A COMPARISON BETWEEN ESTIMATED RENAL FUNCTION FROM POINT-OF-CARE DERIVED CYSTATIN C MEASUREMENTS AND SERUM CREATININE DERIVED MEASUREMENTS

by

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KEYWORDS

Cystatin C

Serum Creatinine

Point-of-care device

Metabolic syndrome

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Renal function

Estimated Glomerular Filtration Rate

ABSTRACT

A COMPARISON BETWEEN ESTIMATED RENAL FUNCTION FROM POINT OF CARE DERIVED CYSTATIN C MEASUREMENTS AND SERUM CREATININE DERIVED MEASUREMENTS

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Background: Serum Creatinine (SCr) is the most commonly used and cost effective biomarker used to quantify estimated glomerular filtration rate. However, creatinine is affected by anthropometric parameters such as muscle mass, age and gender. Cystatin-C (CysC) is a low molecular weight protein which is freely filtered through the glomerulus. It has been said to be as accurate as plasma creatinine and is independent of limitations derived from anthropometric parameters. CysC can also be determined through point of care devices which do not require the phlebotomy or experienced laboratory personnel for its use. There have also been correlations noted in CysC serum concentrations and cardiovascular risk assessment. For this reason it is an alternative biomarker of interest in determining renal function and possibly cardiovascular risk.

Objectives: To compare and observe the viability of CysC point of care testing in the primary healthcare setting as opposed to SCr for determining renal function and to explore correlations between CysC and cardiovascular risk assessment scores in a South African population.

Method: The study followed an explorative, descriptive, and cross-sectional design. Data collection took place over two months at the Bishop Lavis Community Health Center (CHC). The study included 105 participants with metabolic syndrome. Cystatin-C levels of the participants were quantified using a Eurolyser CUBE point-of-care device, and SCr plasma concentrations drawn by nursing practitioners were determined by the National Health Laboratory Services (NHLS). The WHO CVD risk chart was used to determine the risk of cardiovascular disease. The categories of CysC findings were compared to CVD risk using Spearman rank correlation. Analysis was carried out using IBM SPSS and Prism Graphpad 9.

Results: Overall, CysC had a moderately significant correlation with SCr concentration (r= 0.51; 95% CI: 0.3484–0.6409; P 0.0001), which was significantly higher than any

correlation that compared the various eGFR equations. CysC was used to reclassify about 17.5% of the patients from the Cockcroft-Gault equation from Stage III to Stage IV. An intermediate risk of developing a CVD within 10 years was assigned to 49.3% of participants. CysC and CVD risk were found to be significantly correlated (r =0.32; P = 0.007 [0.08299 - 0.5173]), according to Spearman's method for determining correlation between two sets of categorical values.

Conclusion: The NHLS and Eurolyser CUBE POC measurements of [CysC] and SCr correlate, indicating that both of these markers assess the kidney's capacity to excrete them. The correlations between the levels of the biomarkers were higher than any estimated GFR from SCr or [CysC]. When using the Cockcroft-Gault formula, there were significant decreases in eGFR when the ideal bodyweight calculated from each participant's BMI was used. Since muscle cells are not the only source of CysC, it is likely to be more useful in an obese population. Additionally, our study found a correlation between CysC and CDV risk. It was discovered that renal function has no bearing on the correlations between CysC and CVD risk. Our research supports the possibility that the Eurolyser CUBE POC device, in comparison to laboratory testing, could be used as a quicker screening test for renal function, particularly in circumstances where lab tests are not readily available.

November 2022

DECLARATION

I declare that this study, entitled, *A comparison between estimated renal function from point of care derived Cystatin C measurements and serum creatinine derived measurements*, is my own work and has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

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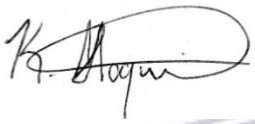
Signature: 

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DEDICATION

I would like to dedicate this thesis to God Almighty for giving me the courage and strength to see this thesis through. Even at times when I was overcome with imposter syndrome, I was reminded that He is faithful. Matthew 6:33; *"Seek first the Kingdom of God and His righteousness and all these things will be added unto you as well"*

LIST OF ABBREVIATIONS AND ACRONYMS

Abbreviation/ Acronym	Description
[CysC]	Cystatin C serum concentration
[SCr]	Serum Creatinine concentration
51Cr-EDTA	51chromium ethylenediaminetetraacetic acid
99mTc-DTPA	diethylene-triamine-pentacetate
ABW	Adjusted Body Weight
AIBW	Adjusted Ideal Body Weight
CHC	Community Health Centre
CHD	Coronary Heart Disease
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CVD	Cardiovascular Disease
CysC	Cystatin C
eGFR	Estimated Glomerular Filtration Rate
GFR	Glomerular Filtration Rate
KDIGO	Kidney disease Improving Global Outcomes
MDRD	Modification of Diet in Renal Disease
MetS	Metabolic Syndrome
mGFR	Measured Glomerular Filtration Rate
NHLS	National Health Laboratory Services
NIH	National Institutes of Health
PAD	Peripheral Artery Disease
POC	Point of care
SCr	Serum Creatinine

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CHAPTER 1: INTRODUCTION

1.1 **Introduction**

The study's background, problem statement, research objectives, and significance are all described in this chapter. This serves to introduce the reader to the researcher's perceived problem and sets out literature to enforce the rationale for this research.

1.2 **Background and Rational for this research**

Renal function is a term used to describe how well the kidneys are working. The kidneys play an integral role in filtering metabolic waste products. The kidneys' ability to filter blood shares a direct correlation with the glomerular filtration rate (Yokota, Benyajati and Dantzler, 1985). Over the past three decades, many different mathematical models derived from various biomarkers have been explored to describe the kidney's glomerular filtration(Sandilands, Dhaun, Dear and Webb, 2013; Lopez-Giacoman and Madero, 2015; Wasung, Chawla and Madero, 2015; Delanaye, Cavalier and Pottel, 2017; Edelstein, 2017; Eiamcharoenying, Kulvichit, Lumlertgul and Chaiwatanarat, 2020; P Ravi, Mitali and S, 2020). Of these, Cystatin C (CysC) and Serum Creatinine (SCr) are actively used in clinical settings to determine glomerular filtration rate. The use of these biomarkers as a standard of care is widespread internationally and has been successful in defining glomerular filtration in a wide array of patients across a variety of different demographics.(KDIGO Guidelines, 2013; Miller *et al.*, 2022)

The use of CysC has been explored in the South African population to some extent and shown to be a potential alternative biomarker (Moodley, Gangaram, Khanyile and Ojwang, 2004; van Deventer, Paiker, Katz and George, 2010; Peer, Lombard, Steyn and Levitt, 2015; Monnet *et al.*, 2019; Fabian *et al.*, 2021).

Adding to the rationale of onsite testing, recent literature (Jernberg *et al.*, 2004; Koenig, Twardella, Brenner and Rothenbacher, 2005; Luc *et al.*, 2006; Deo *et al.*, 2008; Servais *et al.*, 2008; Zethelius *et al.*, 2008; Shlipak *et al.*, 2009) also points to the effectiveness of Cystatin C serum concentrations ([CysC]) as a possible predictor of

underlying atherosclerosis. This capability could hold potential in early detection of coronary heart diseases (CHD).

CHDs are strongly linked to atherosclerosis (Luc *et al.*, 2006). Atherosclerosis is a core component in the development of cardiovascular diseases and their eventual complications are driven by a wide variety of diseases and factors, including: hypertension, diabetes, obesity, sedentary lifestyles, dyslipidaemia, smoking and genes. Virtually all of these risk factors make up or form part of metabolic syndrome (MetS). The therapeutic management of these diseases is essential in reducing morbidity and mortality linked to CHDs.

Cardiovascular diseases (CVDs) is the leading cause of death in the United States of America and the Western World (Gaziano, Bitton, Anand, Abrahams-Gessel and Murphy, 2010). In 2013, CVD was responsible for an estimated 1 million fatalities in Sub-Saharan Africa alone, accounting for 5.5% of all worldwide CVD-related deaths and 11.3% of all deaths in Africa (Mensah *et al.*, 2015).

The development of atherosclerosis is initially asymptomatic and is seldom recognised until symptoms start appearing (Cannon *et al.*, 2004). There is no treatment available at a primary healthcare level within South Africa to reverse or cure atherosclerosis (National Department of Health., 2020). It is a progressive disease that slowly develops and increases the risk of cerebrovascular and cardiovascular emergencies related to CHD such as stroke, myocardial infarction, and pulmonary embolism (National Department of Health., 2020)

Those most at risk of developing atherosclerosis are patients with MetS; in other words, they suffer from at least three of the following: hypertension, diabetes, dyslipidaemia, and obesity (Mathieu, Pibarot and Després, 2006; Grundy, 2008; Guembe *et al.*, 2020). Although the exact number of people suffering from MetS is unknown, a study by Peer *et al.*(2015) found that 30.7% of black people in urban areas in Cape Town suffer from MetS. Their research did not report on any other races. Regardless of the reporting among other races, this research still serves to provide some insight into the incidence of MetS among individuals in the Cape Town metropole.

Patients being treated at the primary healthcare level in South Africa for cardiovascular diseases have six monthly follow up appointments (National Department of Health., 2020). During these different appointment dates, blood samples are to be taken and SCr as well as other laboratory values are meant to be reviewed to direct pharmacological and non-pharmacological therapy (National Department of Health., 2020). Blood samples sent to the National health laboratory services are typically reviewed on a follow up appointment, often 6 months or more after the consultation during which blood samples were taken.

This centralization of laboratory services leads to an increase in patient waiting times and distress caused to the patient (*Eurolyser Cystatin C (GFR) Test Kit | eShop Sysmex Suisse AG*). Decreasing patient waiting times is one of the fundamental goals The National Department of Health wants to improve upon (Mckenzie, Schneider, Schaay, Scott and Sanders, 2017).

With the government's goal of decreasing the waiting times, the researcher explored the possibility of using the Eurolyser CUBE POC measured [CysC] to replace traditional SCr derived eGFR measurements and to assess the value of [CysC] beyond its use as a renal biomarker. This was done by establishing if a link exists between patients' cardiovascular risk score and [CysC].

1.3 **Research Questions**

Can the use of a point-of-care device for determining Cystatin-C serum concentrations be used within a primary healthcare setting to reliably establish eGFR compared to SCr?

1.4 **Aim of study**

To compare and observe the viability of CysC point of care testing in the primary healthcare setting as opposed to SCr for determining renal function and to explore correlations between CysC and cardiovascular risk assessment scores in a South African population.

1.5 **Objectives of study**

Primary Objective

- To correlate the eGFR results obtained from CysC to those obtained from SCr.
- To compare the clinical CKD staging based on the eGFR results obtained from both CystC and SCr.
- To explore the correlation of [CysC] with cardiovascular risk assessment scores.

Secondary Objectives

- To ascertain if a [CysC]obtained from a point-of-care device would add value to patient care in a primary healthcare setting
- To quantify the amount of cases where drug dose-adjustments could be made sooner based on eGFR derived from the Cys C point of care testing on the day patients visit the clinic.

1.6 **Significance of Study**

Exploring different mathematical models for the determination of eGFR derived from SCr and [CysC] will add to the existing body of knowledge on the applicability of various mathematical models within a South African population suffering from MetS. Results from this study will also help determine if eGFR derived from CysC using a point-of-care device can be used to determine a patient's renal function at a clinic setting within the same day by seeing how well the eGFR measurements from [CysC] derived equations correlate with eGFR derived from SCr. This could prompt earlier referral and dosage adjustments of pharmacotherapy. In addition, the results obtained from this research will help identify uses of CysC beyond renal function calculation by exploring the links between coronary heart disease (CHD) disease progression and [CysC].

1.7 **Problem statement**

SCr is currently the only biomarker used in South Africa to predict renal function. This test is performed every six months on patients who visit primary health care facilities. Results from the blood specimen are often only available after the patient has left the facility, as these blood specimens are sent to a centralized off-site laboratory. This

means that any pharmacotherapy related interventions required due to changes in a patient's eGFR can only be made at the patient's next clinic visit, at least a six-month delay with an increased risk for a possible adverse drug reaction. The Western Cape is also heavily burdened with a population suffering from MetS (2015, Peer *et al*). Metabolic syndrome places patients at a higher risk for cardiovascular events. CysC has been successfully used as a biomarker for renal function. Additionally, literature suggests that CysC may be a predictor for cardiovascular risk alongside standard cardiovascular risk assessment tools. The use of a point-of-care device to predict CysC at the primary healthcare level could be beneficial in ensuring prompt therapeutic response to a decreased eGFR and elevations in cardiovascular risk score, over and above the current system's dependence on centralised laboratory reports on SCr.

CHAPTER 2: LITERATURE REVIEW

1.1 **Introduction**

This chapter will explore the literature pertaining to CysC versus SCr in the quantification of estimated renal function, cardiovascular disease, MetS and the use of point-of-care devices in the primary healthcare setting.

1.2 **The Kidney**

The kidneys are two bean-shaped organs that are approximately the size of a human fist(Zheng *et al.*, 2021). They are located just under the rib cage, one on each side of the spine. Every day, the two kidneys filter around 120-150 litres of blood to produce approximately 1-2 litres of urine, which is composed of wastes and excess fluid (Shamsi and Bano, 2017).

The kidneys are important because they maintain the blood's composition, or makeup, which enables the body to function. They help control blood pressure, generate red blood cells (by producing and releasing Erythropoietin), and keep bones healthy by preventing waste and excess fluid build-up in the body, maintaining electrolyte levels such as sodium, potassium, and phosphate, and producing hormones that help control blood pressure and generate red blood cells. The kidney is more than just a large filter. Each kidney is made up of millions of filtering units called nephrons. Each nephron filters a little amount of blood. The glomerulus permits fluid, waste items and drug metabolites to pass through while preventing blood cells and large molecules, typically proteins, from passing through. Following that, the filtered fluid travels through the tubule, which restores essential minerals to the circulation while also eliminating wastes. Urine is the subsequent product. (Murray and Paolini, 2020)

1.3 **Renal function**

Urea was the first recognized biomarker to be used historically to assess renal function (Smith, 1951). New exogenous and endogenous indicators that may be used to measure renal function have been developed over time as a result of new research and in-depth investigation of the human body (Porrini *et al.*, 2019). The most popular and contemporary equations will be utilized for the purposes of this study.

Accurate evaluation of kidney function is a requirement for making well-informed clinical decisions in several domains. The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines recommend measuring the glomerular filtration rate (GFR) using an exogenous filtration marker "in circumstances in which more accurate ascertainment of GFR will influence treatment decisions," such as the dosing of potentially nephrotoxic medications with a narrow therapeutic window or the evaluation of a potential living kidney donor. (KDIGO guidelines, 2013)

1.4 **Glomerular Filtration Rate (GFR)**

A foundation membrane that covered the veins in the uniferous tubes was discovered in 1842 by British surgeon and anatomist William Bowman. He defined it as "...an external tunic of transparent homogeneous tissue lined by epithelium." He gave his name to the glomerular capsule, also known as the Bowman's capsule (Bowman, 1842). According to Jamison (2014), Marcello Malphigi first used a microscope to examine the capillaries near the renal arteries, noticed that large particles do not pass through the capillaries and are thus not expelled, which served as the inspiration for his research. He suggested that the glomerulus was where urine production started. A series of experiments that involved injecting lead acetate and potassium bicarbonate into the renal artery supported this notion.

In the same year, Carl H. Ludwig identified a phenomena that suggested urine was the consequence of reabsorption and ultrafiltration in the glomeruli, challenging Bowman's idea (*Renal Physiology: People and Ideas - Google Books*; Davis, Thurau and Häberle, 1996). Through the use of semi-permeable membranes, Ludwig and his associates were able to detect that blood fluid components flowed through without any proteins. He deduced that the presence of urine was a function of hydrostatic pressure, meaning that this pressure caused filtration and enabled chemical particles from the blood to pass through the membrane's minute holes undisturbed (Davis, Thurau and Häberle, 1996). Then two researchers Wearn and Richards (1924) confirmed this notion by developing a method that allowed them to place a pipette into the glomerulus without ever contacting the capillary tuft or the Bowman's capsule. Ludwig's findings were supported by the swift increase in filtrate volume within the pipette and the discovery that the filtrate was protein-free.

Unbeknownst to these early pioneers, their discoveries would become an integral part of diagnosing kidney disease, staging the diagnosis of renal failure, establishing medication doses and the prognosis for renal related events and death (Lopez-Giacoman and Madero, 2015; Inker and Titan, 2021). The rate at which plasma fluid is filtered into the Bowman's capsule by the glomeruli, a web of capillaries that serves as the kidneys' cleaning mechanism, per unit of time is known as the glomerular filtration rate (Shahbaz and Gupta, 2021). Typically, the rate is calculated by determining how rapidly a substance is cleared from the plasma.

Normal GFR levels might vary depending on anthropometric characteristics; hence, GFR is corrected for body surface area (McIntosh, Möller and Van Slyke, 1928). To calculate the rate, many equations are utilized; these equations frequently consider race, age, gender, and the assessment of endogenous or exogenous renal biomarkers.

A decrease in GFR is routinely used to diagnose kidney failure. A low GFR may indicate cardiovascular illness, an increased risk of hospitalization, and premature death. Because early kidney illness does not cause symptoms, GFR determination is frequently conducted in those who are at a higher risk of developing renal disease (Staples and Wong, 2010).The value of determining renal function is therefore paramount in early detection of renal disease, allowing for the implementation of interventions that would delay the disease's progression (Hsu and Bansal, 2011).

1.5 **Measuring and Estimating GFR**

GFR cannot be measured directly in humans because the filtration process occurs in millions of glomeruli at the same time, while the makeup and volume of the filtrate fluctuates as it passes through the kidney (Soares *et al.*, 2009); therefore there is currently no way to establish the absolute certainty or accuracy without introducing exogenous markers. Measured GFR (mGFR) is calculated by measuring the clearance of an ideal exogenous biomarker (Inker and Titan, 2021). The administration of an exogenous substance for the determination of renal function in patients who have multiple co-morbidities presents an unnecessary risk to their health and is therefore not a method used in clinical practice. As a more practical technique of assessing a patient's renal function, estimated GFR (eGFR) equations that take anthropometric data

into account have been formulated based on filtration and excretion of endogenous biomarkers (Winter, Guhr and Berg, 2012).

