 2013) is that they were derived from a healthy population with normal kidney function. A concern raised by Klein et al (Klein et al. 2019) is whether these reference ranges are valid for QC of GFR tests performed for patients with serious disease e.g. cancer, or in individuals with reduced kidney function. Current work in our unit is attempting to address these questions. Using the dataset of paper 3 (786 3-sample SI-GFR measurements in individuals with various pathologies and GFR values across the full range), the reference range for V_D using 2.5 SD error limits was found to be applicable with only 1.9% of studies giving false positive results for V_D (unpublished data). These results require confirmation however.

In the 2004 BNMS guideline the T½ was noted to be 'typically within' 100-120 min in a person with normal GFR (Fleming et al. 2004), but no guidance was provided on the use of T½ as a QC test if GFR was decreased. Paper 1 provided a GFR-dependent reference range for T½. However, the usefulness of this as a QC test is yet to be evaluated.

^{99m}Tc-DTPA vs ^{51}Cr-EDTA

As a secondary aim in paper 1, we defined reference data for ^{99m}Tc-DTPA GFR. For adults < 40 years, mean GFR was 108 ml/min/1.73 m^2. Thereafter, it decreased at a rate of 1.55 ml/min/1.73 m^2 per year.

In paper 2, we performed a mini meta-analysis of the data of 3 previous studies directly comparing the plasma clearance of ^{99m}Tc-DTPA and ^{51}Cr-EDTA (Holness et al. 2015). The methods were found to agree well, with median bias < 1%, P_{30} = 93%, and P_{10} = 72%.

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The ^{99m}Tc-DTPA GFR reference range defined in paper 1 (Holness et al. 2013) agreed reasonably well with the reference data that was available at that time (Granerus and Aurell 1981; Hamilton et al. 2000; Grewal and Blake 2005a). Limitations of our study though were the small sample size and the lack of individuals > 40 years. In 2017, the data from paper 1 was included in a meta-analysis comprising 5482 healthy, potential kidney donors from 12 independent studies (Pottel et al. 2017). GFR was measured using a variety of tracers (^{51}Cr-EDTA 3667, iothalamate 1305, inulin 332, ^{99m}Tc-DTPA 126, and iohexol 52). Mean GFR was found to be ~107 ml/min/1.73 m^2 in individuals < 40 years, and decreased at a rate of 0.89 ml/min/1.73 m^2 per year in individuals > 40 years. Differences in mean GFR were observed in the 12 included studies, and were thought to be due to a combination of systematic differences in the clearance of the various filtration markers and major differences in methodology and/or calculation method.

Although already in the 2004 BNMS guidelines ^{99m}Tc-DTPA was stated to be an acceptable radiopharmaceutical for GFR measurement, there are still members of the Nuclear Medicine and wider clinical community that question its reliability over alternatives such as ^{51}Cr-EDTA. Furthermore, doubts were raised about the use of ^{99m}Tc-DTPA to measure GFR in a systematic review that compared the plasma and urinary clearance of various exogenous filtration markers to urinary inulin clearance (Soveri et al. 2014). The plasma clearance of ^{99m}Tc-DTPA was concluded to be an inaccurate method. However, this was based on the results of two relatively old and methodologically questionable studies that compared plasma ^{99m}Tc-DTPA clearance to urinary inulin clearance head to head (the requirement for inclusion in the review). Its conclusions were reached without considering literature supporting the use of ^{99m}Tc-DTPA, based on studies that compared ^{99m}Tc-DTPA with other tracers. The results of our mini meta-analysis of data from 3 published studies comparing plasma clearance of ^{99m}Tc-DTPA and ^{51}Cr-EDTA confirmed that there is excellent agreement between them (Holness et al. 2015). This has become particularly relevant recently as production of ^{51}Cr-EDTA has been discontinued by the major global supplier forcing departments to find an alternative. Given its availability, relatively low cost, and the methodology being identical to that of ^{51}Cr-EDTA, it is a simple adaptation for affected centres to change to ^{99m}Tc-DTPA. The availability of reference data for ^{99m}Tc-DTPA plasma clearance (Holness et al. 2013), combined more importantly with the demonstrated comparability with ^{51}Cr-EDTA (Holness et al. 2015; Pottel et al. 2017), provides evidence in support of this transition.