1.6 **Renal Biomarkers**

The National Institute of Health (NIH) defines a biomarker as "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic response to a therapeutic intervention"(Atkinson *et al.*, 2001). An ideal filtration marker should be excreted by the kidneys through glomerular filtration, not be protein-bound, and not be secreted or reabsorbed in the tubules(Inker and Titan, 2021). In Homer William Smith's book - (*The Kidney: Structure and Function in Health and Disease - Homer William Smith - Google Books*), the ideal biomarker is also described as being physiologically inert to avoid any additional effects to the body's physiological homeostasis.

There are currently various renal biomarkers that have been used and explored in the past that will be unpacked in two main categories: Those administered to patients, exogenous markers, and those produced by the body, endogenous markers.

Table 1: Summary of Exogenous and Endogenous renal biomarkers

Exogenous filtration markers	**Endogenous filtration markers**
Inulin (5200 Da)	Urea 60 Da
Iothalamate (with ^{125}I) 640 Da	Creatinine 113 Da
Iohexal 821 Da	Cystatin C 13 300 Da
^{51}Cr-EDTA 372 Da	
^{99m}Tc-DTPA 938 Da	

1.6.1 **Exogenous Markers used for mGFR**

Exogenous biomarkers are used to determine mGFR, this measurement entails administering the exogenous marker intravenously at a constant rate (Lopez-Giacoman and Madero, 2015). The mGFR is then determined by drawing blood or urine samples at specified intervals to ascertain the concentration of the exogenous substance present which represents the body's capacity to eliminate the exogenous substance(Smith, 1951)smith. This is of great value clinically since it can aid in predicting the excretion of renally cleared drugs or substances. The problem with this or similar procedures is

that it is time-consuming, invasive, and difficult, making it unsuitable for therapeutic use. This is especially true in those critically ill, in which the introduction of an exogenous substance is an even more questionable practice, leaving the clinical team otherwise devoid of options to safely predict a patient's ability to excrete exogenous substances. (Hsu and Bansal, 2011)

The mGFR may be determined by measuring the renal clearance of exogenous markers such as inulin, iohexol, technetium-labelled diethylene-triamine-pentacetate (^{99m}Tc-DTPA), and 51chromium ethylenediaminetetraacetic acid (^{51}Cr-EDTA).

1.6.1.1 **Inulin**

The gold standard for assessing mGFR is inulin, which has a mean molecular radius of 1.5 nm and a molecular weight of around 5,200 Da (Smith, 1951). Inulin is not protein bound, not reabsorbed, has no effect on renal function, and is not released or processed by the kidney(Sandilands, Dhaun, Dear and Webb, 2013). The test entails injecting a fluid containing the marker molecule into the veins by intravenous infusion and then timed urine samples are collected over the course of several hours. The urine volumes are recorded, and the inulin content of each sample is measured to determine the GFR (Schwartz and Furth, 2007). Inulin clearance is reported to equal GFR when administered intravenously (Dalton, 1999):

$$Cx = ClI = GFR$$

However, such direct assessment of GFR is time-consuming and intrusive, rendering it unsuitable for day-to-day clinical practice (Hsu and Bansal, 2011). In addition to this, inulin is no longer widely accessible and needs substantial technical support. Radioactive substances such as 51Cr-EDTA, 99mTc-DTPA, 125I or 131I-iothalamate are used as surrogate indicators of GFR. However, there are issues with each of these agents: The renal processing of 99mTc-DTPA might vary depending on the commercial source, and 131I-iothalamate is quickly released by the kidney (Rodby, Ali, Rohde and Lewis, 1992).

1.6.1.2 **Iohexal**

Iohexol is a non-ionic contrast agent with a low osmolarity and molecular weight of 821 Da. It is only removed through the kidneys, where it is filtered but not secreted,

metabolized, or reabsorbed. It binds to protein at less than 2%. As a result, it is an excellent marker for establishing mGFR and a viable alternative to radiotracers, which are incompatible with certain patients and need particular handling, storage, and disposal. (Mx, Shuler, Tarfersall and Golper, 1995)

1.6.1.3 ^{51}Cr-EDTA

Garnett *et al*.(1967) introduced 51Cr-EDTA plasma clearance in 1967 as an alternative way to evaluate GFR, and it has been utilized in clinical nephrology since then. Plasma clearance of 51Cr-EDTA is accomplished with a single intravenous injection followed by blood collection at 2, 3, 4, 5, or 24 hours. The higher the concentration of ^{52}Cr-EDTA in the blood stream at later intervals; the lower the mGFR. Several possible problems are connected with the procedure, including failure to inject the dosage, errors in creating the standard dilution, pipetting, and in blood sampling (Granerus and Aurell, 1981).

1.6.1.4 ^{99m}Tc-DTPA

One of the technetium radiopharmaceuticals used in renal imaging is ^{99m}TcDTPA, which is generally used to calculate the mGFR. ^{99m}Tc-DPTA is quickly cleared from the circulation by glomerular filtration after intravenous infusion and is eliminated unaltered into the urine. (Arnold, Subramanian, McAfee, Blair and Thomas, 1975)(Gates, 1982)

1.6.1.5 ^{125}I or ^{131}I-iothalamate

Iothalamate is a radiopaque medium that is water-soluble and iodinated. The pharmacokinetics of ^{125}I-iothalamate (IOT125I) and ^{131}I-o-iodohippurate (OIH131I) are that they both conform to the two-compartment model after intravenous injection. Because of the strong relationship between renal function and clearances, iothalamate may be utilized as a marker of renal function (Welling, Mosegaard, Dobrinska and Madsen, 1976).

Exogenous biomarkers for mGFR are tough substances to work with, and the operations are intrusive. As a result of these drawbacks, alternative clearing techniques that use endogenous markers are employed.

1.6.2 **Endogenous Biomarkers used for eGFR**

Endogenous filtration biomarkers' serum levels are determined by their generation, renal excretion (filtration, secretion, and reabsorption), and extrarenal elimination. Estimating equations incorporate easily measurable clinical indicators as surrogates for estimating these unmeasured physiological processes, resulting in more accurate estimates than serum concentrations alone (Stevens *et al.*, 2006). Estimates of renal function based on endogenous filtration markers may provide a more accurate picture of average renal function over a longer period of time; days to weeks, which is the underlying physiologic parameter of interest. Only in the steady state are serum levels of endogenous filtration biomarkers, and eGFR derived from these biomarkers, likely to provide a reliable estimate of mGFR (Kassirer, 1971).Endogenous GFR markers include serum and urinary concentrations of creatinine [SCr] and Cystatin C [CysC].

Table 2 summarises the various formulas used to determine eGFR using endogenous biomarkers that are discussed in this section.

Table 2: Representative eGFR equations based on endogenous biomarkers in adults through different equations.

Renal Biomarker	Equation	References
Serum creatinine	**Cockroft-Gault** CrCl = (140 – age)/SCr) × weight (× 0.85 if female)	(Cockcroft and Gault, 1976) Requires patient weight, gender and serum Creatinine (sometimes not readily attainable by laboratory staff)
	Cockroft-Gault ABW CrCl ABW=[(140 – age)/(72SCr)] × weight(ABW) (× 0.85 if female)	(Levey *et al.*, 1999)
	MDRD GFR = 175 × $SCr^{-1.154}$×$age^{-0.203}$×1.212 (if patient is black)×0.742 (if female)	(Levey *et al.*, 2009)
	2009 CKD-EPI creatinine eGFR =141 × min(SCr/κ,1)$^{\alpha}$ × max(SCr/κ,1)$^{-1.209}$ × 0.993^{age} [× 1.018 if female] [× 1.159 if black] If female: κ = 0.7, α = -0.329 If male: κ = 0.9, α= -0.411	(Inker *et al.*, 2021)
	2021 CKD-EPI creatinine eGFR= 142 X min(SCr/κ,1)α X max(SCr/κ,1)-1.200 X 0.9938Age X 1.012 [if female]	
Cystatin C	**2012 CKD-EPI Cystatin C** eGFR = 133 × min(CysC/0.8, 1)$^{-0.499}$ × max(CysC/0.8, 1)$^{-1.328}$ × 0.996^{age} [× 0.932 if female]	(Lesley A. Inker *et al.*, 2012)
	Le Bricon eGFR = [(78) x (1/CysC)] + 4	(Le Bricon *et al.*, 2000)
	Larsson eGFR = 77.239 x $CysC^{-1.2623}$	(Larsson, Malm, Grubb and Hansson, 2004)
	Hoek GFR = −4.32 + (80.35 × 1/CysC)	(Hoek, Kemperman and Krediet, 2003)
Hybrids	**2012 CKD-EPI creatinine-cystatin C** eGFR = 135 × min(SCr/κ,1)$^{-\alpha}$× max(SCr/κ,1)-0.601 × min(CysC/0.8, 1)-0.375 ×	(Lesley A. Inker *et al.*, 2012)

	max(CysC/0.8, 1)-0.711 × 0.995age [× 0.969 if female] [× 1.08 if black] If female: κ = 0.7, α = -0.248 If male: κ = 0.9, α = -0.207	(Inker *et al.*, 2021)
	2021 CKD-EPI Creatinine-Cystatin C eGFR= 135 X min(SCr/κ,1)α X max(SCr/κ,1)-0.544 X min(CysC/0.8,1)-0.323 X max(CysC/0.8,1)-0.778 X 0.9961Age X 0.963 [if female]	

CrCl; Creatinine Clearance, SCr; Serum creatinine,CysC; Cystatin C, ABW; Adjusted Body Weight, MDRD; Modification of Renal Disease, CKD-EPI; Chronic Kidney Disease Epidemiology Collaboration

1.6.2.1 **Creatinine**

Creatinine is a small molecule with a molecular weight of 113 Da (Bakker, Gemke, Wijk, Hubeek and Stoffel-wagner, 2018).In skeletal muscle, creatinine is a catabolic product of creatinine phosphate. It is synthesised based on a patient's muscle metabolism and nutrition (meat and creatinine supplements). During muscular contraction, creatine converts high energy phosphate, adenosine diphosphate (ADP), to adenosine triphosphate (ATP). From the conversion of ADP to ATP, creatine's metabolite, creatinine, is released into the bloodstream. During the day SCr is removed from the bloodstream via glomerular filtration. (Hosten, 1990)

According to the findings of Delanghe and Speeckaert (2011), Jan Horbaczewski (1854–1942, professor of medicinal chemistry at the Czech medical faculty in Prague) was the first person to synthesize creatinine. A discovery made a few years later by German pharmacologist Max Jaffe in 1886 caused a breakthrough in biomedical research. He discovered the Jaffe reaction, a colorimetric technique used in clinical chemistry to detect creatinine levels in blood and urine (Jaffe, 1941). Unfortunately, Jaffe may not have been aware of creatinine testing's diagnostic use in nephrology (Delanghe and Speeckaert, 2011). Despite the development of more accurate enzymatic tests, the Jaffe principle's exceptional simplicity guaranteed that this test had a long-standing influence. Creatinine was often quantified using the Neubauer reaction toward the close of the nineteenth and beginning of the twentieth centuries (adding an alcoholic solution of zinc chloride to a creatinine-containing solution, yielding a complex with two molecules of creatinine and one molecule of zinc chloride). The quantity of precipitated creatinine was determined by weighing the precipitate and multiplying the result by 0.642. (Neubauer, 1866)

Creatinine's importance as a renal marker molecule was only realized thanks to the pioneering work of Poul Kristian Brandt Rehberg (1895–1989). In 1926, Rehberg (University of Copenhagen's Zoophysiological Laboratory) postulated that creatinine was filtered through glomeruli and concentrated in the tubules, without being reabsorbed or excreted (Rehberg, 1926). He was the first to propose the use of creatinine for exogenous administration. Endogenous creatinine could not be relied on since there were numerous compounds in the serum that were not creatinine but caused Jaffe's response. (Cook, 1975)

Total daily creatinine excretion has long been used to estimate muscle mass in humans and animals (Kalantari and Kline Bolton, 2013). This is because practically all of the body's creatine is located in skeletal muscle and is converted to creatinine nonenzymatically and at a steady pace (Kalantari and Kline Bolton, 2013). In people with normal kidney function, the only route of elimination for creatinine is renal excretion, which is supposed to be constant and equal to its production at steady state(Kalantari and Kline Bolton, 2013). Creatinine clearance (CrCl) and GFR can both be determined using [SCr] in blood and urine (Shahbaz and Gupta, 2021).Overall, the relationship between [SCr] and GFR is inversely exponential, not linear (Stevens *et al.*, 2006; Jin *et al.*, 2008). Before plasma levels of creatinine increase over the upper normal reference range, the true GFR may fall to around half of normal.

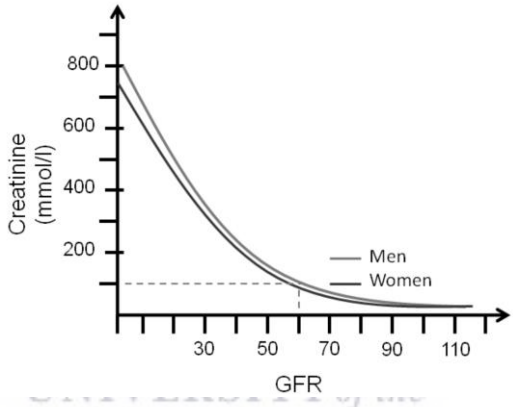

Figure 1: Relationship between creatinine and GFR

Adapted from Levey et al.(1999)

1.6.2.1.1 **Serum creatinine**

The quantity of creatinine in your blood is measured by a SCr test, which serves to quantify the amount of SCr present in the bloodstream (Cockcroft and Gault, 1976). The decrease in [SCr] is due to the marker's quick transit from the plasma into its volume of distribution (fast component) and renal excretion (slow component). This is best calculated using a two-compartment model that necessitates early blood collection (Schwartz and Furth, 2007). The glomerulus filters most of the SCr before it is reabsorbed in the proximal tubule, which accounts for 10–20% of the excreted amount. When renal production and filtration are at steady state, SCr measurements are mostly utilized. (Schwartz and Furth, 2007)

The reference values for healthy CrCl levels differ depending on age and gender. In general, men's reference values vary from 97 to 137 ml per minute, whereas women's values range from 88 to 128 ml per minute (Shahbaz and Gupta, 2021).

Clearance is calculated by dividing a drug's excretion rate by its plasma concentration Clearance is measured in millilitres per minute and is commonly standardized to a standard 1.73 m^2 idealized adult body surface area (Clearance in millilitres per minute per 1.73 m^2) using the formula 1.73/body surface area (BSA), where BSA is the tested subject's body surface area (in square meters). (Schwartz and Furth, 2007)

1.6.2.1.2 **Urinary creatinine**

Creatinine is tested from a 24-hour urine sample when a less intrusive approach of assessing renal function is needed. A urine creatinine test measures the total quantity of creatinine in all urine produced over the time period of 24 hours. Women have a normal creatinine range of 6 to 13 mmol/24 hours, whereas males have a range of 7 to 14 mmol/24 hours (Hosten, 1990). Clearance is calculated by multiplying the urine concentration of creatinine by the volume of the timed urine sample and dividing the result by the average plasma concentration for the same time period. Timed urine samples are susceptible to inaccuracy due to imprecise time-keeping and incomplete urine collection. (Rowe, Andres, Tobin, Norris and Shock, 1976)

1.6.2.1.3 **SCr formulas used in clinical practice for adult patients in South Africa**

Cockroft-Gault (Cockcroft and Gault, 1976)

The first formula to estimate CrCl from [SCr] was devised by Donald W. Cockcroft and Henry Gault. The formula was developed based on observations collected on a cohort of 249 male patients ranging in age from 18 to 92 years. The formula required patient weight, age, gender and SrCr. A correlation coefficient of 0.83 was found to exist between the predicted and measured means of CrCl when the equation was applied.

MDRD (Levey *et al.*, 1999)

To simplify GFR prediction, (Levey *et al.*, 1999) devised the Modification of Renal Disease (MDRD) equation. A cross-sectional study of GFR, CrCl, [SCr], and demographic and clinical characteristics was performed. Stepwise regression was used to develop the prediction equation. The equation was then compared to other validation sample equations. Only demographic and serum variables were used to predict GFR. The MDRD Study equation estimated GFR more accurately than CrCl or other equations.

CKD-EPI

The National Institutes of Diabetes, Digestive and Kidney Diseases developed the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. A study authorized by the institutional review boards of all participating institutions was conducted to develop the equation. Using a data base collected from ten trials, the CKD-EPI equation was designed to predict GFR in people using [SCr]. Additional data from 16 studies were amalgamated to verify the CKD-EPI equation and demonstrate that it is more accurate than the MDRD Study equation. The CKD-EPI equation is believed to have decreased bias, particularly when estimated GFR is more than 60 mL/min/1.73 m^2, although accuracy remains restricted. The CKD-EPI equation's increased accuracy overcomes some of the shortcomings of the MDRD Study equation. (Levey *et al.*, 2009)

In 2021, Inker *et al.*(2021) developed a new creatinine equation which increased population estimates of CKD prevalence among black people while yielding similar or lower prevalence among non-Blacks when compared to the current creatinine equation.

1.6.2.1.4 **SCr derived eGFR calculations in obese patients**

Cockroft-Gault using Adjusted Body Weight (ABW) (Winter, Guhr and Berg, 2012)

To assess the influence of various body weights and [SCr] on the bias and accuracy of the Cockcroft-Gault CrCl equation compared with measured 24-hour CrCl, an alternative Cockcroft-Gault equation was developed. According to the adjusted body weight correction, a 40% correction factor should be used in the Cockcroft-Gault equation if a patient's actual weight is 30% or more than their ideal body weight. Adjusted body weight = 0.4 (actual body weight - ideal body weight) + ideal body weight.

Salazar Corcoran Equation (Salazar and Corcoran, 1988)

An animal model of obesity was used as the starting point for studies into estimating CrCl in obese patients. In both obese and normal animals, the mean CrCl was found to vary in direct proportion to fat-free body mass. By reviewing studies that have been published and that detail the rates of CrCl and excretion in obese and normal people, it was determined whether this observation has any bearing on human renal function. When normalized to fat-free mass, measured CrCl showed a strong correlation with age (r = 0.960) and well with estimated fat-free body mass (r = 0.772, p 0.02). These observations were used to create a formula that forecast CrCl at steady state in obese patients, often referred to as Salazar Corcoran's equation.