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mGFR as the ground truth

In studies that validate the performance of eGFR equations, mGFR serves as the reference technique against which eGFR is compared. Irrespective of the tracer or method used, mGFR is regarded as the gold standard. Consequently, there is an assumption that errors in eGFR are solely due to failure of the estimating equations in that population. The contribution of errors in mGFR and biological variation are seldom considered. A reason for this may be that eGFR validation studies are frequently conducted by clinicians who may have limited understanding of the intricacies of GFR measurement.

As GFR cannot be measured directly in humans, 'true' GFR is never known (Levey and Inker 2016). Thus, whether GFR is assessed using clearance methods (mGFR) or estimating equations (eGFR), the error compared to true GFR cannot be quantified. It is well known that the urinary clearance of inulin provides the most accurate measure of GFR (Brenner and Levine 2008), and that there are systematic differences in the clearance of other tracers compared to inulin (Soveri et al. 2014). The use of a simplified technique (e.g. SI-GFR or SS-GFR) is likely to result in a further deviation from true GFR. It follows that in studies developing new estimating equations, in order to approximate true GFR as closely as possible, the most accurate mGFR method available should be selected, and the measurements performed with the utmost precision. However, even with the most accurate technique, there will always be some systematic and random error against the ground truth.

In eGFR validation studies, error is measured as eGFR-mGFR. This difference, typically used as a proxy for eGFR error, overestimates eGFR errors relative to the ground truth and is really a combination of 3 independent sources of error:

1. eGFR error (relative to the true GFR)
2. mGFR error (relative to the true GFR)
3. biological variation of the true GFR

There are two components to eGFR error: (i) error in Scr measurement and (ii) error in the model. Error in Scr measurement is both systematic and random, however it is relatively small in magnitude. Routine standardization of Scr assays has minimised the large systematic differences that existed previously between different laboratories (Myers et al. 2006). Random errors vary depending on the method (enzymatic vs. Jaffe), however these are quantifiable and

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values are readily available from the laboratory (Delanaye et al. 2017). For example, the analytical imprecision of the Roche Cobas 6000 analyser (enzymatic) used in our laboratory is 1.9% at Scr=90.5 µmol/L, and 1.3% at 327.9 µmol/L. On the other hand, the error in the model is difficult to quantify and an estimate can only be made after consideration of all other sources of error.

There are also two main components to error in mGFR: (i) measurement errors and (ii) errors inherent to the method. In paper 3 measurement errors were found to have a relatively small impact on GFR, however they had an increasingly significant effect as GFR decreased. In a prospective eGFR validation study, it is plausible to keep measurement errors to a minimum. This is achieved by strict adherence to protocols, use of high-precision equipment and, in particular, use of a dedicated, well-trained research team. In contrast, there is frequently little control over mGFR methodology in retrospective studies or in validation studies initiated by a department that does not perform the GFR measurements itself.

Errors inherent to the method include the choice of tracer and calculation technique. At this point it must be emphasized that the importance of these errors lies in the *differences* in tracer or technique between the validation and development studies, rather than deviations from true GFR. For the development of the existing eGFR equations a variety of tracers and techniques were used. Those assessed in this dissertation are listed in Table 7.1. Use of a tracer different to the tracer used in the development study will result in systematic errors. In most cases, this bias has been quantified previously, either by direct comparison, or indirectly as in the systematic review by Soveri et al (Soveri et al. 2014).

Use of a different calculation technique will introduce both systematic and random errors. The errors will vary in magnitude depending on the technique used. In paper 5, the results of previous studies conducted in adult cancer patients were not compared for this reason. For example, in two of the more recent papers (Lauritsen et al. 2014; Hartlev et al. 2012) the single-sample technique described by Groth and Aasted was used (Groth and Aasted 1981). Subsequently, this technique has been shown to be inaccurate when compared to GFR measured from the full area under the plasma clearance curve with median bias of 11 ml/min/1.73 m^2, and 24% of measurements differing from the reference GFR values by > 20% (McMeekin et al. 2016a).