1.6.2.2 **Cystatin C**

CysC is a low molecular weight (13kDa) protein which is endogenously produced at a constant rate and positively charged at physiological pH which suggests that it is easily filtered via the glomerulus (Daniel *et al*., 2009). It is not reabsorbed nor secreted by the renal tubules. It has been said to be as accurate as SCr and is independent of body composition. CysC therefore meets the key criteria of an ideal endogenous glomerular filtration rate marker (Seape, Gounden, Van Deventer, Candy and George, 2016). CysC belongs to the family of papain-like cysteine proteinase inhibitors (Grubb, 2000). The concentration of hCC in the blood correlates with the GFR (Grubb *et al.,* 2014). They are widely spread and may be detected in almost all bodily fluids. In addition to being inhibitors that impede the action of thiol proteases; they also play an important role in a number of clinical disorders (Shamsi and Bano, 2017).

CysC's history started in 1961, when Jorgen Clausen discovered a "cerebrospinal fluid-specific" protein in human cerebrospinal fluid (CSF), which he termed γ-CSF. Since then, γ-CSF was renamed as CysC and has been discovered in urine, human plasma, ascetic fluid, and pleural fluid. Löfberg and Grubb, (1979)also discovered a γ-trace in human bodily fluids. Since then γ-CSF and γ-trace have been identified as CysC. Now well known as a reliable endogenous biomarker used in estimating glomerular filtration. Serum, saliva, seminal, synovial, and cerebral fluids all been identified to have significant levels of CysC. CysC secretion has a strong association with GFR when compared to the gold standard exogenous GFR indicators, such as Cr-EDTA and inulin. (Van Deventer, George, Paiker, Becker and Katz, 2008)

Recently, there have been findings of a connection between CysC and variables unrelated to GFR. As GFR decreases with age, a link between CysC and age is anticipated (Helmersson-Karlqvist, Flodin, Hansson and Larsson, 2013). Therefore, distinct CysC reference levels have been established for normal renal function in young adults and those over the age of 50 (Finney, Newman, Thakkar, Fell and Price, 2000).There are contradictory findings on whether CysC levels change by gender, however after age 60, there is no difference between men and women. Protein consumption has no effect on [CysC] (Tangri *et al.*, 2011).

It has been theorised that the serum CysC level is a more accurate indicator of the GFR than the SCr level because it has no relationship to sex or muscle mass (Stevens *et al.*, 2009). However, it has been proven to be affected by extra-renal factors; higher production in patients with hyperthyroidism, MetS and underlying atherosclerosis and lowered in patients with hypothyroidism and taking those oral glucocorticosteroids. (Zethelius *et al.*, 2008; Shlipak *et al.*, 2009).

1.7 **CysC derived eGFR**

2012 CKD-EPI Cystatin C (Inker et al., 2012)

The CKD-EPI working group published a new CKD-EPI equation that incorporates [CysC]. They developed estimating equations based solely on CysC using cross-sectional analyses. They validated that the new equation was superior to existing equations across the GFR range and in relevant subgroups. Even participants with an extreme body mass index (BMI) of less than 20kg/m^2 could benefit from the use of this equation.

Le Bricon (Le Bricon *et al.*, 2000)

Le Bricon's equation was developed as part of an investigation into whether CysC was more accurate at predicting glomerular filtration rate three months after kidney transplantation than 24-hour CrCl and SCr. The study found that GFR derived from CysC may have been underestimated by 14% in the three months following transplantation compared to SCr due to the usage of glucocorticosteroids. Therefore indicating possible limitations in patients on glucocorticosteroids.

Larsson (Larsson, Malm, Grubb and Hansson, 2004)

In order to give clinicians accurate and accessible GFR data based on discrete measurements of [CysC], the Larsson equation was created. This was done in order to overcome the lack of a formula for converting CysC expressed as mg/L to GFR expressed as mL/min. This study compared iohexol clearance to SCr and CysC levels. The relationship between CysC and iohexol clearance shared a significant correlation over the correlation between [SCr] and iohexal (p 0.0001, r2 = 0.84).

Hoek (Hoek, Kemperman and Krediet, 2003)

Using the Cockroft-Gault equation (which estimates GFR/1.73 m^2 based on information on body weight, age, height, and sex as well as [SCr]), Hoek *et al*.(2003) investigated the accuracy and precision of the eGFR calculation when using the [CysC] results in the same manner. The equation provided a good estimate of GFR that was more accurate and precise than the Cockroft-Gault equation and displayed a strong correlation with mGFR.

SCr and CysC Hybrid formulas

The combination of SCr and CysC equation is said to be beneficial as a confirmatory test for chronic renal disease. Such an equation was found to perform better than equations that were based on either of these indicators alone (Inker et al., 2012).

In 2021, a new eGFR equations free of race was created. Using both established and new equations, the prevalence of chronic kidney disease (CKD) and GFR stages were predicted in a sample of adults. With less variation between racial groups, new SCr and CysC equations were more accurate than new SCr equations. (Inker *et al.*, 2021)

1.8 **CysC versus SCr for eGFR**

SCr is convenient and cost-effective to use (Daniel *et al.*, 2009). However, creatinine is insensitive to asymptomatic acute changes in the filtration function and a 24-48 hour delay in creatinine response to underlying functional change (Nei *et al.*, 2021). One significant disadvantage of creatinine as a GFR measure is that it is not a sensitive marker of early CKD. CysC has become an alternative biomarker of interest with stronger and more linear risk relationships to that of SCr (Astor *et al.*, 2013). Unlike creatinine, CysC is not released by renal tubular epithelial cells, however they reabsorb and catabolize it, preventing it from returning to the circulation. (Reed, 2000)

Clinical uses of CysC in comparison with SCr

Several studies have stated that CysC has several advantages over SCr. Globally, CysC is used as a confirmatory test for renal impairment. The 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guideline recommends that clinicians use the creatinine-based eFR (eGFRcr) as an initial test and CysC-based eGFR (eGFRcys) or SCr and CysC based eGFR (eGFRcr-cys) or measured clearances as confirmatory tests for the detection of CKD (Levin and Stevens, 2014). In South Africa, the use of CysC as a renal biomarker is still being explored.

1.9 **Global and local application of Cystatin C as a renal biomarker**

CysC's use as a replacement to SCr in determining eGFR has been explored since 1995(Jung and Jung, 1995).The independence from height, gender, age, and muscle mass is considered to be advantageous. Select patient groups such as children, the elderly, and patients with reduced muscle mass benefit in particular. Filler *et al.*(2005) found that CysC is at least equal if not superior to SCr as a marker of GFR.

In around 80% to 85% of instances, projected GFR-values derived from CysC or SCr equations are within 30% of mGFR derived from invasive gold standard techniques using exogenous markers. However, the greatest proportion of predicted GFR-values within 30% of observed GFR is attained by using GFR-prediction equations based on both CysC and SCr. (Grubb, 2010; Grubb, Nyman and Björk, 2012)

In research that compared exogenous and endogenous biomarkers used to calculate mGFR and eGFR to four outcomes, all-cause mortality, cardiovascular disease mortality, and kidney failure, composite outcome of kidney failure and all-cause mortality, mGFR was not superior to eGFR in predicting any of the four outcomes. (Hsu and Bansal, 2011)

A cross sectional study was conducted in black men and women living in Cape Town, South Africa. The aim was to determine the prevalence of CKD determined by five eGFR formulas which included CysC based equations. CKD prevalence was higher when CysC based equations were used compared to creatinine based equations. In this study the majority of participants who were diagnosed as having CKD using CysC

based equations did not have CKD according to creatinine based equations. The authors suggest that the current eGFR marker, creatinine is not the best estimator for eGFR in black South Africans. They also concluded that the prevalence estimates can vary within a population due to different eGFR equations used. (Peer *et al.*, 2009)

According to a study by Herget-Rosenthal *et al.*(2005), CysC detects rapid GFR declines one to two days before SCr does. Another study by Vaidya and Aeddula (2022) discovered that 85% of people experienced a GFR decline of 4-5 mL/min/year on average. After the initial discovery of an abnormal value, a daily increase in a renal biomarker suggests at least a portion of an ongoing acute process. In contrast, a renal biomarker that shows little to no change over a period of weeks to months may indicate the presence of CKD. The frequency of laboratory monitoring should be determined by the severity of CKD, rate of eGFR decline, changes in clinical symptoms, and other factors (such as the dependability of patient follow-up). Overall, early retesting to establish a baseline should be prompted by an eGFR decline of more than 25%, or 5 mL/min/1.73 m^2, per year. (KDIGO guidelines, 2013) The ability of GFR classifications (see Table 3) to predict CKD progression allows for earlier detection of the disease's progression, which may improve CKD management.

Table 3: Classification of CKD (Inker *et al.*, 2014)

Stage	Description	GFR (mL/min/1.73m^2)
1	Normal	≥90
2	Mildly decreased	60 – 89
3	Moderately decreased	30 – 59
4	Severely decreased	15 – 29
5	Kidney failure	<15 (or dialysis)

1.10 **Chronic Kidney Disease**

Chronic kidney disease (CKD) is a clinical illness caused by the irreversible alteration of the function and/or structure of the kidney and is defined by its slow and gradual progression. The pathology is also associated with an increased risk of complications and death, particularly cardiovascular-related. (Lamb, Levey and Stevens, 2013)

Epidemiology

The Global Burden of Disease Study 2015 showed that CKD ranked 12th globally for leading causes of death (Wang *et al.*, 2016). The number of patients suffering from

CKD has been on the increase, in part because of the growth in the prevalence of risk factors including obesity and diabetes mellitus. It is estimated that 843.6 million people throughout the globe were afflicted by CKD in 2017 (Jager *et al.*, 2019). The results of a 2018 meta-analysis that examined 98 432 individuals from 98 studies in Africa reported a prevalence of CKD stages 1–5 of 15.8% (95% CI 121–199) and a prevalence of CKD stages 3–5 of 46% (33–61) in the general population (Kaze, Ilori, Jaar and Echouffo-Tcheugui, 2018).It has also been found that the incidence of CKD is much greater in females than in men (Carrero, Hecking, Chesnaye and Jager, 2018).

In addition to its high prevalence, chronic kidney disease (CKD) also predisposes patients to an increased risk of cardiovascular disease (CVD), with an increased severity and mortality in those with co-morbid CVDs (Hill *et al.*, 2016).

1.10.1 **Impact of CKD on medication prescribing**

Patients who already have kidney disease are vulnerable to additional kidney damage and metabolic disturbances from medications, which can exacerbate the condition. The chance to reduce medication-related patient safety threats is lost if patients with CKD are not recognized (Whittaker, Miklich, Patel and Fink, 2018). A study by Chertow *et al.*(2001) focussing on dosage adjustments based on renal function discovered that 15% of patients had at least 1 dosing parameter modified. The study concluded that guided medication dosing for patients with renal insufficiency appears to result in improved dose and frequency choices.

The following medication should be avoided or stopped when kidney failure has been established in a patient (Aggarwal, Harber and Laing, 2014):

- Angiotensin-converting enzyme inhibitors (ACEI) and angiotensin-II receptor Blockers (ARBs)
- Non-steroidal anti-inflammatory (NSAIDs)
- Cyclo-oxygenase-2 (COX-2) inhibitors
- Biguanides i.e. Metformin
- Some analgesics (e.g. renally cleared opioids)
- Disease-modifying antirheumatic drugs (DMARDs) (e.g. methotrexate)

The following medications require dose adjustment or frequent monitoring when administered in patients with confirmed CKD (Aggarwal, Harber and Laing, 2014):

- Aminoglycosides
- Anticoagulants
- Anticonvulsants (e.g. phenytoin, gabapentin)

- Antivirals (e.g. acyclovir, ganciclovir)
- Antiretroviral agents (e.g. lamivudine, tenofovir)
- Digoxin
- Immunosuppressants (e.g. cyclosporin)
- Hypoglycaemic medicines (e.g. metformin)

Many of these drugs are prescribed according to the primary healthcare standard treatment guidelines for primary healthcare and are contained within the essential drug list (National Department of Health., 2020).

1.10.2 **Treatment and management of CKD**

The goals of treatments for CKD include lowering the risk of CVD, slowing the progression of CKD, treating complications of CKD, and, if possible, controlling the underlying cause (Turner, Bauer, Abramowitz, Melamed and Hostetter, 2012).

The fact that CKD is an independent, potent, and potentially modifiable risk factor for CVD is the most significant aspect of the condition (Muntner, He, Hamm, Loria and Whelton, 2002). According to the Chronic Kidney Disease Surveillance System, CVD is the main cause of morbidity and mortality in people with CKD. The results of research reported in the medical literature suggest that all patients with CKD should be considered to be at high risk for CVD, evaluated based on "traditional" and "non-traditional" risk factors for CVD, and treated for the reduction of modifiable cardiovascular risk factors. (Levin, 2003)

1.11 **Cardiovascular Disease**

The term cardiovascular disease refers to a broad range of conditions, some of which include illnesses that affect the cardiac muscle as well as the vascular system that supplies the heart, brain, and other essential organs (Gaziano, Reddy, Paccaud, Horton and Chaturvedi, 2006).

Epidemiology

CVDs are thought to be the leading cause of death worldwide. In 2019, an estimated 17.9 million people died from CVDs, accounting for 32% of all global deaths. Heart attacks and strokes were responsible for 85% of these deaths. More than three-quarters of CVD deaths occur in low- and middle-income countries. CVDs were responsible for

38% of the 17 million premature deaths (under the age of 70) caused by non-communicable diseases in 2019. (Roth *et al.*, 2020; Amini, Zayeri and Salehi, 2021) CVDs, are the second leading cause of mortality in Africa, particularly in Sub-Saharan Africa (SSA), where they account for 74% of global deaths and 80% of all premature deaths each year (Nzali, Temgoua, Tochie and Choukem, 2021).

1.11.1 **Types of Cardiovascular Diseases**

In Sub-Saharan Africa, the three most common causes of CVD death are Coronary Heart Disease (CHD), stroke, and hypertensive heart diseases. (*VizHub - GBD Results*; Mensah, 2013)

Coronary Heart Disease

Coronary heart disease is the leading cause of death in developed countries and one of the primary contributors to the disease burden in developing countries (Gaziano, Bitton, Anand, Abrahams-Gessel and Murphy, 2010). The two most common symptoms of CHD are angina and a sudden myocardial infarction.

The ACCESS (ACute Coronary Events - a multinational Survey of current management Strategies) registry data from 615 South African patients admitted to hospitals with ischemic heart disease showed a 12-month case-fatality rate of 5.7%, with higher mortality seen in persons under the age of 70, those who also had type 2 diabetes, or those who had a history of cerebrovascular disease. (Schamroth, 2012)

Stroke

Globally, stroke is the main cause of disability, dementia, and death. A disruption or reduction in the blood flow through the brain's blood vessels, leading to insufficient perfusion of brain tissue, is what constitutes a medical emergency. The three main types of stroke are ischemic stroke, haemorrhagic stroke, and transient ischemic attack. Contrary to the global trend, which saw a sharp decline in age-standardized stroke mortality rates between 1990 and 2016, this did not happen in Africa, where the annual rate was around 316 per 100,000, with a prevalence of 1460 per 100,000 and a mortality incidence of >80% within 3 years of disease onset (Zeleňák *et al.*, 2021). Low- and middle-income countries, including those in Africa, were responsible for about 70% of all fatalities and 87% of all injuries between 1970 and 2010. (Zeleňák *et al.*, 2021) Ghana's death rate for stroke patients continues to be 40%, and

Mozambique's is 70% due to insufficient implementation of evidence-based guidelines for stroke care in Africa (Baatiema, Chan, Sav and Somerset, 2017). Between 5.5% and 11% of people in African populations die from stroke overall (Akinyemi *et al.*, 2021).

Hypertensive Heart disease

Hypertension is one of the risk factors for CVD that is associated with the strongest evidence for causation and has high exposure prevalence. In research and clinical practice, normal blood pressure levels have typically been described as being much higher than what is considered to be biologically normal (120mmHg systolic and 80 mmHg diastolic) (Fuchs and Whelton, 2019).

From 1999 to 2013, a systematic review of 33 studies from 15 Sub-Saharan African countries found a prevalence range of 15-70% for hypertension (Addo, Smeeth and Leon, 2007). The systematic review also highlighted the region's lack of hypertension awareness, treatment, and control (Ataklte *et al.*, 2015). According to current WHO estimates, the prevalence of hypertension in all African regions is seemingly shifting towards the upper end of historical figures (typically 35-45% of adults), and will be a major contributor to highly preventable CVD events in the foreseeable future. A 2005 Gabonese population study involving 736 patients aged 40 years found that CVD was largely caused by high rates of hypertension, with high blood pressure being predictive of stroke and peripheral arterial disease (PAD) (Ngoungou *et al.*, 2012).

1.11.2 **Risk Factors**

Several risk factors play a big role in how likely it is that you will get a CVD. The main factors that place individuals at risk for CVD include smoking, high blood pressure, high blood glucose, lipid abnormalities, being overweight, and inactivity. Rates of CVD change around the world due to these known risk factors changing over time (Gaziano, Reddy, Paccaud, Horton and Chaturvedi, 2006). Smoking cessation and dietary changes are key secondary preventive targets for CVD. Cardiac rehabilitation, including exercise, benefits a broad spectrum of CHD patients and lowers future vascular events by roughly 15%. Exercise alone is said to reduce vascular mortality by 24% and vascular endpoints by 15%. (Brinks, Fowler, Franklin and Dulai, 2017)

1.11.3 **Cardiovascular risk score assessments**

Investigators at Framingham, Massachusetts, came up with the concept of coronary risk factors. They identified age, hypertension, smoking, diabetes, and dyslipidaemia as the primary variables that determine coronary heart disease by studying large cohorts across multiple years. The research that started in Massachusetts has propagated across the world and generations. (Doyle, Dawber, Kannel, Kinch and Kahn, 1964; Kannel, Wolf, Benjamin and Levy, 1998; Dawber, Moore and Mann, 2015). These risk factors and the control patients had over their individual values resulted in the creation of variables and population-based coefficients. These could then be used to individualise and calculate the eventual standardized risk scores, estimating a patient's chances of having a stroke or myocardial infarction within the next 5-10 years. The use of calculations and formulae integrating these variables and coefficients has become widespread and has helped to identify the relative chances each patient has to undergo a cardiovascular event/accident for many patients across the world which are used for evaluating the risk of cardiovascular disease. (Wood *et al.*, 1998; Expert Panel on Detection, 2001)

The World Health Organization (WHO) has recognized the critical significance of investing in the prevention of non-communicable diseases (NCDs) as well as in community screening programs. This is important not only for the ability to reach large portions of the population in a manner that is efficient but also for the development of community-based models of care for disease management. This is essential to achieving success in the reduction and management of NCDs. ('NCD Alliance's submission to the first WHO consultation on the updated Appendix 3 of the Global action plan for the prevention and control of NCDs 2013-2030', 2022)

Models that estimate the risk of cardiovascular disease are very useful for both the prevention and treatment of cardiovascular conditions. There are a variety of different methods for estimating risk. (Conroy *et al.*, 2003; Woodward, Brindle and Tunsfall-Pedoe, 2007; Hippisley-Cox *et al.*, 2008; Jahangiry, Farhangi and Rezaei, 2017)

One of the most significant challenges in performing laboratory-based screening in developing countries is a lack of financial and physical capacity to conduct the large-scale laboratory testing required to implement established laboratory-based risk scores.