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Other differences in mGFR methodology between development and validation studies, such as the method to calculate BSA or to correct for the missing first exponential (SI-GFR), will also result in systematic and random errors. These errors would best be modelled using full AUC reference data. However, use of the dataset of paper 3 provides an idea of their magnitude. Three-point SI-GFR (2, 3, 4 h), using the Haycock formula to correct for BSA (Haycock et al. 1978) and the mean Bröchner-Mortensen equation to correct for the one-pool assumption (Fleming et al. 2004), served as the reference for each of the 786 GFR measurements. A widely used alternative BSA formula, the DuBois and DuBois equation (DuBois and DuBois 1989), resulted in small, insignificant systematic and random errors in GFR, with a median error of -1.0 ml/min/1.73 m^2 and IQR of 0.3 ml/min/1.73 m^2. Use of alternative formulae to correct for the one-pool assumption (Bröchner-Mortensen 1972; Bröchner-Mortensen et al. 1974; Jodal and Bröchner-Mortensen 2009) also resulted in small systematic errors ranging between -1.5 and 1.1 ml/min/1.73 m^2. However, the random errors were larger (IQRs ranging from 1.9 to 13.6 ml/min/1.73 m^2).

The third component of the error in eGFR-mGFR is the intra-individual biological variation between Scr and GFR measurements. It contributes to the imprecision of GFR estimates. For Scr the CV for biological variation is 4.3-6.0% (Delanaye et al. 2017). For GFR the biological variation is not known. Values of 7.5-12.2% reported in the literature are measures of repeatability (Bröchner-Mortensen and Rödbro 1976; Wilkinson et al. 1990; Blake et al. 1997; Grewal and Blake 2005; Delanaye et al. 2008a; Bird et al. 2008). However, in paper 3, using the calculated values for measurement error, the biological variation of GFR was estimated to be 2.8-11.0%. The contribution of biological variation to error in eGFR is applicable only to studies in which Scr and GFR are measured on different days, as in paper 5. Ideally, in validation studies GFR and Scr should be measured simultaneously. However, this is not always feasible and is impossible to ensure in retrospective studies.

Like many studies in the literature, papers 4, 5 and 6 made the assumption that errors in GFR estimation are solely due to errors in the model (eGFR equation). The contribution of errors in mGFR, biological variation, and Scr measurement was not quantified in these papers. Supporting this assumption are the results of paper 6. The errors in mGFR, biological variation and Scr measurement are expected to be similar in magnitude for the non-cancer and cancer groups, therefore the finding of significant differences in bias and precision between the groups is consistent with poor performance of the model in the cancer group. However, it would be a

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useful adjunct to existing literature to perform a more rigorous evaluation of the effects of error in mGFR, biological variation and Scr measurement in eGFR evaluations.

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Table 7.1 Methods for measuring GFR in equation development studies

Equation	Filtration marker	Sampling and calculation algorithm
MDRD	Iothalamate urinary clearance	Subcutaneous injection without epinephrine 1 h equilibration period 4 consecutive urine samples & 5 plasma samples
CKD-EPI	Iothalamate urinary clearance	Various (multiple databases)
Cockcroft-Gault	Creatinine clearance	24-hour urine collection Creatinine measured using continuous flow analysis
Janowitz Williams	^{51}Cr-EDTA plasma clearance	3 plasma samples Details not specified
Schwartz formula	Iohexol plasma clearance	Full AUC Sample times: 10, 30, 120, 300 min (Schwartz et al. 2006) HPLC to measure plasma concentrations
Flanders metadata equation	^{51}Cr-EDTA plasma clearance	SS-GFR (Ham and Piepsz 2008) Sample time: 120 min
Schwartz-Lyon equation	Inulin urinary clearance	Priming dose, then continuous infusion 3-4 urine and plasma samples Enzymatic method to measure plasma & urinary concentrations (Dubourg et al. 2010)
Simple height independent equation	^{51}Cr-EDTA plasma clearance	SS-GFR (Ham and Piepsz 2008) Sample time: 120 min
Q(age) and Q(height)	^{51}Cr-EDTA plasma clearance	SS-GFR (Ham and Piepsz 2008) Sample time: 120 min
CKiD univariate equation	Iohexol plasma clearance	Group 1: full AUC Sample times: 10, 30, 120, 300 min (Schwartz et al. 2006) Group 2: Technique described by Ng (Ng et al. 2011) Sample times: 120, 240, 300 min
Gao's quadratic formula	Inulin urinary clearance	Details not specified Anthrone test to measure plasma & urinary concentrations
Japanese equation	Inulin urinary clearance	Priming dose, then continuous infusion 2 urine samples: 60, 120 min Enzymatic method to measure plasma & urinary concentrations
FAS(age) and FAS(height)	(i) Iohexol plasma clearance* (ii) Inulin urinary clearance	(i) SI-GFR with Brochner-Mortensen correction* Sample times: 120, 180, 240 min (ii) Technique described by Dubourg (Dubourg et al. 2010)

*Tracers and methods used in the paediatric databases only.

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Abbreviations: **MDRD**, Modification of Diet in Renal Disease; **CKD-EPI**, Chronic Kidney Disease Epidemiology Collaboration; **CKiD,** Chronic Kidney Disease in Children; **FAS**, full age spectrum; **AUC**, area under the curve; **HPLC**, high performance liquid chromatography; **SS-GFR**, single-sample glomerular filtration rate; **SI-GFR**, slope-intercept glomerular filtration rate.

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eGFR in South African populations

In paper 4, the performance of the MDRD and CKD-EPI equations was evaluated in a mixed ancestry adult population comprising healthy individuals and patients with CKD. Both equations performed reasonably well, with no significant difference between the two. The P_{30} of the MDRD equation was 80% and the CKD-EPI 72.5%.

Consistent with previous publications (van Deventer et al. 2008; Stevens et al. 2011; Madala et al. 2011), inclusion of the ethnicity factors derived for African Americans resulted in a decrease in accuracy of both equations.

In paper 5, the performance of four creatinine based GFR estimating equations was evaluated in 435 adults with cancer. The agreement of all the equations with measured GFR was poor, and all the equations over-estimated mGFR. The over-estimation suggests lower Scr levels on average in this population, which is largely secondary to the cancer itself. However, the degree of over-estimation which is greater than that found in previous non-South African studies, is likely to reflect poorer nutrition (lower protein intake), possibly combined with greater muscle wasting due to more advanced disease. Three of the equations had moderate accuracy (60-80%) in this population, while the accuracy of the fourth was poor (< 60%). The Janowitz-Williams equation, developed specifically for cancer patients, was the most accurate.

In paper 6, eleven GFR estimating equations were evaluated in non-cancer and cancer paediatric populations. In both groups all the equations over-estimated GFR, with a larger bias in the cancer group. None of the equations had good (> 80%) accuracy. In the non-cancer group, the accuracy of 7/11 equations was moderate, while in 4/11 it was poor. In the cancer group 9/11 equations had poor accuracy.

It was hypothesized that the GFR estimating equations would perform poorly in *all* the studied populations and that ethnicity would be the major predictive factor. Consequently, the results of paper 4 were surprising. The MDRD and CKD-EPI equations performed reasonably well in this population. The accuracy of the MDRD equation was in fact comparable to some of the largest international validation studies (Levey et al. 2009; Earley et al. 2012). It is clear that in some populations ethnicity *is* important. The most well-established of these is in African-Americans in whom ethnicity has been shown to be an independent predictor of higher GFR

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(Levey et al. 1999). However, for our mixed ancestry adult population the results suggest that despite their diverse ethnicity, equations derived from pathologically similar, largely White populations are still useful. This is interesting in light of the generally poorer results in Black and Indian South Africans (Madala et al. 2011; Moodley et al. 2018).

Pathology and mean GFR are two factors that have been shown to affect the performance of eGFR (Stevens et al. 2010). Ideally, a multivariate analysis looking at the accuracy of eGFR in various categories (e.g. GFR, pathology, and BMI) would have been performed, but this was not possible due to the relatively small sizes of each of the subgroups. The fact that the population in paper 4 closely resembled the original MDRD equation development set, with mean GFR < 60 ml/min/.73 m^2 and CKD being present in the majority of participants, is the most likely reason for the good performance of the MDRD equation in particular.