Screening tests, such as the Framingham, require HDL and total cholesterol levels, which cannot be tested in every patient.

With these limitations in mind, the WHO and the International Society for Hypertension (ISH) created separate risk charts for developing world regions that include and exclude laboratory measures (i.e., cholesterol values). The non-laboratory-based charts, in particular, only require age, gender, smoking status, systolic blood pressure, and diabetes history to estimate total CVD risk.

The results of a study by Pandya *et al*.(2011) showed that non-laboratory-based risk scores and laboratory-based risk scores had a high level of agreement in terms of describing risk. This finding suggests that in resource-constrained settings, the non-laboratory-based score can serve as a reliable substitute for Framingham or SCORE functions.

1.12 **Metabolic syndrome**

Haller *et al*.(1975) coined the phrase "metabolic syndrome" to refer to a condition that occurs when a person has dyslipidaemia, diabetes, high blood pressure (hypertension), and obesity all at the same time. It makes the likelihood of developing coronary heart disease, stroke, and other illnesses that impact the blood vessels much higher.

Since that time, numerous international organizations and expert groups, such as the World Health Organization (WHO) (Alberti and Zimmet, 1998), the European Group for the study of Insulin Resistance (EGIR) (Balkau and Charles, 1999), the National Cholesterol Education Program Adult Treatment Panel III (NCEP:ATPIII) (Expert Panel on Detection, Evaluation and Treatment of high Blood Cholesterol in Adults; 2001), the American Association of Clinical Endocrinology (AACE) (Einhorn *et al.*, 2003), the International Diabetes Federation (IDF) (Alberti, Zimmet and Shaw, 2005), and the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) (Grundy, Brewer, Cleeman, Smith and Lenfant, 2004), have endeavoured to incorporate these findings into their respective guidelines and recommendations.

The NCEP: ATPIII definition is one of the criteria for MetS that is utilized the most often. It considers the most important aspects of hyperglycaemia and insulin resistance, visceral obesity, atherogenic dyslipidaemia, and hypertension. It does this by making use of measures and test findings that are easily accessible to doctors, which makes its implementation in clinical and epidemiological settings much simpler. It is also easy to remember due to its simplicity. It is important to note that it does not need that any particular criterion be satisfied; all that is required is that at least three out of five requirements be met. As a result, the definition of MetS does not include any preconceived notions about the underlying cause of the condition, whether that reason is insulin resistance or obesity.

1.12.1 **Epidemiology**

In order to ascertain the prevalence of MetS, a cross-sectional study was conducted in the community of Bellville South in Cape Town. MetS was defined using the IDF, ATP III, and the Joint Interim Statement (JIS) from 2009. In contrast to the IDF definition's value of 60.6% and the NCEP: ATP III value of 55.4%, the JIS definition of MetS had a crude prevalence of 62.0%. (Erasmus *et al.*, 2012)

According to the JIS definition (Alberti *et al.*, 2009), a person is considered to have MetS if three out of the five criteria are present in their body: high blood pressure, high triglyceride levels, low high-density lipoprotein levels, central obesity, and hyperglycaemia.

There is growing evidence that the prevalence of MetS is not only concerning on a global scale, but also prevalent in the South African population. According to the findings of Kruger & Nell, (2017), the prevalence of MetS is quite substantial in South Africa's Western Cape province.

The main causes of MetS are said to be related to inactivity and a diet high in fats and carbohydrates are the primary contributors to the development of MetS, which has two primary clinical characteristics: central obesity and insulin resistance. According to the findings of a research, females may be at a greater risk than males of acquiring the MetS. (Kruger and Nell, 2017)

1.12.2 **Management and Prevention**

All current guidelines for the management of the individual components of MetS emphasize lifestyle modification (weight loss and diet modification) as the first-line treatment. Despite the fact that therapeutic lifestyle modification is the first-line treatment for MetS and therefore warrants initial consideration, drug therapy may be required in many patients to achieve the recommended goals. Risk assessment in patients with MetS is essential for establishing therapeutic goals. (Grundy, 2008; National Department of Health., 2020)

1.13 **MetS and the link to cardiovascular risk**

MetS is associated with an increased risk for cardiovascular risk (Guembe *et al.*, 2020). Evidence shows that even patients without Type II diabetes mellitus are still at high risk for cardiovascular events. The presence of MetS can therefore be used to identify patients who are possibly at higher risk for cardiovascular events (Mottillo*et al.*, 2010).

In South Africa, two main cardiovascular risk assessment tools are recommended by the Department of Health; the BMI based risk assessment and the Framingham risk score. Combined, both these scores assess the patient's weight, blood pressure, lipid profile and presence of diabetes to determine the percentage risk a patient faces over a 10 year period towards experiencing a cardiovascular event. (National Department of Health., 2020)

In a European community, having MetS, however one chooses to define it, is linked with a risk of incident cardiovascular morbidity and death that is about two times higher than average (Dekker *et al.*, 2005).

1.14 **Cystatin C and Cardiovascular disease risk**

Research by Parikh *et al.*(2008) explored the correlations between CysC levels and cardiovascular risk factors. There was an independent correlation between cardiovascular risk and CysC levels, the risk factors most closely related to CysC levels were: BMI, low HDL, tobacco use and microalbuminuria. These findings are in line

with findings across multiple studies spanning across multiple countries and demographics (Jernberg *et al.*, 2004; Koenig, Twardella, Brenner and Rothenbacher, 2005; Luc *et al.*, 2006; Ix, Shlipak, Chertow and Whooley, 2007; Deo *et al.*, 2008; Servais *et al.*, 2008; Zethelius *et al.*, 2008; Shlipak *et al.*, 2009). A more recent study by Hassan, Aboelnaga and Al-arman (2021) found strong correlations between diabetic nephropathy and [CysC], adding to the various correlations that have already been identified.

Another study by Zethelius *et al.*(2008) found that the addition of various biomarkers for cardiovascular or renal function in elderly men with or without cardiovascular disease improves the risk stratification for death due to cardiovascular causes. Such biomarkers include CysC, microalbuminuria, HDL and glucose levels.

Shlipak *et al.*(2005) found that CysC was a better predictor of cardiovascular events and death than creatinine or estimated GFR and concluded that "If this finding is supported by additional research, CysC could be a useful prognostic tool in the evaluation of elderly patients".

In summary, CysC appears to be the a probable estimation of GFR for patients with early kidney disease and MetS (Madero and Sarnak, 2009). It may be of significant benefit as a prognostic marker in patients at high risk for developing CKD and CVD, such as patients with diabetes mellitus, hypertension, obesity and Dyslipidaemia (Parikh *et al.*, 2008).

1.15 **The use of Point of Care Devices**

It is possible that using point-of-care (POC) diagnostics to assess kidney function in patients who are at risk for CKD might offer a safe and cost-effective alternative to the standard procedure. These tests can provide results much more rapidly.

A technique for quick examination of blood glucose was established in 1962 (Clark & Lyons, 1962), which was the first introduction of point-of-care devices. Rapid pregnancy tests were developed not long after that, in 1977 (*Pregnancy Test - A Thin Blue Line The History of the Pregnancy Test - Office of NIH History and Stetten*

Museum, 1978), which were the first of their kind and helped to pave the way for individualized diagnostic testing. As a result of these advancements, POC tests and technologies have been introduced into clinical settings and laboratories all over the world, transforming the diagnostics industry. POC devices are useful in a variety of settings, including critical care units, outpatient clinics, and personal care settings.

Low to middle income countries are faced with a high burden of non-communicable diseases: cardiovascular disease and MetS. Primary Health Care facilities play an integral role in the promotion and prevention of disease in order to reduce mortality. However, the majority of these primary health care facilities lack adequate resources for diagnosis and monitoring. The time it takes for primary health care personnel to obtain patient results from physical laboratory services can be lengthy and often require patients to return to the facility at a later stage for interpretation of results. Disease progression is most likely to occur during lengthy waiting periods (Vetter *et al.*, 2021). To circumvent the long waiting time, delayed diagnosis and interventions to dosage adjustments or therapy, POC devices can be introduced (Alseed*et al.*, 2021). POC devices are able to improve patient triage, expedite patient care, provide instantaneous results, supporting the provision of timely patient care by better informed health care personnel.

A POC device is defined as a test designed for use at or near the site where the patient is located that does not require permanent designated space and where testing is performed outside a physical laboratory setting (Glen T Hansen, 2019). The major prospects of POC devices include portability, user-friendliness, robustness, inexpensiveness and the ability to generate quick results (Pandey *et al.*, 2018).

WHO ASSURED criteria for evaluating POC devices in resource limited environments adapted from (Kosack, De Kieviet, Bayrak, Milovic and Page, 2017)

- Affordable
- Sensitive
- User-friendly
- Rapid and robust
- Equipment-free

- Deliverable to end users

Uses of POC in renal function , adapted from (Duarte *et al.*, 2021)

- Checking for CKD in patients at high risk.
- Keeping an eye on renal function in people with known CKD.
- Dosage adjustments for drugs that are excreted by the kidneys in CKD patients.
- Detection of relapse in patients who have had a kidney transplant.
- AKI and acute-on-chronic renal failure found in sick patients who visit their physician.

1.15.1 **Time efficiency**

In terms of analytical performance and turnaround time, POC usage is superior to the central laboratory reference technique. Creatinine use at POC decreases delay outcomes, possibly enabling healthcare personnel to make quicker clinical judgments and shorten patient wait times. (Bargnoux *et al.*, 2018)

In the vast majority of the trials that were looked at, point-of-care (POC) creatinine testing devices were shown to be a reliable and speedy alternative to laboratory testing. Nevertheless, a number of investigations have shown that some devices have a propensity to underestimate renal impairment. (Schnabl *et al.*, 2010; Shephard *et al.*, 2010; Morita, Suzuki, Masukawa and Ueno, 2011)

1.16 **Eurolyser CUBE POC device**

Various patient care protocols in the emergency department may require rapid assessment of renal function in order to triage the patient. In addition, many emergency department therapies need timely administration of medications and adjustment of drug dosage based on renal function. Eurolyser Diagnostica is a medical technology company from Salzburg, Austria that has been a player in the point-of-care testing business for more than 15 years. (Eurolyser Diagnostica, 2022) The company has released a CysC point-of-care device into the market which is said to accurately test renal function within 10 minutes. The device uses an assay that can be used particularly on young or old patients (< 18 and >70 years) with diabetes, hypertension or myocardial diseases. (Eurolyser Diagnostica, 2013)

CHAPTER 3: METHOD

2.1 **Introduction**

This chapter describes the study design used to conduct the study, the setting at which the study took place, the inclusion and exclusion criteria which made up the population including how the sample size was determined. This section also describes how the study was conducted at the chosen facility.

2.2 **Study Design**

This was a single centre exploratory, descriptive, cross-sectional study designed to determine and compare the eGFR of patients with MetS using SCr and [CysC]. The study took place over a period of two months at Bishop Lavis CHC, a day hospital in the Tygerberg Western Health District of the Metro Region.

Exploratory research is frequently conducted when a thorough understanding of a novel concept is desired. Exploratory research's primary objective is to investigate a topic to produce knowledge and understanding for a more in-depth analysis, as the name suggests. There is also not a clear hypothesis for this research, since it is impossible to determine which eGFR measurement was more accurate/closer to the actual mGFR.

In order to gather information that will help characterize a research topic effectively, the descriptive research design requires the use of a variety of qualitative and quantitative research methodologies. Both qualitative and quantitative research can use this design.

Cross-sectional studies are a subcategory of observational studies that focus on information gathered from a population at a specific time. They are frequently used to identify the prevalence of health outcomes, comprehend the variables that affect health, and describe the characteristics of a population. Contrary to other kinds of observational studies, cross-sectional studies don't follow participants over a long period of time. They are frequently inexpensive and easy to complete. Cross-sectional studies can be classified as descriptive or analytical, depending on whether the outcome variable is analysed for possible links with exposures or risk factors. The purpose of

cross-sectional descriptive studies is to simply define the prevalence of one or more health outcomes in a specific population.

2.3 **Study Site**

The study was conducted at Bishop Lavis Community Health Centre in Cape Town. On the Cape Flats in the province of the Western Cape is where you'll find the low-socioeconomic community of Bishop Lavis. Bishop Lavis is a residential neighbourhood that is 15 kilometres to the east of the city's central business district and is close to Cape Town International Airport.

During the time of apartheid in South Africa, the ruling government of the nation constructed a number of townships, including Bishop Lavis, in the province of the Western Cape. Bishop Lavis is only one of these townships. During this time period, non-white inhabitants were forcibly removed from their farms and houses throughout the region and relocated into these townships when those areas were suddenly classified as being exclusive to whites.

The community has a total population of 54 006 people, the majority (92%) of whom self-identify as being coloured. Most coloured people are Afrikaans speakers and make up the largest ethnic group in the Western Cape region (50.2%). According to the results of the National Census conducted in 2011, this community has a rate of unemployment of 26.6%, and 47.0% of the community members have a monthly income of less than R3200. The census also revealed that 15.4% of this population has either not finished elementary school or has no education at all, whilst just 5.9% of this population has higher level credentials (City of Cape Town-2011 Census Suburb Bishop Lavis, 2013).

2.4 **Study population and sampling**

The study population included patients who visit the Bishop Lavis Community Health Centre (BLCHC), which is a public healthcare facility. It primarily provides services to the Bishop Lavis community, which is one of the underprivileged communities in the Cape Metropole and is plagued by social issues such as high unemployment rates, high rates of crime and gangsterism, and high rates of domestic violence as well as substance abuse (City of Cape Town-2011 Census Suburb Bishop Lavis, 2013). MetS was found to have 30% prevalence in the coloured community of the Cape Town

metropole. This demonstrates that it is the most notable among sub-Saharan African communities. (Erasmus *et al.*, 2012)

2.5 **Inclusion and exclusion criteria**

The study included adults aged ≥18 years who required laboratory investigations for SCr, patients who had been diagnosed with MetS according to the NCEP: ATPIII definition and provided informed consent.

The study excluded patients who had acute kidney injury (AKI),according to the findings of Royakkers *et al.*(2011), [CysC] is not a reliable indicator of AKI. Patients taking oral corticosteroids or who were taking oral corticosteroids two weeks prior were excluded. According to the findings of Zhu et al.(2019), corticosteroids have the ability to stimulate the formation of CysC in a variety of bodily tissues, which leads to increased levels of CysC in plasma. Patients diagnosed with either hypothyroidism or hyperthyroidism were also excluded. The [CysC] in the blood is impacted by thyroid dysfunction, which may in turn alter the rate at which the protein is produced (Manetti *et al.*, 2014). Pregnant patients were also excluded, since pregnancy also effects the [CysC] (Saxena *et al.*, 2011).

The exclusion of patients younger than 18 years was evidence based. The American Academy of Paediatrics (AAP) issued its guidelines in December 2007, in which they said that urinalysis tests should not be performed on children at any age throughout childhood (Walker *et al.*, 2007). Because of "obstacles facing clinical laboratories for reporting paediatric eGFR, including the lack of height information on test requisition forms", practitioners are encouraged to "focus eGFR reporting for adults aged ≥18 years", according to the research that has been published. (McDonough, 2007)

2.6 **Sample selection**

The following formula was used to determine the sample size (*Daniel, W.W. (1999)* . The sample size equation was selected based on previous research which deems it to be suitable for cross-sectional studies (Naing, Winn and Rusli, 2006; Pourhoseingholi, Vahedi and Rahimzadeh, 2013).

Where n is the sample size, Z is the confidence level statistic, P is the predicted prevalence (obtained from previous studies), and d represents precision (corresponding to effect size).The desired degree of confidence is a 95% which translates to 1.96 when plotted into the equation confidence interval (CI).

z=1.96

p=0.3

d=0.05

$$n = \frac{1.96^2 \times 0.3(1 - 0.3)}{0.05^2}$$

The estimated sample size required was 323 patients to meet the criteria of 95% confidence interval (CI) and a 5% margin of error (ME). This is based on the 54 006 (City of Cape Town-2011 Census Suburb Bishop Lavis, 2013) population residing in Bishop Lavis. The p value was evidenced by the prevalence of MetS in the coloured community within the Cape Town metropole (Erasmus *et al.*, 2012).

2.7 **Sampling Design**

The sampling process took place over a period of two months from 20 June to 19 August 2022. The facility stipulated no additional disruptions or demands on the facility's staff could be made in order for the study to proceed. Due to this, participants were chosen to participate in the study when they were due for their routine blood tests, which included SCr, as well as those who were referred for blood work by the facility's general practitioners on the day of their biannual follow-up. If they matched the

inclusion criteria, these individuals were included in the study. As a result, the study followed a convenience sampling approach.

Convenience sampling is based on the availability of access to participants. The researchers have almost little say in the selection of the pieces that make up the sample, and the decision is made solely based on proximity rather than representativeness. Convenience sampling is used in settings when there are restrictions placed on the available resources, such as the preliminary phases of this research.

Prior to having their blood drawn, participants who were sent for blood work were required to turn in their clinic folders to the blood room. On the basis of the patient's medical history, the folders could then be examined to check for MetS. Participants who met the inclusion were then approached one by one to confirm the inclusion and exclusion criteria obtained from the folders. Patients who met the requirements received information on the study. Written and verbal informed consent was obtained before the study was continued.

It should be noted that all SCr tests were performed by a single laboratory.

2.8 **Data collection tool**

A team of pharmacists created the data collection tool (see Appendix A) with the aid of reviewed literature. Microsoft Office Excel 2010 was used to create the data collection tool. The screening parameters included in the tool were [SCr], [CysC], total cholesterol, age, height, weight. After screening the tool also included information on demographics and comorbidities. To enable the use of eGFR prediction using particular equations that require race as a factor, race was included in the demographics. To evaluate the ease of use of the data collection tool, a pilot data collection process was conducted at BLCHC in their screening room. The study supervisor then reviewed the data collection tool and amendments were made before the study could commence.

2.9 **Validity and reliability**

To ensure validity, instructions for the use of the Eurolyser CUBE POC were followed in a step-wise manner (see Appendix F).