In general, the performance of eGFR in adults and children with cancer was worse than in the non-cancer groups. Reasons for the greater bias and poorer precision in the cancer groups have been discussed in the respective papers. If pathology was the only contributing factor, it is likely that *all* the equations derived from non-cancer populations would have performed better in populations without cancer. However, this was not the case, and in both adult and paediatric populations, there were some exceptions. In the adult non-cancer population (paper 4), the CKD-EPI equation was found to have a P_{30} value of 72.5%, similar to the 69.7% found in the adult cancer population (paper 5) (p=0.6). Similarly, in children (paper 6), the P_{30} values of Gao's QF and the CKiD equation did not differ significantly between the non-cancer and cancer groups (p=0.8 and p=0.1 respectively). While differences may have become apparent with greater statistical power, it is likely that there are other factors that these three equations are better able to correct for. In our populations differences in mean GFR is the most likely secondary cause. The mean GFRs were significantly higher in the adult and paediatric cancer groups compared to the non-cancer groups. Looking at the origins of these equations may explain why they are better suited than other equations. The CKD-EPI and CKiD equations are modifications that were developed to improve the performance of the MDRD and Schwartz equations respectively, in individuals with normal or high GFR (Stevens et al. 2007a; Levey et al. 2009; Pottel et al. 2010; Schwartz et al. 2012). Likewise, Gao et al (Gao et al. 2012) developed their equation to improve performance across the entire GFR range.

A recurring message in papers 4-6 is that interpreting an eGFR is not necessarily straightforward and is highly dependent on the clinical setting. In some settings, such as in the

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management of patients with CKD, an eGFR result based on an equation with P_{30} = 80% is adequate (KDIGO 2013). Based on the results of paper 4, for detection of individuals with GFR < 60 ml/min/1.73m^2, both equations had sensitivities of 97% and specificities 83-88%. However, the same degree of uncertainty would be unacceptable in a potential kidney donor. Similarly, in cancer patients binary decisions are frequently made based on the GFR result. For example, cisplatin is usually only administered if the GFR exceeds 60 ml/min/1.73 m^2. Based on the data from these studies, relying on eGFR will result in a large proportion of patients being misclassified and either being exposed to high risk of nephrotoxicity, or being denied potentially life-saving treatment. In these cases, there is no satisfactory alternative to measuring GFR. It can be argued that providing a 95% CI alongside every GFR estimate would provide more clarity on the information it provides. However, clinicians are unlikely to find this useful. The 95% CIs in two of our populations were extremely wide, which means for example that only when the eGFR result is high (> 90 ml/min/1.73 m^2) can impaired kidney function reliably be excluded.

Estimated GFR is also frequently used in epidemiological studies to determine the prevalence of CKD in a population. Based on the adult non-cancer study (paper 4), the sensitivity of eGFR for detecting low GFR (< 60 ml/min/1.73 m^2) was > 97%. However, these values were based on a population with a high prevalence of CKD, whereas epidemiological studies are conducted in populations with low prevalence. In this context, the result of the adult non-cancer study thus needs to be interpreted with caution. To determine the true utility of eGFR as a screening test for CKD, a large study comparing eGFR to mGFR in a healthy, non-hospital population would be required. However, logistically this would be very difficult. Pooling CKD patients with potential kidney donors, while controlling for confounders, would be a more practical alternative.

Adapting eGFR for local populations

In paper 4, where the existing MDRD and CKD-EPI equations both provided reasonable estimates of mGFR, the equations were not adapted.

Adapting the equations for adults with cancer significantly improved their accuracy. The P_{30} values improved from 57-74% to 78-83%, with 3/4 of the adapted equations having good (> 80%) accuracy. However, despite adaptation, all equations still had poor sensitivity (< 50%)

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for detecting individuals with GFR < 60 ml/min/1.73 m^2, an important requirement in the clinical management of these patients. The 95% confidence intervals of the adapted estimates were wide. Using the original equations, 28-46% of patients would have received carboplatin doses that were incorrect, with only a modest improvement using the adapted estimates.

Adapting the equations for paediatric populations resulted in significant reductions in bias in 3/4 equations in non-cancer patients, and 4/4 equations in cancer patients. In both patient groups, accuracy of 3/4 adapted equations was good, and moderate in 1/4.

Before using eGFR in clinical practice, the performance of the equation/s should be validated in that population. In some of the larger independent validation studies, or in their own validation populations, the P_{30} values of the MDRD, CKD-EPI and CKiD equations ranged between 80% and 84% (Stevens et al. 2007a; Levey et al. 2009; Stevens et al. 2011; Schwartz et al. 2012). It is not expected that the accuracy will exceed these values in populations elsewhere, but it is reasonable to use them as an indication of optimal performance. If the accuracy of the tested equation is found to be inadequate, adapting the parameters of the equation can be reasonably easily achieved using a Microsoft® Excel-based tool. The methodology for this has been described in papers 5 and 6. In paper 4, where the original MDRD and CKD-EPI equations were found to perform adequately, adaptation offered no advantage (results not given in the published paper). However, in the adult cancer, paediatric non-cancer and paediatric cancer groups, adaptation offered a significant improvement and resulted in good accuracy of 3/4 equations in each group.

The use of unvalidated and potentially invalid eGFR equations is unacceptable in populations in which published equations are unlikely to perform well. However, in many such centres the routine use of mGFR or the setting up of large studies to develop local versions of eGFR equations is unrealistic. In this scenario a relatively simple adaptation requiring modest amounts of data may provide a practical alternative. In our cancer population, the minimum number of cases required for a satisfactory adaptation was found to be 40-50 (unpublished data). Unfortunately, the centres that rely on eGFR exclusively are frequently also those without any access to GFR measurement, and validating and adapting equations requires at least *some* access. A solution may be collaboration between state and private institutions serving similar patient populations.

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Conclusion and future directions

The intention of GFR measurement or estimation is to provide a value that is as close to the patient's true GFR as possible. Many factors, including the tracer used, the choice of calculation method, and errors in measurements, may cause deviations from true GFR.

GFR measurement

The first part of this dissertation focused on improving GFR measurement.

Reference data were defined for two QC parameters that may be used to check the result of SI-GFR. However, the low sensitivity of all conventional post-calculation QC tests shown by McMeekin et al (McMeekin et al. 2016)has raised the question of whether QC tests should be used at all and if so, which ones?

Reference data for ^{99m}Tc-DTPA GFR were defined. The data from this study were included in a subsequent meta-analysis (Pottel et al. 2017).

Re-analysis of data from direct comparisons of ^{51}Cr-EDTA and ^{99m}Tc-DTPA demonstrated good agreement, thus providing evidence to support a change from ^{51}Cr-EDTA to ^{99m}Tc-DTPA. This is particularly relevant currently considering to the global unavailability of ^{51}Cr-EDTA.

Paper 3 provided a comprehensive assessment of the impact of measurement errors on GFR:

- The sources of measurement errors that occur during GFR measurement were identified, and their relative importance was assessed.
- The overall impact of measurement errors on the GFR result was determined and compared to expected biological variation in GFR.
- Practical guidance was provided in the form of recommendations for maximum allowable measurement errors, and ways to minimise measurement error.
- The most robust GFR calculation methods were identified, under different circumstances.
- The interpretation of changes in GFR over serial studies was discussed.

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These results, in conjunction with the BNMS guideline recommendations (Burniston et al. 2018), are likely to improve the precision of GFR measurements. However, it must again be noted that this study did not assess the systematic errors, often larger in magnitude, that arise from use of a simplified method to calculate GFR, as opposed to calculation of GFR from the area under the full plasma clearance curve, as this has been addressed previously (Bird et al. 2007; McMeekin et al. 2016a).

Estimated GFR

The performance of estimated GFR is known to be population specific. The second part of this dissertation evaluated eGFR in local populations and demonstrated a simple technique for adapting the estimating equations to improve performance:

The need to first validate eGFR in a population before using it clinically was highlighted.

Estimated GFR was shown to perform adequately in a local, mixed ancestry renal population. This study was instigated in response to a request from nephrologists to validate eGFR locally as it is used routinely in their clinics. The results are therefore reassuring.

In local adult cancer and paediatric populations eGFR was shown to perform poorly. These papers are yet to be submitted for publication, but the intention is to disseminate the results to local oncologists and paediatricians who use eGFR, with the intention of improving local practice.

A technique for adapting existing estimation equations was demonstrated with an improvement in performance being shown in most cases. This methodology may offer a realistic alternative to developing new equations in other centres where existing equations are shown to be invalid.

Practical suggestions were provided for interpreting an eGFR result taking cognisance of the inherent uncertainty associated with GFR estimates.

A limitation of the three eGFR studies is that being based in specific local populations limits the generalisability of the results. However, these studies were initiated in response to questions raised by clinicians about the validity of eGFR in their populations. The results are therefore likely to influence and enhance local practice.

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Future research

This work raises new questions that require further research.