This study only used one kind of POC device to measure CysC, so it is not possible to say whether all POC devices would produce the same results. In order to maintain viability, the buffer solution used for the POC had to be kept between 2 and 8 degrees Celsius. The solutions were kept in the pharmacy refrigerator at the facility, which is checked twice daily to ensure that the temperature stays between 2 and 8 degrees Celsius. Prior to use, each solution had to be warmed to room temperature (20 to 25 degrees Celsius). There was no reliable way to tell if, as per the manufacturer's instructions, the precise temperature had been reached after 10 minutes. The reliability may have been impacted by this.

To ensure the most precise [CysC] were obtained, the portable Eurolyser CUBE POC device was calibrated daily in accordance with the manufacturer's specifications. In order to rule out any confounding variables that might affect patients on different days, blood samples for the patients' [SCr] and [CysC] were collected on the same day. This ensured that the same blood was used for all laboratory tests, negating the possibility that a patient's diet, level of physical activity, or evolution of their disease over time could affect the results.

2.10 **Data Collection Process**

Patient folders were retrieved from the blood room of Bishop Lavis CHC, where they were examined for patients who might have met the criteria for inclusion based on their medical histories. Then, each of these patients was approached individually to confirm that they met the criteria. Patients' verbal and written informed consent was obtained after they had been informed of the potential advantages, risks, and study procedures (see Appendix E). The data collection tool was completed by obtaining baseline demographic data, anthropometric measurements, and comorbidities. Following that, each patient was given a study code that would be used to record patient data for the duration of the study. A different document was used to record patient information that included patient folder numbers. Initial testing for CysC was performed on patients using the Eurolyser CUBE POC device in accordance with the manufacturer's instructions:

Solutions from the test kit were removed from the refrigerator and allowed to sit at room temperature for 10 minutes. Using a capillary, 20uL of blood was drawn from a needle-finger prick. The blood sample was then placed in a Tris-buffer solution-filled

ERS cuvette. The cuvette was then sealed with a CysC antibody reagent-filled cap. The cuvette was then inserted into the receiving port of the Eurolyser CUBE POC device for CysC determination. The blood draw was conducted under the direct supervision of a licensed nurse practitioner.

The nursing staff then drew blood samples for laboratory investigations on the same day to ensure that the same blood was used to calculate eGFR using SCr and CysC. The samples of blood were sent to the national health laboratory services (NHLS) for SCr assessment. After 24 hours, SCr results were posted and reviewed on the NHLS website by the researcher. eGFR for SCr and [CysC] was determined using the various equations.

Patients whose screening results indicated a high risk of disease progression were referred to the appropriate physician or clinic nurse for additional intervention. In accordance with South African standard treatment guidelines, recommendations were provided to physicians and clinic nurses following any significant findings. In order to preserve renal function or prevent medication toxicity, these recommendations included dosage adjustments or the use of alternative medications. Recommendations were acknowledged and documented.

2.11 **Data entry and analysis**

Analysis was carried out using the Statistical Product and Service Solutions-IBM SPSS Statistics for Windows, Version 25.0 (Armonk, NY, USA) and PRISM Graphpad 9.0.0. The mean ± standard deviation (SD) of the different studied variables was calculated using SPSS as well as Microsoft excel 2010. The differences were reported as absolute values with 95% confidence intervals. P values of 0.05 or less were considered statistically significant.

Both [CysC] and [SCr] were required for each participant in order to perform an appropriate correlation, participants who did not have both were not compared (see Figure 7). eGFR was calculated using the MDRD, CKD-EPI, Salazar-Corcoran, and Cockroft-Gault Equations based on SCr. Applying the CKD-EPI 2012, Hoek, and Larsson equations allowed us to determine eGFR based on [CysC] (see Table 2). The Eurolyser CUBE POC device has a built-in calculator to determine eGFR based on

[CysC] levels. To calculate the standardised body surface area value and measure it in (mL/min/1.73m^2), the resulting eGFR was divided by 1.73. This was how the Eurolyser CUBE POC eGFR results were all shown. CKD staging was done on the basis of eGFR. Staging could only be compared if participants had both CysC and SCr levels (see Table 9).

Pearson's correlation analysis was utilized to investigate the link between SCr and [CysC], as well as the eGFR calculations based on the different equations used.

Pearson's correlation, which produces a score ranging from 1 to + 1, is used to determine the similarity of two objects by comparing them attribute by attribute, usually summing the squares of the magnitude differences for each attribute, and using the calculation to compute a final outcome known as the correlation score. Two factors that have a high score (near + 1) are nearly identical/influenced by the same process. In this research that would be the elimination rate of SCr or [CysC]. A Pearson correlation of near 0 would indicate that the two factors are unrelated, measuring different processes or have different variables responsible for their differences. Two negatively linked objects (one dropping as the other rising) would have a Pearson score close to -1 to -0.01. (Berman, 2016)

The WHO CVD risk chart (Appendix B) was used to determine the risk of cardiovascular disease. The categories of [CysC] findings were compared to CVD risk using Spearman rank correlation. Only 71 participants had all the parameters documented required to calculate CVD risk (total cholesterol, systolic blood pressure, age and smoking history). Patients who did not have all the parameters were not included in the correlation. For the purpose of comparing CVD risk scores, CysC values were categorized into seven levels to facilitate comprehension of the findings (see Table 10).

Spearman rank correlation is the nonparametric version of Pearson and is used to assess the degree and direction of relationship between two ranked variables. It essentially provides a measure of the monotonicity of the connection between two variables, i.e. how effectively the relationship between two variables can be described by a monotonic function. (Schober and Schwarte, 2018)

It is important to note that participant results were compared based on the attainment of all the necessary variables for particular equations. Participants were not included in the comparison if they lacked specific criteria.

Correlation values were interpreted according to the following table:

Table 4: Conventional Approach to Interpreting a Correlation Coefficient

Correlation Coefficient	Interpretation
0.00 – 0.10	Negligible correlation
0.10 – 0.39	Weak correlation
0.40 – 0.69	Moderate correlation
0.70 – 0.89	Strong correlation
0.90 – 1.00	Very strong correlation

(Schober and Schwarte, 2018)

A narrative revolving around the use of the Eurolyser CUBE POC device served to address the secondary objectives in a descriptive manner.

2.12 **Ethical considerations**

2.12.1 Permission

Both the Biomedical Research Ethics Committee at the University of the Western Cape (UWC) (Appendix B, reference number: BM22/4/6) and the Health Research Committee within the Western Cape Government Health department (reference number: WC_2002206_004) gave their approval for the research to be carried out after it was presented to them for approval. After this, permission to proceed with the investigation was sought from, and received from, the facility manager (Appendix C).

3.12.2 *Informed consent*

Prior to the screening starting, participants gave their informed consent. First, consent was obtained verbally from participants in a language they could understand. The study was explained to the participants verbally because the majority of them spoke Afrikaans. Participants also received a sheet of information that contained the following:

- Research purpose
- Procedures to be undertaken
- Any risks that may arise

- Benefits the research will provide society and possibly to the participants
- Expected involvement time of the participants
- Contactable person should any questions or concerns be voiced
- Statement signifying that participation is voluntary
- Statement addressing participants' right to confidentiality
- Statement addressing participants' right to withdraw from the research at any given point in time exclusive of any consequences

2.12.2 Confidentiality

Patients were identified through their folder numbers during data collection, but data was anonymized before data analysis. To ensure that no one other than the researcher and supervisor will ever have access to the raw data, the generated anonymized data was stored in an excel spreadsheet compressed into a password-protected zip file. The University of the Western Cape will retain ownership of the data for a period of five years.

2.13 **Funding**

The study was funded by the Clinical Pharmacy Grant from the department of higher training.

2.14 **Summary**

The methodology of the study was covered in this chapter. The findings will be presented in the next chapter. These methodologies were used in the collection and interpretation of the results in chapter 4. These results together with the literature review of Chapter 2 complement one another to ensure the reader would be able to independently interpret and verify the validity and reliability of the results obtained from this research project. It also allows the reader to synthesise a conclusion of their own.

CHAPTER 4: RESULTS AND DISCUSSION

3.1 **Introduction**

Within this chapter, all the data and discussion of the possible implications and reasons behind the findings are portrayed. The statistically relevant findings will be highlighted and unfolded in an attempt to best address the study's aim and objectives.

3.2 **Baseline Characteristics/ demographics**

The study included 141 participants in Bishop Lavis CHC whose CysC levels were measured over a three-month period (27 June 2022 to 06 September 2022). Thirty-six samples had remained outside of the recommended temperature ranges for storage and were subsequently omitted from our results. This meant there were 105 participants whose eGFR data was analysed and interpreted.

Seventy-eight (74.3%) participants were female and 27 (25.7%) were male. The majority (97%) of the participants were coloured. All participants had MetS as per the NCEP ATP III definition(Expert Panel on Detection, 2001), which was diagnosed during their follow up consultation at the clinic or a previous one. Participants' age ranged from 40 to 88 years old with a mean of 62.1, SD = 10 (Table 1).In the sample, no significant difference in mean age between males and females was found (p=0.13).There were more participants between the 60-69 years for both males and females, which made up 38.1% of the overall sample size. Other baseline characteristics are summarised in Table 6.

Table 5: Age Demographics

Gender	Number of patients, n (%)	Mean age, years ±SD	Median age	Minimum age, years	Maximum age, years	Categories				
						Age 40-49 years (%)	Age 50-59 years (%)	Age 60-69 years (%)	Age 70-79 years (%)	Age >80 years (%)
Female	78(74.3)	62,5 ±10.1	62,5	40	86	7 (9)	24 (30.8)	28 (36)	15 (19.2)	4 (5.1)
Male	27(25.7)	61,2 ±9.9	63	42	88	4 (14.8)	7 (25.3)	12 (44.4)	3 (11.1)	1 3.7)
Total	105	62,1 ±10	63	40	88	11 (10.5)	31 (29.5)	40 (38.1)	18 (17.1)	5 (4.8)

Table 6: Clinical information captured for all participants

Variable	All	Median	Female	Male
N	105		78	27
Age (years)	62,14± 10.03	63	62.5	62.2
Weight (kg)	83,62±16.49	79	82.09±16.97	87.84±14.6
Height (m)	1,59 ±0.08	1.6	1.56±0.07	1.67±0.07
BMI (kg/m^2)	33,09 ±5.67	32.19	33.61±5.8	31.69±5.16
SBP (mmHg)	147.48 ±21.46	147	147.3±20.77	148.05±24.07
DBP (mmHg)	87.05 ±9.5	88	86.69±9.31	88.19±10.41
CysC mean (mg/L)	0,76	0.7	0.76±0.29	0.78±0.24
SCr mean (µmol/L)	76,3	71	67.79±19.71	97.39±30
CVD risk(%) n=71				
<5	3(4.2)		2	1
5-10	23(32.4)		16	7
10-20	35(49.3)		25	10
20-30	9(12.7)		7	2
>30	1(1.4)		1	0
Comorbidities				
T2DM and HPT	13		10	3
HPT and Dyslipi.	46		35	11
T2DM and Dyslipid.	0		0	0
T2DM, HPT and Dyslip.	33		23	10
CKD	2		0	2
Other*	53		40	13
Smoker	17		11	6
Cardiovascular disease	7**		5	2
Race				
Coloured	102		76	26
Black	1		1	0
White	2		1	1

BMI; Body Mass Index, SBP; Systolic Blood Pressure, DBP; Diastolic Blood Pressure, CysC; Cystatin C, SCr; Serum Creatinine, CVD; Cardiovascular Disease, CKD; Chronic Kidney Disease, T2DM; Type 2 Diabetes Mellitus, HPT; Hypertension, Dyslip.; Dyslipidaemia.. Results are represented as mean ±SD(standard deviation) for age, weight, height, BMI, SBP, DBP, CysC and SCr. Results for CVD risk are represented as the number of participants whose CVD risk % fell within the category and the (%) of participants within the population. The number of patients for each variable is used to depict the remaining findings.*Other comorbidities included osteoarthritis, rheumatoid arthritis, asthma and COPD.**Cardiovascular event included three cerebrovascular accidents, two coronary heart disease, one congestive cardiac failure and one unstable angina.

3.3 **Primary Objectives**

3.3.1 **Performance of estimating equations**

Among our primary objectives was to compare the eGFR results from CysC derived equations and SCr derived equations. This objective is necessary to establish if the Eurolyser CUBE POC device would be useful within clinical practice settings and if [CysC] eGFR results are comparable to currently used SCr derived eGFR measurements. The NHLS report eGFR based on the MDRD and CKD-EPI formula from results measured within their laboratories.

The lowest calculated eGFR was obtained from the CysC eGFR Eurolyser (mean = 92.96, SD = 40.16 mL/min/1.73m^2), whereas the highest eGFR was obtained using the Larsson equation (mean = 124.19, SD = 41.4 mL/min/1.73m^2). eGFR using [CysC] based equations appeared to be higher in comparison with SCR derived eGFR for females compared to males except when utilizing the CKD-EPI 2012 Cystatin C equation . In contrast to the MDRD and CKD-EPI 2009 equations (mean = 81,4,l/min, SD=26.55 mean = 81.99ml/min SD =20.56 respectively), which produced lower eGFR values with comparable standard deviations, the eGFR calculated for SCr using the CKD-EPI 2021 equation produced higher eGFR results (mean = 85.4, SD = 20.52 mL/min/1.73m^2). The lowest mean eGFR calculation was estimated when using the Cockcroft-Gault ABW equation (mean = 53.77ml/min, SD = 38.2 mL/min).

eGFR was higher for females than males for all equations except CKD-EPI 2012 Cystatin C. The CKD-EPI 2012 Cys C + SCr eGFR (mean = 92.8, SD = 22.31mL/min/1.73m^2), was greater when utilizing the hybrid equation, CKD-EPI 2021 Cys C + SCr (mean = 95.6ml/min, SD = 23.58).

At a glance it seems that renal function calculated using [CysC], measured by the Eurolyser CUBE POC device, are higher in comparison to the SCr derived formulae measured by the centralised NHLS. Even though there is a moderate correlation between Scr and [CysC] with a Pearson's correlation coefficient of 0.49 (p-value <0.0001), all of the eGFR results obtained from the different formulae, comparing [CysC] derived eGFR to SCr derived eGFR, shared a weaker correlation than the biomarkers they are derived from.

The strongest correlation between eGFR derived from [CysC] and SCr formulae were: between the CysC based calculated by the Eurolyser CUBE POC device and the MDRD (SCr) with a Pearson's correlation coefficient of 0.36and a P-value of 0.0009. All other correlations between eGFR formulae derived from SCr and eGFR from [CysC] shared correlation values below a Pearson's correlation Coefficient of 0.36. If all the formula used should have been accurate one would expect the eGFR values from the different formula to closely follow the correlation between [CysC] and [SCr], but the correlations were all significantly weaker compared to that of the biomarkers.

Table 7: Mean Values of all participants' eGFR derived from different formulae (n = 80)

	All Participants	**Female**	**Male**
	CysC based Equation		
eGFR CKD-EPI 2012 Cys C (ml/min/1.73m^2)	104,67±16.23	103.17±16.4	109±15.1
eGFR EurolyserCys C (ml/min/1.73m^2)	92,96±40.16	94.53±41.8	88.4±35.1
Le BriconCys C (ml/min/1.73m^2)	116,54±30.3	117.9±31.1	112.7±27.9
Hoek Cys C (ml/min/1.73m^2)	111,61±31.2	113±32.1	107.7±28.8
Larsson Cys C **(**ml/min)	124,19±41.4	126.1±42.7	118.8±37.5
	SCr based Equation		
eGFR Cockroft-Gault (mL/min)	81.25±38.28	85.5±40.2	70.8±31.3
Cockroft-Gault$_{ABW}$ (mL/min)	53,77±38.2	60.5±40.6	38.2±26.6
eGFR MDRD (ml/min/1.73m^2)	81,47±26.55	84±27.1	75.3±24.6
eGFR CKD-EPI 2009 SCr (ml/min/1.73m^2)	81,99±20.56	84.1±20.3	76.9±20.7
eGFR CKD-EPI 2021 SCr (ml/min/1.73m^2)	85,36±20.52	87.2±20.2	80.8±21
Salazar-Corcoran (ml/min/1.73m^2)	83,01±39.4	92.9±40.3	59.8±25.7

CysC and SCr Hybrid Equations			
eGFR CKD-EPI 2012 Cys C +SCr (ml/min/1.73m^2)	92,8±22.31	93.1±22	92.1±23.6
eGFR CKD-EPI 2021 Cys C +SCr (ml/min/1.73m^2)	95,6±23.58	98.2±22.4	89.2±25.6

Results are represented as mean ±SD; standard deviation, eGFR; estimated Glomerular Filtration Rate, CKD-EPI; Chronic Kidney Disease Epidemiology Collaboration, MDRD; Modification of Diet in Renal Disease, Cys C; Cystatin C, ABW; Adjusted Body Weight, SCr; Serum Creatinine

In line with the findings of Poggio *et al.*(2009), females had higher eGFR values compared to the male participants. This might point to the inaccuracy of both biomarkers in calculating eGFR or be a phenomenon limited to the study population under investigation. All the relevant adjustments were made according to gender for all the formula used in calculating eGFR. This means that the differences in eGFR between male and female participants are more likely attributable to the formulae being used to calculate the eGFR over a real difference in the gender of the study population. In support of this statement a study conducted on a healthy Pakistani population, did not find a difference in inulin clearance between the sexes (Jafar *et al.*, 2011). Additionally, the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines do not recommend having different thresholds for what is considered a normal GFR for males and females. Another possible reason for difference in eGFR measurements among male and female participants could also be due to the small sample of male patients attending Bishop Lavis CHC.

According to the findings of a study carried out by Kakde *et al.*(2018), the eGFR CKD-EPI 2012 CysC and SCr equation has the highest accuracy possible while still maintaining an acceptable level of bias in both males and females. They concluded that if a gender-specific equation is going to be used, the eGFR CKD-EPI 2012 CysC equation ought to be favoured in females, while the eGFR CKD-EPI 2009 SCr equation ought to be preferred in males. This was not explored further since the sample size is unevenly distributed toward female participants and it was not one of the objectives of this study, but serves to inform future research.

eGFR Cystatin C CKD-EPI 2012

Because this research is attempting to explore the feasibility of clinically implementing the CysC derived eGFR from the Eurolyser CUBE POC device, the focus was on the eGFR derived from the CKD-EPI 2012 using the [CysC] from the POC device. Subsequent comparisons and correlations were drawn between the CKD-EPI formula's eGFR and eGFR measurements derived from other [CysC] and SCr based formulae. Pearson correlation coefficients were calculated for each pairing and graphs drawn to visually represent the correlation between the different eGFR quantification methods.