1. QC of mGFR

Klein et al (Klein et al. 2019) recommended a series of QC checks, that are not biologically based, that their department has found useful. These include an assessment of duplicate sample count differences, the correlation coefficient, and the counts-activity-ratio (CAR), as well as a batch of range checks for measurements such as BSA, injected activity, residual activity in syringes etc. Initially, local normal ranges for these QC parameters need to be defined using a departmental GFR database. This would be expected to further improve the accuracy of the GFR measurements being performed in a department. Thereafter, it would be useful to establish the sensitivity of these tests for detecting significant errors in GFR.

When defining a reference range for any QC parameter, practical consideration needs to be given as to what the optimal range should be. Broader ranges (e.g. 2.5 or 3 SD of the mean) tend to increase the specificity of the test but at the expense of sensitivity. An approach suggested by Klein et al (Klein et al. 2019) is a two-tiered system of *warning* and *error* ranges. The ranges for their QC parameters were based on false positive rates of 1:20 for a warning and 1:1000 for an error. A GFR measurement in which one of the QC parameters falls outside its error range requires repeating, whereas a QC parameter that falls outside the warning range requires review but is accepted if no error can be found. This may be a useful approach but requires further validation.

A well-designed study analysing the sensitivity of V_D, T½ and other ancillary QC checks specifically for detecting type 2 (whole curve) and type 3 (individual point) measurement errors is required. The results may assist nuclear physicians/physicists in deciding what method to use in their departments for measuring GFR. If for QC purposes, they do indeed prove to have poor sensitivity, this would provide further support for the decision to switch to methodologies that do not provide these QC parameters.

We have begun to address this issue in a post-hoc analysis of V_D. Using the dataset of paper 3 (786 3-sample GFR measurements), we introduced errors of varying size to (i) the standard counts (type 2) or (ii) one of the sample counts (type 3). Only “plausible” studies (i.e. GFR between 0 and 160 ml/min/1.73 m^2 and sample counts progressively decreasing with time)

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were included. GFR errors > 20% were considered significant. Cases in which V_D in litres was beyond our 2.5 SD reference range (Holness et al. 2013) were considered positive. GFR changed significantly for type 2 errors > 20%. For gross errors in the standard (< -50% or > 120%) V_D had a sensitivity of > 80%. For significant type 3 errors, the correlation coefficient (r < 0.985) was an excellent QC check, far more sensitive than V_D. For the 2-sample SI-GFR (where the correlation coefficient cannot be used), V_D had a sensitivity of > 80% for large errors of the 2- or 3-hour samples (< -50% or > 40%). These early findings suggest that for SI-GFR measurements based on only 2 samples, or performed without injection site imaging, V_D is of some value for the detection of large type 2 and type 3 errors that may not be detectable by other means (unpublished data). While this preliminary work suggests that V_D may only be sensitive for larger errors, this may not equate to V_D being of little utility as a QC check as, in our experience at least, the real world blunders that give rise to type 2 and 3 errors are often crude enough to be flagged by an abnormal V_D, without being detectable from looking at the incorrect mGFR result. While these events should be infrequent, they are probably impossible to completely eliminate, and can be potentially devastating for the affected patient.

The 2004 BNMS guideline (Fleming et al. 2004) suggested a minimum value of 0.985 for the correlation coefficient, whereas McMeekin et al (McMeekin et al. 2016) in a receiver operating curve (ROC) analysis found no cut-off value to be adequate for detecting inaccurate SI-GFR values. However, Klein et al (Klein et al. 2019) highlighted the dependence of the correlation coefficient on GFR and suggested further work to define a GFR-dependent lower limit. This would be worth exploring.

In the updated BNMS guideline (Burniston 2018) the possibility was raised of using eGFR as a QC check for GFR measurements. An advantage of this interesting approach is that eGFR is independent of mGFR and can therefore be used to check any method. Further research could provide insights into *how* to use it optimally, and how effective it would be.

2. Measurement error at low GFR (< 50 ml/min/1.73 m^2)

The 2018 guideline suggests taking blood samples for SS-GFR at 6 h (when GFR is 25 to 50 ml/min/1.73 m^2) or at 24 h (when GFR < 25 ml/min/1.73 m^2) (Burniston 2018). In paper 3, measurement errors were found to have a significant effect on SS-GFR when GFR fell below 50 ml/min/1.73 m^2. However, SS-GFR based only on earlier sampling times (3 h and 4 h) was assessed in this study. It is possible that sampling at the recommended times will address the

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significant errors in SS-GFR, but this will require further research. Similarly, later blood sampling has been recommended for SI-GFR in cases of low GFR (Fleming et al. 2004). Whether this would reduce the impact of measurement errors on the GFR result would also require further research.