Figures 2 to 4 represent the $eGFR_{CysC}$ CKD-EPI 2012 plotted on the X-axis and differing eGFR methods plotted along the Y-axis. Dot's represent where each participants x-value (eGFR from [CysC] meets the eGFR, Y-value, derived from other methods). In Figure 2 (A=MDRD eGFR as Y, B= SCr derived eGFR CKD-EPI 2009 as Y, C= SCreGFR CKD-EPI 2021 as Y). In Figure 3 (A = $CrCl_{Cockroft\text{-}Gault}$ as Y, B = $eGFR_{Salazar\text{-}Corcoran}$ as Y, C = $CrCl_{Cockroft\text{-}Gault\ ABW}$ as Y). In Figure 4 (A = $eGFR_{SCr\text{-}CysC}$CKD-EPI 2012 as Y, B = $eGFR_{SCr\text{-}CysC}$CKD-EPI 2021 as Y)

$eGFR_{CysC}$ CKD-EPI showed a weak but significant correlation with $eGFR_{MDRD}$ (r=0.31 [95% CI 0.099 – 0.4979]; P = 0.0048) (Figure. 2A).$eGFR_{CysC}$ CKD-EPI showed an insignificant but weak correlation for both eGFRSCr CKD-EPI 2009 (r=0.17 [95% CI -0.0342 – 0.3619]; P = 0.1018) and $eGFR_{SCr}$ CKD-EPI 2021 (r=0.17 [95% CI -0.0391 – 0.3576]; P = 0.1119) (Figure. 2B and C).$eGFR_{CysC}$ CKD-EPI showed a negligible correlation with $eGFR_{Salazar\text{-}Corcoran}$(r=0.08 [95% CI -0.1564 – 0.3008]; P = 0.4991) (Figure. 3B). A weak correlation was observed between $eGFR_{CysC}$ CKD-EPI and $CrCl_{Cockroft\text{-}Gault}$ (r=-0.23 [95% CI -0.3876 – -0.0611]; P = 0.0082) compared to a moderate correlation with $CrCl_{Cockroft\text{-}Gault\ ABW}$ (r=0.22 [95% CI -0.0075 – 0.4309]; P < 0.001) (Figure. 3A and C). $eGFR_{CysC}$ CKD-EPI showed an insignificantly weak correlation with $eGFR_{SCr\text{-}CysC}$CKD-EPI 2012 (r=0.21 [95% CI 0.0032 – 0.3939]; P = 0.0467) and a weak correlation with $eGFR_{SCr\text{-}CysC}$CKD-EPI 2021 (r=0.17 [95% CI -0.0402 – 0.3607]; P = 0.1133) (Figure. 4A and B)

eGFR Eurolyser CUBE POC

Figure 5. A represents the eGFR derived from the Salazar-Corcoran on the X-axis and the Eurolyser CUBE POC device plotted along the Y-axis. Figure 4.B represents the

eGFR derived from the MDRD equation on the X-axis and the Eurolyser CUBE POC device plotted along the Y-axis.

eGFR derived from the Eurolyser CUBE POC device showed a significant but weak correlation for both $eGFR_{MDRD}$ (r=0.36 [95% CI 0.1577 – 0.5411]; P = 0.0009) and $eGFR_{Salazar\text{-}Corcoran}$(r=0.26 [95% CI 0.0399 – 0.4637]; P = 0.0218). (Figure. 5A and B).

CrCl vs MDRD

To further explore the differences between different formulas used, I also compared the MDRD formula to that of the simplified Cockroft-Gault equation, since the MDRD attempts to make provision for adipose tissue and the simplified Cockroft-Gault equation uses total weight. The results are detailed below.

Figures 6 A represents the eGFR derived from the MDRD equation on the X-axis and the CrCl derived from the Cockroft-Gault equation plotted along the Y-axis.

$CrCl_{Cockroft\text{-}Gault}$ showed a strong and significant correlation to $eGFR_{MDRD}$ (r=0.78 [95% CI 0.6897 – 0.8487]; P <0.0001). (Figure. 6A)

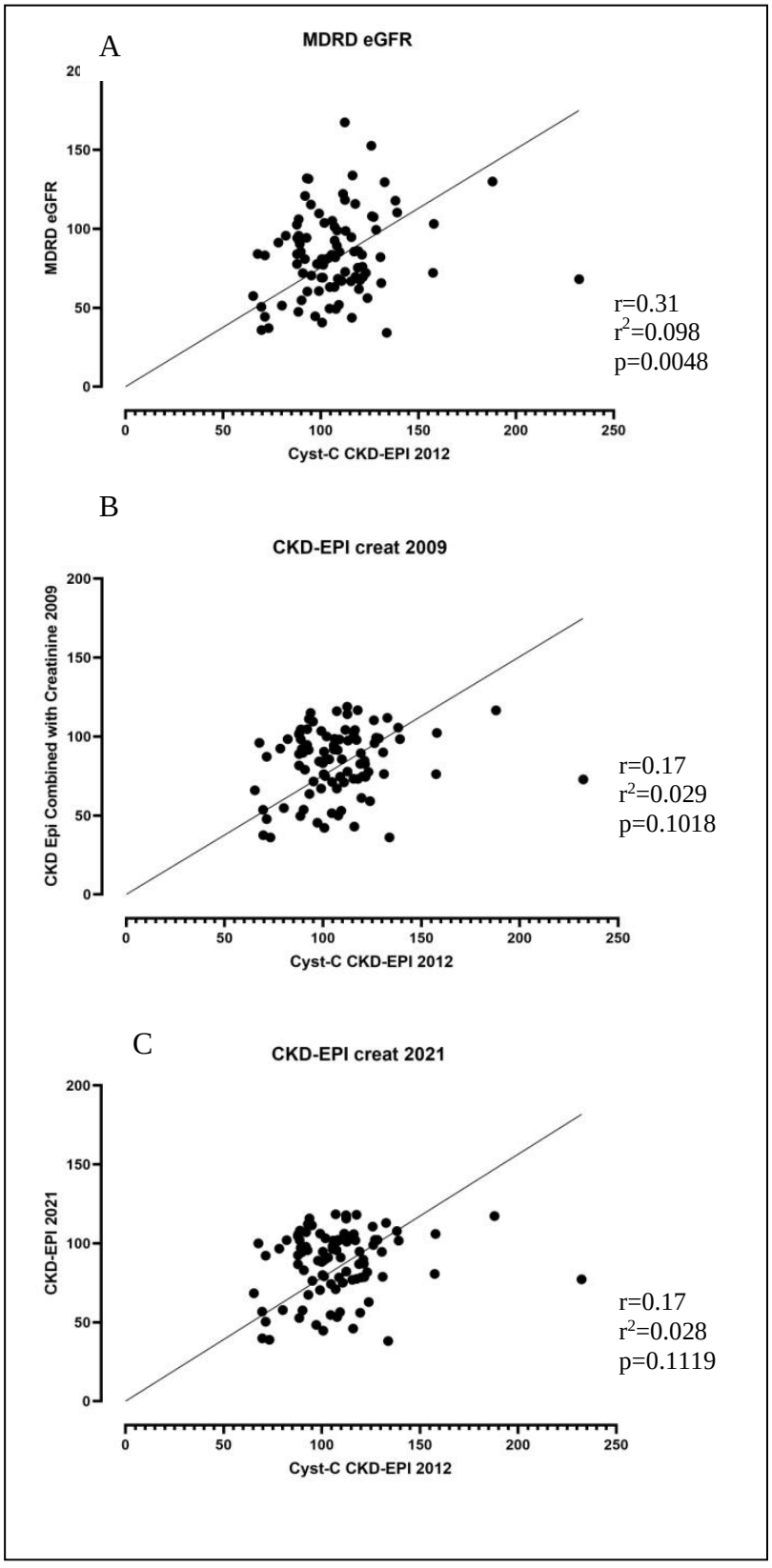

Figure 2. MDRD, CKD-EPI 2009 and CKD-EPI 2021 vs CysC CKD-EPI 2012. (A) MDRD eGFR derived from SCr along Y-axis and eGFR derived from [CysC] using the CKD EPI 2012 formula plotted along the X-axis. (B) eGFR derived from SCr using CKD-EPI 2009 formula along Y-axis and eGFR derived from [CysC] using the CKD EPI 2012 formula plotted along the X-axis. (C) eGFR derived from SCR using CKD-EPI 2021 formula along Y-axis and eGFR derived from [CysC] using the CKD EPI 2012 formula plotted along the X-axis.

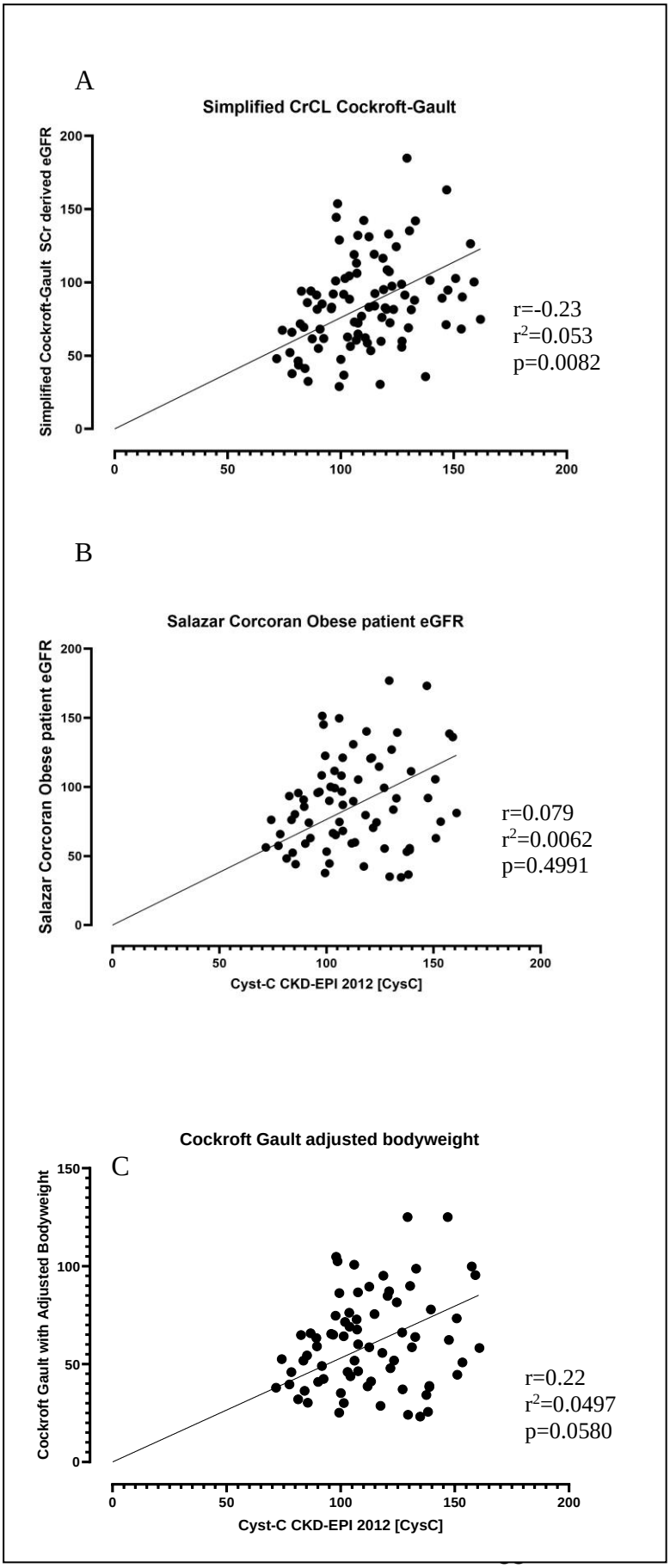

Figure3.Correlation of weight-based equations. (A) eGFR derived from SCr using the Cockroft-Gault equation (ideal body weight) formula along Y-axis and eGFR derived from [CysC] using the CKD EPI 2012 formula plotted along the X-axis. (B) eGFR derived from SCr using the Salazar-Corcoran formula along Y-axis and eGFR derived from [CysC] using the CKD EPI 2012 formula plotted along the X-axis. (C) eGFR derived from SCr using the Cockroft-Gault ABW formula along Y-axis and eGFR derived from [CysC] using the CKD EPI 2012 formula plotted along the X-axis

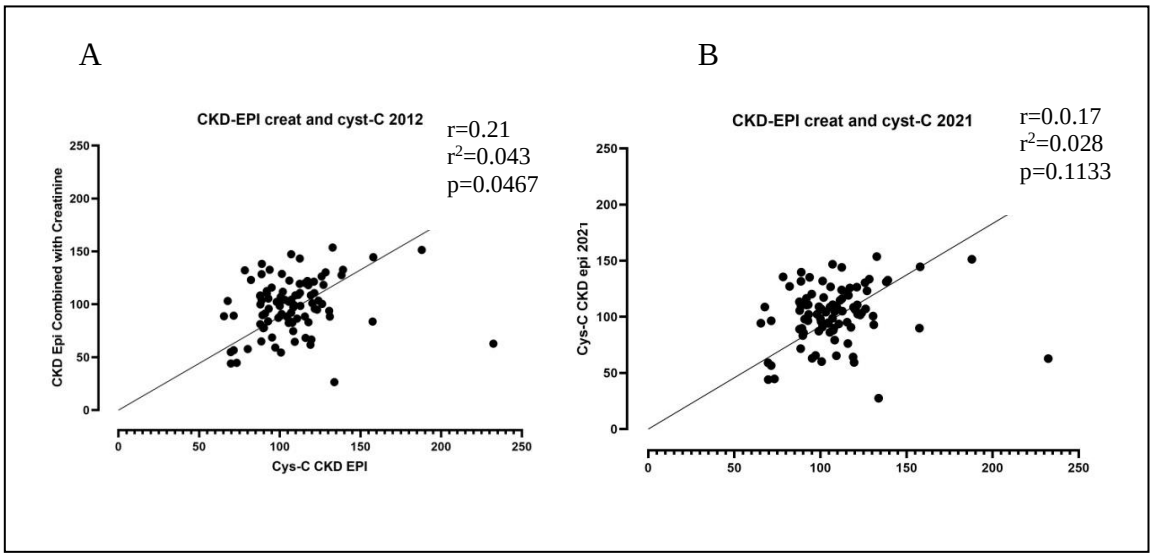

Figure 4. CysC Hybrid Equations vs CysC CKD-EPI 2012. (A) eGFR derived from SCr and [CysC] 2012 formula using the CKD-EPI formula along Y-axis using CKD-EPI 2012 formula plotted along the X-axis.. (B) eGFR derived from SCr and [CysC] 2021formula along Y-axis using the CKD-EPI 2012 formula plotted along the X-axis.

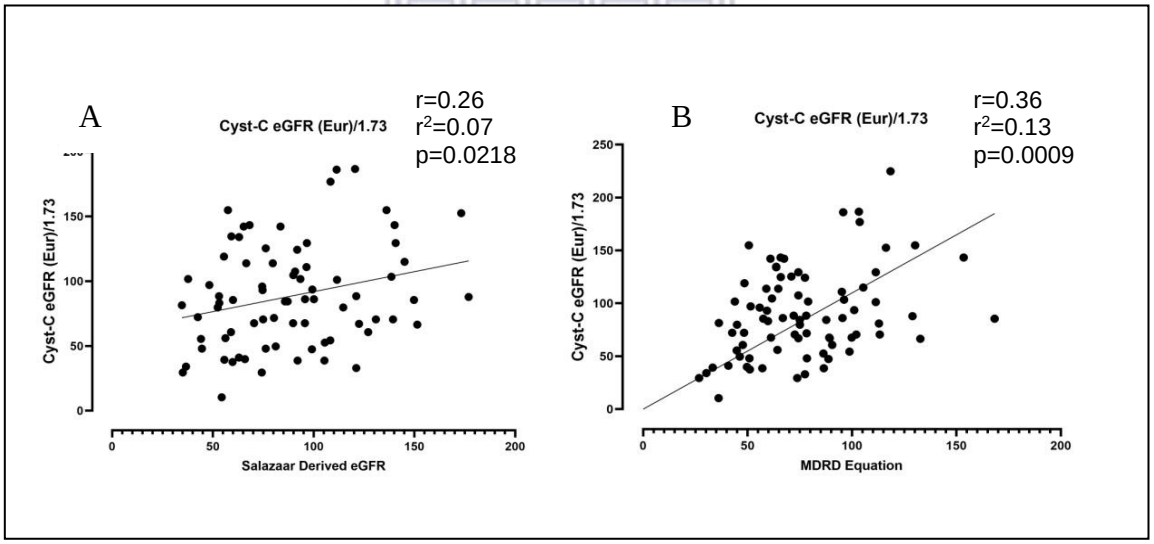

Figure 5.Euolyser Cube POC eGFR correlations. (A) eGFR derived from [CysC] based on the Eurolyser CUBE POC built in calculator along Y-axis and eGFR derived from SCr using the Salazar-Corcoran formula along the X-axis. (B) MDRD eGFR derived from SCr along X-axis and eGFR derived from [CysC] based on the Eurolyser CUBE POC built in calculator along the Y-Axis.

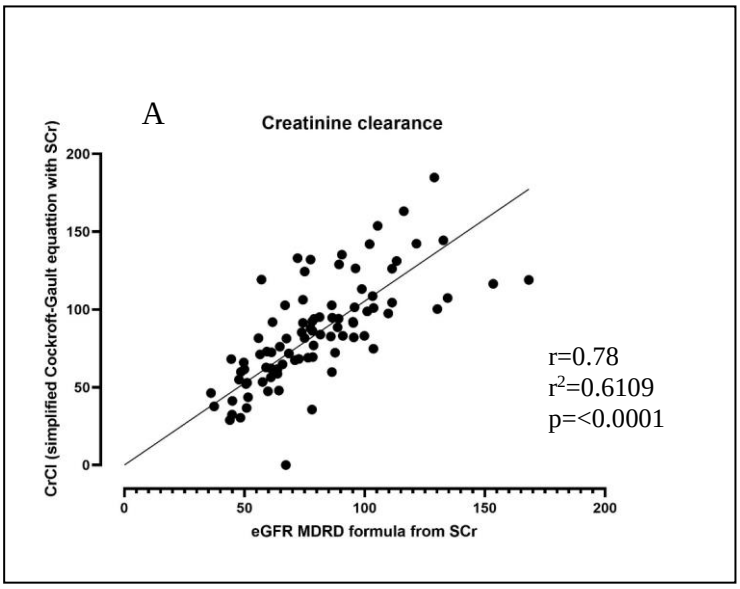

Figure 6.Cockroft-Gault vs MDRD equation. (A) eGFR derived from SCr using the Cockroft-Gault simplified equation formula along Y-axis and eGFR derived from SCr using the MDRD formula plotted along the X-axis.

The Cockroft-Gault$_{ABW}$ equation produced a significantly lower CrCl (mean = 53.77, SD = 38.2 mL/min) than the other equations. This may be related to the majority of patients being classified as Obese Class I due to the average BMI being high (mean = 33.09, SD = 5.67 kg/m^2). (Table 7)

In a cohort of obese patients, Bouquegneau *et al.*(2016) discovered that the Cockroft-Gault equation is imprecise, biased, and overestimates GFR in all CKD subgroups. They concluded that because of this, it is not the most appropriate equation for estimating the GFR in order to adapt the dosage of medications to the renal function of obese patients. However, using the adjusted ideal body weight (AIBW) instead of the Cockroft-Gault equation significantly improved the performance of this equation, which may warrant further investigation in future research. If no other diagnostic marker is considered such as in this study, the Cockroft-Gault$_{ABW}$ consistently estimated lower eGFR values across all the study participants. If the results from Bouquegneau *et al.*(2016) are extrapolated to our findings it implies that the study population have lower renal functions than is reported currently in clinical practice.

The results in Figures 2 to 4 show that there is generally a positive correlation between eGFR derived from CysC based and SCr based equations. However; the correlation ranged from weak to moderate with a negligible correlation when the Salazar-Corcoran equation was used. It should be noted that the strength of a correlation coefficient does not imply that the equations are the most accurate version of the CysC- and SCr-based method for GFR calculation. The correlation coefficient values should only be

regarded as the existence of a link or relationship between the two variables (Bland and Altman, 1986). This is also further enforced by no validated estimation of renal function based on either [CysC] or SCr within the population group being investigated.

When comparing CKD-EPI equations, our study found similar results to a study by Delanaye *et al.*(2013), who discovered a positive correlation between the difference in equations and the mean of the two equations, implying that the differences between equations increase slightly with eGFR levels. This correlation was weak and linear (r^2 between 0.02 and 0.04, P= 0.001). When MDRD was compared to CKD-EPI and CKD-EPI Mix, a clear nonlinear relationship with a merge around x = 80 mL/min/1.73 m^2 was discovered. This also implies that the differences between the MDRD and the CKD-EPI are systematic and always in the same direction. When comparing the MDRD formula and the CKD-EPI cyst formula, we too found a significant clear nonlinear relationship r=0.31 [95% CI 0.099 – 0.4979]; P = 0.0048) (Figure. 2A).

According to the findings of (Khalid *et al.*, 2020)study, equations based on $eGFR_{SCr-CysC}$CKD-EPI had the highest correlation (r=0.984) with mGFR at all stages of CKD. Additionally, Tidman *et al.*(2008)came to the same conclusion that combining SCr and CysC equations might produce more accurate results.

According to a study (Kilbride *et al.*, 2013), the $eGFR_{SCr-CysC}$ equation had the lowest bias (median difference = 0.8) and highest accuracy when compared to MDRD, CKD-EPI_{SCr}, and CKD-EPI_{CysC}.

The results of all of these studies seem to indicate that the hybrid equation performs significantly better than either the CysC or the SCr equations on their own. Within the design of this study it is not possible to definitively conclude which one formula worked best or which biomarker performed better since we do not have any measured GFR values to compare the eGFR results with.

Within this study the different eGFR formula derived from SCr had strong correlations above 0.85. There was however an exception when using the simplified Cockroft-Gault formula and comparing the eGFR results to that of the MDRD formula. This can help explain the difference in using different formula for the same patient population. In this case the simplified CrCl does not take the adipose tissue into account and estimates the SCr production based on the patients weight, not excluding the adipose tissue.

4.3.2 **Serum Creatinine concentration vs Cystatin C concentration**

Overall, [CysC] had a moderate significant correlation with [SCr] concentration(r= 0.51 [95% CI 0.3484 – 0.6409]; P <0.0001) (Figure. 7).Participants with the highest average mean fell within the 70-79 years old age category (CysC mean = 0.85 ± 0.26 mg/L, SCr mean = 88.33 ± 27.87 µmol/L). The [CysC] mean was 0.76 overall which was similar for both male and female counterparts (mean = 0.78, SD =0.24; mean = 0.76, SD = 0.29 respectively). [CysC] and [SCr] had a better correlation compared to any of the eGFR equation correlations derived between them in Figure 2 to 5.

Table 8: Data for participants stratified by Age Groups (n=95).

	Mean Age				
	Age 40-49 years ±SD	**Age 50-59 years ±SD**	**Age 60-69 years ±SD**	**Age 70-79 years ±SD**	**Age >80 years ±SD**
Cystatin C mg/L	0,62±0,13	0,73±0,21	0,78±0,35	0,85±0,26	0,83±0,26
Serum Creatinine µmol/L	78±23,40	72,5±31,78	73,45±23,85	88,33±27,87	79,25±19,67

Data are presented as mean ±SD

Four patients (3.8%) had concentrations of CysC which were less than 0.5 mg/L. Sixteen patients (15.2%) had concentration values of CysC that were greater than 1mg/L, 10.5% of which were females and 4.8% were males. CysC in the Eurolyser CUBE POC has a clinical range of 0.33 mg/L to 8.0 mg/L. Some researchers have indicated that the usual healthy range of [CysC] is 0.5 - 1.03 mg/l. As a result, utilizing 1 mg/L as a disease management cut-off threshold is crucial.(Villa, Jiménez, Soriano, Manzanares and Casasnovas, 2005)

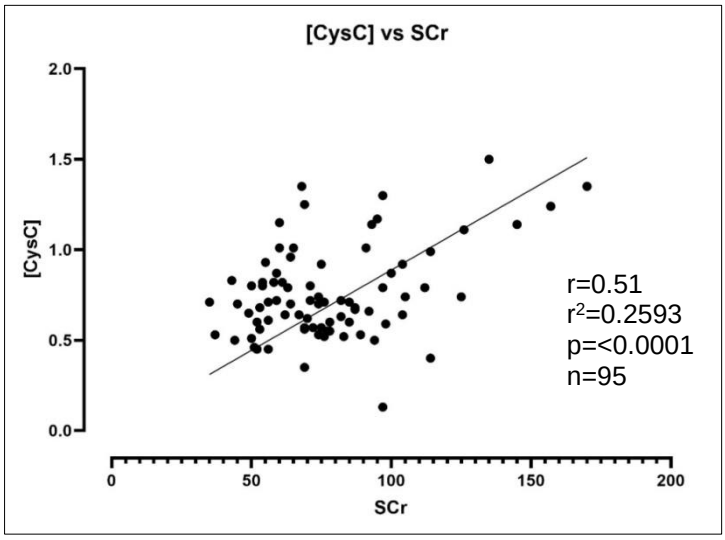

Figure 7. [CysC] vs SCr concentration

In a study by Stephan *et al.*(2019), participants ranged in age from 50 to 107 (60% of participants were women, mean age = 69.36, SD = 9.54) . CysC was retested four years later. At the start of the trial and four years later, a higher subjective age was associated with a higher likelihood of exceeding the CysC clinical threshold. The study found a link between perceived age and renal dysfunction risk that extends beyond chronological age. Odden *et al.*(2010) combined individual-level cross-sectional data from 18,253 participants in one or more of four studies: the Cardiovascular Health Study, the Health, Aging and Body Composition Study, the Multi-Ethnic Study of Atherosclerosis, and the Prevention of Renal and Vascular End-Stage Disease cohort. CysC was used to measure renal function. Age and CysC levels correlated non-linearly throughout all ages. This link was significant even among persons without clinical risk factors for renal disease; mean Cystatin C levels in participants aged 80 and beyond were 46% higher than those aged 40 (1.06 vs 0.72 mg/L, P 0.001). In this study there was not a clear positive correlation between [CysC] and age (r= -0.03988). This is contrary to many other studies (van den Brand *et al.*, 2011; Delanaye *et al.*, 2012; Lesley A Inker *et al.*, 2012; McCullough *et al.*, 2012; Schaeffner *et al.*, 2012). This could also be due to our inclusion criteria, which limited the sample collection to only those with MetS. These are typically older patients at BLCHC, which our mean participant age of 63 years also suggests.

In 106 CKD patients, CysC had a significantly higher correlation (r=0.9735) with measured GFR than creatinine, according to a study conducted in South India (Kumaresan and Giri, 2011). This would imply that CysC is a more reliable predictor of renal function once patients' have co-morbid CKD.

In elderly patients, muscle mass is decreased and adipose tissue is increased, which would explain why CysC correlated so much better with creatinine clearance after all the fat was subtracted and age played a larger role in the calculation. It also supports the study by Kumaresan and Giri, (2011).

3.3.3 **Clinical CKD staging**

Comparing a new clinical measuring technique to an existing one is necessary to assess whether the results generated are congruent with each other in order for a suggested alternative to take place(Bland and Altman, 1986).The Cockroft-Gault Equation, CKD-EPI and MDRD Equations are currently well established equations in South Africa and were used to compare the staging of CKD with the CysC CKD-EPI 2021 and SCr + CysC CKD-EPI 2021.Based on Table 9CysC containing equations appear to underestimate the CKD staging compared to Serum Creatinine based equations which could possibly be overestimating the CKD staging. Based on the CysC CKD-EPI 2012 Equation, only one patient would have been classified as CKD stage III compared to the serum creatinine based equations where at least 14 (17.5%) patients had an eGFR less than 60 mL/min/1.73m^2.

Approximately 17.5% of patients from the Cockcroft-Gault equation were reclassified from Stage III to Stage IV when using CysC. Of the patients within the CysC group, 85% of were considered as having an eGFR greater than 90, meaning that approximately 27.5% of patients were reclassified into Stage I CKD from the SCr CKD-EPI 2021 equation which had 50% of patients classified within Stage I.

Table 9: Comparison of CKD Staging using different equations (n = 80)

Variable	CKD Staging				
	Stage V <15	Stage IV 15-29	Stage III 30-59	Stage II 60-89	Stage I >90
			mL/min/1.73m^2		
Serum Creatinine Equations					
Cockroft-Gault equation (mL/min)	0	1(1.3)	15(18.8)	28 (35)	32(40)
Cockroft-Gault adjusted BW (mL/min)	5(6.3)	5(6.3)	33(41.3)	24(30)	10(12.5)
Salazar Cochrane	5(6.3)	0	17(21.3)	19(23.8)	34(42.5)
MDRD	0	0	14(17.5)	39(48.8)	27(33.8)
SCr CKD-EPI 2009	0	0	13(16.3)	32(40)	34(42.5)
SCr CKD-EPI 2021	0	0	14(17.5)	26(32.5)	40(50)
Serum Cystatin C Equations					
Cystatin C CKD-EPI 2012	0	0	1(1.3)	15(18.8)	62(85)
Le Bricon Cystatin C	0	0	1(1.3)	18(22.5)	61(84)
Hoek Cystatin C	0	1(1.3)	3(3.8)	16(17)	59(82)
Larsson Cystatin C	0	1(1.3)	5(6.3)	13(14)	61(84)
Hybrids					
SCr and Cystatin C CKD-EPI 2012	0	1(1.3)	6(7.5)	23(28.8)	46(57.5)
SCr and Cystatin C CKD -EPI 2021	0	1(1.3)	6(7.5)	16(17)	54(67.5)

Results are represented as the number of patients within the particular staging and (%)

Luis-Lima *et al*.(2019) studied the reliability of CysC and/or SCr-based formulae in defining CKD phases in 882 patients with diverse clinical circumstances and glomerular filtration rates. Misclassification was consistent for all 61 equations studied, averaging 50% for creatinine-based formulas and 35% for Cystatin C-based equations. Approximately 10% of patients skipped a stage and were categorised two stages above or below their original level. According to the findings of the study, using equations based on Cystatin C did not result in any clinical benefits over using equations based on creatinine. According to the findings of the study, the categorization of CKD stages based on estimated GFR is arbitrary.

Kakde *et al.*(2018)evaluated the diagnostic performance of several different formulas to discriminate values of GFR below 90 ml/min. Within this study, the number of individuals who were improperly classified with respect to their measured DTPA GFR was evaluated. The CKD-EPI$_{SCr-CysC}$ equation performed the best, as only 1.1% of the individuals were misclassified with CKD-EPI$_{SCr-CysC}$, while 6.6% were misclassified with MDRD.

Because we did not have a comparison to the gold standard, the findings of Luis-Lima *et al.*(2019) were comparable to ours. This was due to the fact that we were unable to determine whether CysC or SCr was more accurate when it came to staging CKD. It is not possible to determine which result was more accurate in terms of the CKD-staging predictions because there is not a validated formula for either CysC or SCr within the study population. If the current practice, using eGFR values derived from [SCr] using CKD-EPI and MDRD formula are in fact accurate, the Eurolyser CUBE POC device would have underestimated the renal dysfunction in the study population. This does support earlier research stating that POC devices have a propensity to over-estimate eGFR results (Schnabl *et al.*, 2010; Shephard *et al.*, 2010; Morita, Suzuki, Masukawa and Ueno, 2011). This would also help make sense of our results in the context (Peer *et al.*, 2009), who in 2009 found that CysC reacted faster to declines in renal function. The existing evidence seems to suggest that the Eurolyser CUBE POC device could likely have overestimated eGFR values in our study population.

3.3.4 **Cystatin C and Cardiovascular risk**

Seventy-one patients who had both CysC and SCr as well as total cholesterol values were included in this comparison. Spearman's correlation was used to define the correlation between two sets of categorical values. Spearman's method for determining correlation between two sets of categorical values showed a significant correlation between CysC and CVD risk (r=0.32; [95% CI 0.08299 – 0.5173]; P = 0.007).

According to the updated WHO CVD risk chart (Appendix B), most of the patients (49.3%) had an estimated CVD risk of 10 - <20%, 35.2% of which were females.

Table 10: Cystatin C and Cardiovascular risk score categories (n=71)

Categories	**All**	**Female**	**Male**
Cystatin C (mg/L)			
<0.5	4(5.6)	4(5.6)	0(0)
0.5-0.59	14(19.7)	9(12.7)	5(7)
0.6-0.69	12(16.9)	7(9.9)	5(7)
0.7-0.79	13(18.3)	9(12.7)	4(5.6)
0.8-0.89	10(14.1)	10(14.1)	0(0)
0.9-0.99	5(7)	4(5.6)	1(1.1)
>1	13(18.3)	8(11.3)	5(7)
Cardiovascular Risk Score (%)			
<5	3 (4.2)	2 (2.8)	1 (1.4)
5-<10	23 (32.4)	16 (22.5)	7 (9.9)
10-<20	35 (49.3)	25 (35.2)	10 (14.1)
20-<30	9 (12.7)	7 (9.9)	2 (2.8)
>30	1 (1.4)	1 (1.4)	0 (0)
<5	3 (4.2)	2 (2.8)	1 (1.4)

Results are represented as the number of patients within each category and (%)

Of the participants within the Bishop Lavis community 49.3% are classified as having an intermediate risk of experiencing a CVD within 10 years. According to Curry *et al*.(2018) individuals with a 10-year CVD event risk more than 20% are deemed high

risk individuals, those with a risk less than 10% are deemed low risk, and those with a risk of 10% to 20% are deemed intermediate risk.

In a study by P Ravi *et al*.(2020), an assessment of eGFR by CysC revealed a significant and inverse relationship with a lipid profile that incorporated total cholesterol (r = -0.19; p 0.05).

CysC has been established as a possible predictor for cardiovascular disease complication (Jernberg *et al.*, 2004; Koenig, Twardella, Brenner and Rothenbacher, 2005; Luc *et al.*, 2006; Deo *et al.*, 2008; Servais *et al.*, 2008; Zethelius *et al.*, 2008; Shlipak *et al.*, 2009). In this research project there was no correlation between participant age and [CysC] (r = -0.03988 and P-value 0.6424). This points to the possibility that [CysC] is measuring underlying atherosclerosis. Should there have been a significant correlation between age and [CysC] it would have helped eliminate the possibility of CysC measuring atherosclerosis, since age weighs the heaviest among the different factors determining one's risk for a CVD complication.

3.4 **Secondary Objectives**

3.4.1 **Eurolyser CUBE POC Cystatin C device value to patient care in a primary healthcare.**

The Eurolyser CUBE POC device was used to perform the test, and the results were obtained within ten minutes of the test being carried out. When broken down into its component parts, the procedure was not difficult to comprehend and carry out (see Appendix F). On the other hand, a few difficulties were identified: It was difficult to determine whether or not the reagents were actually at room temperature because the study was carried out in South Africa during the winter months, when it is typically colder. In order to guarantee that the reagents were at room temperature when it came time to draw a patient's blood, they were taken out of the refrigerator five at a time and placed on a tray. Sometimes patients were unable to provide blood samples or needed to be referred to another facility, which meant that reagents could not be returned to the refrigerator. As a result, they had to be discarded because the manufacturer had not specified whether or not samples could still be used again if they had already been allowed to warm up to room temperature.

3.4.2 **Dose adjustments**

Two patients had elevated CysC levels corresponding to CKD Stage 3 (see Table 3) when calculated using the Eurolyser CUBE POC and CKD-EPI 2012. The relevant health care practitioners were informed as both patients were on Metformin doses that were greater than 1500mg daily. According to (SEMSDA, 2017; Lalau *et al.*, 2018), Metformin doses should be at a maximum of 1g/24hr in patients with eGFR levels between 30-44 mL/min/1.73m^2 were identified as having CKD and required dose adjustments on the day of their visitation. The relevant physicians were informed and dose adjustments made accordingly.

According to calculations made with the Eurolyser CUBE POC and CKD-EPI 2012, two patients had elevated CysC levels that were consistent with CKD Stage 3 (see Table 3). According (SEMSDA, 2017; Lalau *et al.*, 2018), Metformin doses should be administered at a maximum of 1g/24 in patients who are identified as having CKD and eGFR levels between 30-44 mL/min/1.73m^2. Due to the fact that both patients were taking doses of Metformin greater than 1,5g per day, the appropriate healthcare professionals were informed. Dosage adjustments were made by the practitioners after confirming SCr based eGFR with previous NHLS results. These results were actively searched for by the prescribing physicians after they received the CysC results, which allowed for intervention before leaving Bishop Lavis CHC. Under normal circumstances, the SCr results would not have been expedited in the same way.