Although the radiochemical purity of a ^{99m}Tc-DTPA preparation is usually very high (> 97%), one of the impurities that exists is free pertechnetate ($^{99m}TcO_4^-$). Its presence in a plasma sample in these low levels is unlikely to have a significant effect on the final GFR result. Sampling at 24 h assumes in-vivo stability of the radiopharmaceutical in which case the effect of free pertechnetate will remain negligible, particularly in cases of poor kidney function. Modern ^{99m}Tc-DTPA kits are likely to be stable, however it is prudent to prove this as there were reports of instability of some earlier kits (Millar 1983). This is also applicable to GFR measurements for patients with normal kidney function but expanded ECV (e.g. due to oedema or ascites) as the methodology requires 24 h blood sampling (Burniston 2018; Wickham et al. 2013; Wickham et al. 2015).

3. Repeatability of GFR

In paper 3, the CV of repeat GFR measurements was explored. Values ranging between 7.5% and 12% were found in the literature (Bröchner-Mortensen and Rödbro 1976a; Wilkinson et al. 1990; Blake et al. 1997a; Grewal and Blake 2005b; Delanaye et al. 2008b; Bird et al. 2008). A consequence of these high values is that GFR has to decrease by > 20% before the change can be regarded as significant (Fleming et al. 2004), and decreases of < 20% necessitate repeat measurements after a few months for confirmation. However, this recommendation and the evidence on which it was based raises a couple of issues. Firstly, a 20% change translates to a decrease of only 5 ml/min/1.73 m^2 if the GFR is 25 ml/min/1.73 m^2, whereas for a GFR of 100 ml/min/1.73 m^2, it would be 20 ml/min/1.73 m^2. It may be more useful to express significant changes in absolute terms. Secondly, in one study exercise and a lack of fasting were found to increase the variability of repeat GFR measurements (Wilkinson et al. 1990). In South Africa, transport to and from the hospital is a challenge for the majority of patients, and frequently entails waking up early and walking many kilometres. Restricting exercise and food prior to GFR measurement as recommended in the guidelines is thus often difficult to implement. A study measuring the intra-patient variability of GFR in a South African population, without

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restricting diet or exercise, and expressing the variability in absolute terms, may therefore be a useful contribution to local practice at least.

4. Analysis of error in eGFR

A study quantifying and/or reviewing the contribution of errors in mGFR and Scr measurement to the observed systematic and random errors in GFR estimates would allow more meaningful interpretation of eGFR validation study results and possibly also lead to an improvement in the quality of GFR measurement in validation studies. More sophisticated assessments of eGFR performance, incorporating our knowledge of mGFR errors and biological variation, would be expected to provide better measures of eGFR error. Development of these techniques is an area of potential future research.

5. Utilisation of eGFR as a clinical tool:

In some clinical settings the use of eGFR is well established e.g. in the follow-up of patients with CKD, however there are specific clinical scenarios where further research is required to critically evaluate the use of eGFR given the uncertainty that accompanies the estimates, even for equations that are well suited to the population in which they are applied. The first of these is when binary treatment decisions are made based on whether eGFR is above or below 60 ml/min/1.73 m^2 e.g. for the administration of cisplatin in cancer patients. Another scenario is in the follow-up of cancer patients being treated with nephrotoxic chemotherapy. Previous studies have shown eGFR to be insensitive for detecting changes in kidney function (Hartlev et al. 2012; Lauritsen et al. 2014). Further work is required to confirm or refute this in local populations, and to determine the sensitivity of locally adapted equations for detecting changes. Finally, although the MDRD and CKD-EPI equations were shown to perform reasonably well in adults with CKD, further work is required to determine the sensitivity of eGFR as a screening test for CKD and its accuracy for determining the prevalence of CKD in community-based epidemiological studies.

6. Local adaptation of eGFR equations:

A relatively simple technique for adaptation of eGFR to local populations was introduced in papers 5 and 6 that was able to provide improved performance in independent data. However,

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it requires more extensive and statistically rigorous evaluation of its potential limitations and practical implementation e.g. minimum patient numbers.

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