Should these patients have continued to receive incorrect doses of Metformin the possibility of an adverse drug reaction would have increased.

CHAPTER 5: CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

5.1 Introduction

An overview of the study's conclusions, which were conceptualized in line with its goals and research question, is presented in this chapter. The limitations of the study are then discussed. The chapter is concluded with suggestions for future research and practice-based suggestions derived from the study's findings.

5.2 Conclusion

In the absence of a measured eGFR, based on exogenous biomarkers such as inulin, it is not possible to conclude which renal marker could most accurately predict eGFR when using existing renal function formulae. The correlations between the [CysC] and SCr measured by the Eurolyser CUBE POC and the NHLS point to the fact that both of these markers measure the kidney's ability to excrete them. Interestingly, the correlations between the biomarker concentrations were higher than any estimated GFR from SCr or [CysC]. This adds to the case that both biomarkers need a validated formula for the population group that was studied, since the ideal formulas for SCr-derived eGFR and [CysC]-derived eGFR should have been close to the concentrations of the biomarkers in the population. [CysC] derived eGFR did tend to estimate GFR values higher than the corresponding [SCr] derived eGFR values, which was contrary to other research which suggested CysC derived eGFR would likely be lower than SCr derived eGFR (Peer *et al.*, 2009) (Herget-Rosenthal *et al.* 2005).It is likely that the Eurolyser CUBE POC device under estimated [CysC], which would be in line with conclusion by other researchers stating that POC devices tend to over-estimate eGFR (Schnabl *et al.*, 2010; Shephard *et al.*, 2010; Morita, Suzuki, Masukawa and Ueno, 2011). This research cannot beyond reasonable doubt conclude that the POC device utilised over-estimated eGFR, since without mGFR it is impossible to definitively say if SCr or CysC derived equations were more accurate in their predictions.

In light of this, determining which formula is the most appropriate to use within this population is questionable. All the formulas used to calculate eGFR during this research have been validated in different population groups. Our population group, those suffering from metabolic syndrome, has no one eGFR formula that has been

tested and validated beyond reasonable doubt. Based on the knowledge that SCr is confined to muscle cells, a reasonable deduction could be that formulas that take only ideal body weight into consideration are likely to be more accurate. Many of the formulas used to calculate the eGFR do not make provisions to exclude mass from adipose tissue. In this research, the use of ideal bodyweight derived from each participant's BMI led to significant reductions in eGFR when using the Cockcroft-Gault formula. Since Cystatin C does not rely on muscle cells alone for its production, it seems likely that it might be of more value within an obese population. However, this remains inconclusive without a reference mGFR that can guarantee an accurate eGFR to compare to.

This study also concludes that a correlation between CysC and CDV risk does exist. The correlations between CysC and CVD risk were found to be independent of renal function and age, as there were significant positive or negative correlations between the cardiovascular risk score to age, SCr or eGFR derived from SCr. The moderate correlation of CysC further reinforces that [CysC] concentrations may be useful in determining cardiovascular risk and even be useful as a coefficient that could aid in developing a non-invasive method for the quantification of atherosclerosis in the future.

Our study confirms that it is possible that the Eurolyser CUBE POC device could serve as a quicker screening test for renal function when compared to laboratory testing, particularly in situations where lab tests are not readily available. By measuring eGFR at appropriate intervals, more people who have a higher risk of CKD can be identified, which opens the door for more appropriate treatment options to be put into action. That is, preventative measures might be taken in some cases. Estimations of [CysC] by POC devices should be reduced should a medical practitioner wish to more closely follow estimate SCr derived eGFR using [CysC].

5.3 Limitations

The research had three substantial limitations, the first being that it was a single-centre study. Secondly the study could not meet its calculated sample size of 323 participants due to not having enough CysC reagents. Third, the participants in our research who had their GFR estimated were chosen based on the presence of metabolic syndrome rather than at random.

Another limitation of this cross-sectional investigation is that we only measured the serum parameters once for each participant. This indicates that the results might be overestimated or underestimated. We lacked a marker that could be regarded as the "gold standard" because of the intrusive and time-consuming methods required to do inulin or iothalamate assays, which are not appropriate for study subjects. We did not include other criteria like albuminuria in this study due to the limited reagent availability.

The study's usage of the POC device was also subject to other method limitations:

- Since the research was carried out in the winter, it's possible that certain days it took longer for the solutions to reach room temperature.
- Some blood samples were turned down by the NHLS, making it impossible to determine the SCr levels.
- Some samples that were still in the POC device at the time were lost due to power outages in the region.
- The potential of human mistake is significant, and it was impossible to know for sure whether the right procedure was always used. Since NHLS does not presently test for CysC, any findings from the POC cannot be verified by a licensed laboratory.

5.4 Recommendations

5.4.1 Recommendations for future research

In the same participant group, CysC and CVD risk should be retested to see if there has been any progression and, if so, whether there has been any correlation with the progression. Determine the use of CysC in patients with severe muscle wasting brought

on by TB and HIV by comparing it to SCr. A multi-centred approach with a larger sample size would support the findings even more. Use of an exogenous gold standard marker is required for conducting preliminary research.

5.4.2 Recommendations for practice

The Eurolyser CUBE POC can be used as a tool for CKD screening. Patients may be referred for additional monitoring, particularly in rural areas without readily available laboratory services. It would be advantageous to print out the results for practitioners in the facilities where the device is used so they can put them in the patient folders.

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APPENDICES

A. Data collection tool

B. WHO Cardiovascular Risk Chart

C. The University of the Western Cape Biomedical and Research Ethics Committee – Ethics Approval

D. Bishop Lavis Approval Letter

E. Consent Forms and Information Sheets

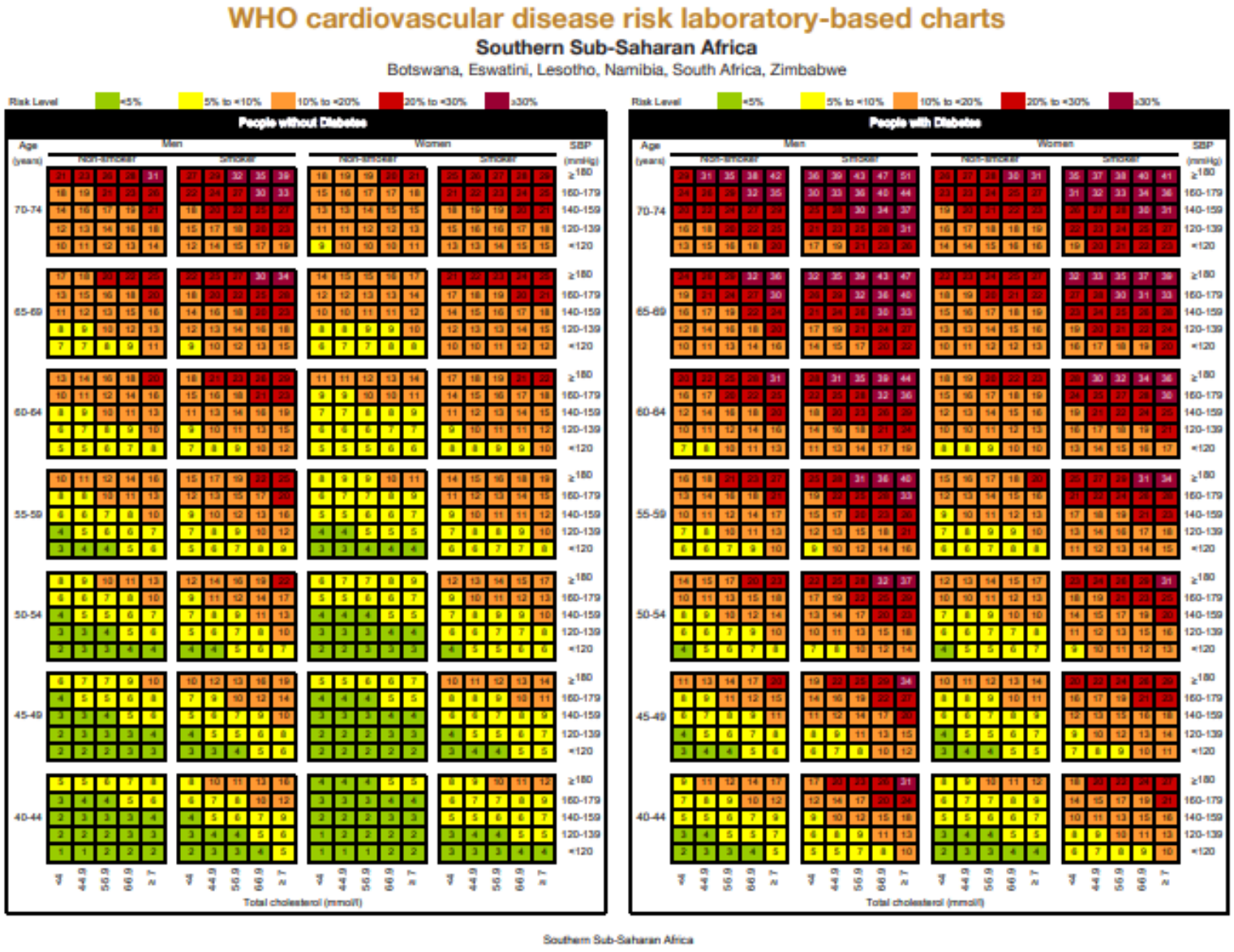
F. Eurolyser CUBE POC Device Steps

APPENDIXA: DATA COLLECTION TOOL

Comparing Cystatin C to Serum Creatinine in determining renal function derived from a point-of-care device and investigating its correlation to cardiovascular risk										
Facility name	**Bishop Lavis Community Health Center**									
Data collector's name		**Signature:**			**Date collected:**					
Folder number										
Participant number										
Gender										
Age (years)										
Pregnancy										
Time of screening										
Comorbidities										

Screening parameters										
BMI (kg/m^2)										
Height (m)										
Weight (kg)										
10 year CVS risk %										
Smoker										
Blood Pressure (mmHg)										
Total cholesterol										
HbA1C (%)										
Serum creatinine (umol/mL)										
Cystatin-C (mg/L)										
eGFR (mL/min/1,73m^2)										

APPENDIX B: WHO CARDIOVASCULAR RISK SCORE CHART



APPENDIX C: THE UNIVERSITY OF THE WESTERN CAPE BIOMEDICAL AND RESEARCH ETHICS COMMITTEE – ETHICS APPROVAL

UNIVERSITY of the WESTERN CAPE

60 YEARS of hope, action & knowledge

01 June 2022

Ms KL Mogakane
School of Pharmacy
Faculty of Natural Sciences

Ethics Reference Number: BM22/4/6

Project Title: Investigating the correlation between cardiovascular risk score and Cystatin C serum concentrations derived from a point of care device compared to serum creatinine.

Approval Period: **30 May 2022 – 30 May 2025**

I hereby certify that the Biomedical Science Research Ethics Committee of the University of the Western Cape approved the scientific methodology and ethics of the above-mentioned research project and the requested amendment to the project.

Any further amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

Please remember to submit a progress report annually by 30 November for the duration of the project.

For permission to conduct research using student and/or staff data or to distribute research surveys/questionnaires please apply via:
https://sites.google.com/uwc.ac.za/permissionresearch/home

The permission letter must then be submitted to BMREC for record keeping purposes.

The Committee must be informed of any serious adverse event and/or termination of the study.

Ms Patricia Josias
Research Ethics Committee Officer
University of the Western Cape

NHREC Registration Number: BMREC-130416-050

FROM HOPE TO ACTION THROUGH KNOWLEDGE.

APPENDIX D: BISHOP LAVIS APPROVAL LETTER

Western Cape Government
Health

STRATEGY & HEALTH SUPPORT
Health.Research@westerncape.gov.za
tel: +27 21 483 0866: fax: +27 21 483 6058
5th Floor, Norton Rose House,, 8 Riebeek Street, Cape Town, 8001
www.capegateway.gov.za)

REFERENCE: WC_202206_004
ENQUIRIES: Dr Sabela Petros

University of the Western Cape
Private Bag x17
Bellville
7535

For attention: Ms Khomotso Lesedi Mogakane, Mr Edward Upton

Re: Comparing Cystatin C to Serum Creatinine in determining renal function derived from a point-of-care device and investigating its correlation to cardiovascular risk

Thank you for submitting your proposal to undertake the above-mentioned study. We are pleased to inform you that the department has granted you approval for your research.
Please contact the following people to assist you with any further enquiries in accessing the following sites:

Bishop Lavis CDC	**Ms Rachel Carelse**	**021 927 1165**

Kindly ensure that the following are adhered to:

1. Arrangements can be made with managers, providing that normal activities at requested facilities are not interrupted and the constraints caused by the Covid-19 epidemic above are respected and adhered to.
2. Researchers, in accessing provincial health facilities, are expressing consent to provide the department with an electronic copy of the final feedback (**Annexure 9**) within six months of completion of research. This can be submitted to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).
3. In the event where the research project goes beyond the *estimated completion* date which was submitted, researchers are expected to complete and submit a progress report (**Annexure 8**) and an updated ethics clearance letter to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).
4. The reference number above should be quoted in all future correspondence.
5. You are required to notify the substructure office when you commence with your study at the above-mentioned facility(ies) and inform them when you have completed the study at the facility. **Northern- Tygerberg Substructure:** Ms Terri Lemmetjies - 021 815 8559 Terri.Lemmetjies@westerncape.gov.za.

Yours sincerely

PROF. V ZWEIGENTHAL
DIRECTORATE: HEALTH INTELLIGENCE
DATE: 27 June 2022
CC

APPENDIX E: CONSENT FORMS AND INFORMATION SHEETS

UNIVERSITY of the WESTERN CAPE

School of PHARMACY
Faculty of Natural Sciences
Private Bag X17
Bellville
7535

Comparing Cystatin C to Serum Creatinine in determining renal function derived from a point-of-care device and investigating its correlation to cardiovascular risk

Khomotso Lesedi Mogakane
3678282

STUDY INFORMATION SHEET

Dear Participant,

Thank you for your willingness to participate. This is a research study is intended for postgraduate degree purposes. This research study aims to assess the use of a Cystatin C point of care testing in a primary healthcare setting as opposed to serum creatinine for determining renal function and to explore a possible relationship between Cystatin C and cardiovascular risk assessment scores in a South African population.

If you have any questions please do not hesitate to ask me or use the contact details at the end of this letter.

Project Title:
Investigating the correlation between cardiovascular risk score and Cystatin C serum concentrations derived from a point of care device compared to serum creatinine

Special Instructions**:**
This information and consent form may contain words that are new to you. If you read any words that are not clear to you, please ask the person who gave you this form to explain them to you.

Purpose:

You are being asked to actively take part in a research study that aims to determine whether the use of a Cystatin-C point-of-care device would be beneficial as an additional or alternative biomarker in determining renal function and predicting cardiovascular risk.

Procedures:
If you agree to participate in this study; a health care professional will take a blood sample from you by means of a finger prick. This blood sample will be used to determine Cystatin-C levels using the point-of-care device.

Risks/Discomforts:
There may be some discomfort during the duration of the finger prick procedure, you will be provided with the necessary care to ensure that the discomfort is minimal. If you do experience consistent discomfort, please inform the health care professional. The researcher performing the finger prick will be required to wear gloves and any personal protective equipment required.

Benefits:
As an active participant of this research study, your renal function will be tested with the use of Cystatin-C point-of-care device, meaning that any required interventions based on your renal function can be made by the clinic sister or physician at the facility on the same day. Cardiovascular screening will also take place during your assessment, this means that your risk of experiencing a cardiovascular event such as a heart attack or stroke in the next 10 years will be calculated. This is important because early detection means early treatment and prevention.

Confidentiality:
Your confidentiality will be protected during the project. You will be identified by a code which will be linked to the data sets gathered from surveying you but not your name. The data collector will not ask you for your name. The informed consent form that you sign will be stored in a secure cupboard that is inaccessible to others. Data will also be stored electronically and will be password protected, which will only be accessible to researchers.

Voluntary Participation/Withdrawal:
Your decision to partake in this project is completely voluntary. You will not receive any remuneration for your participation. You may refuse to take part or you may withdraw from the project at any time without penalty. Any personal information that identifies you will be concealed and removed. However; once your information has been collected and the data collection period ends, we will no longer be able to remove your results from the pool of the data this research generates.

What if you have questions?
If you have any questions about the project, please contact any of the following persons:

Ms Lesedi Mogakane
School of Pharmacy
University of the Western Cape

Private Bag X17, Bellville 7535
Tel: +27 (0) 79 581 0544
Email: 3678282@myuwc.ac.za

Should you have any questions regarding this research and your rights as a research participant or if you wish to report any problems you have experienced related to the project, please contact the supervisor, Mr Edward Upton.

Mr Edward Upton
School of Pharmacy
University of the Western Cape
Private Bag X17, Bellville 7535
Tel: +27 (0) 82 321 1224
Email: eupton@myuwc.ac.za

Alternatively, you can contact the Director of the School of Pharmacy, Professor Sarel Malan.

Prof Sarel Malan
School of Pharmacy
University of the Western Cape
Private Bag X17, Bellville 7535
Tel: +27 (0)21 959 3190
Email: sfmalan@uwc.ac.za

The Biomedical Research Ethics Committee (BMREC) provided approval to conduct the study. The approval number (*to be inserted upon receiving the number*).

Biomedical Research Ethics Committee (BMREC)
Research Development
Room 28 C Block New Arts Building
University of the Western Cape
Robert Sobukwe Road
Bellville, 7535
Tel: +2721 9592988
Email: research-ethics@uwc.ac.za

UNIVERSITY of the WESTERN CAPE

School of PHARMACY
Faculty of Natural Sciences
Private Bag X17
Bellville
7535

Comparing Cystatin C to Serum Creatinine in determining renal function derived from a point-of-care device and investigating its correlation to cardiovascular risk

Khomotso Lesedi Mogakane
3678282

CONSENT FORM

Statement of Your Consent:

- I have read the above description of this research study intended for degree purposes.
- I have been informed of the risks and benefits involved, and all my questions have been answered to my satisfaction. I voluntarily agree to take part in this research study.
- I understand that all efforts will be made to conceal my identity.
- I understand I will receive a copy of this consent form.
- I understand that I will not be remunerated for my participation in the study.

I hereby give consent for the information gathered from this project to be used for education and training purposes, publication in journals, textbooks, or conference material. I understand that my consent or refusal will in no way affect my employment or healthcare. I give consent for my medical records and laboratory results to be recorded for this study.

Printed Name of Participant _____

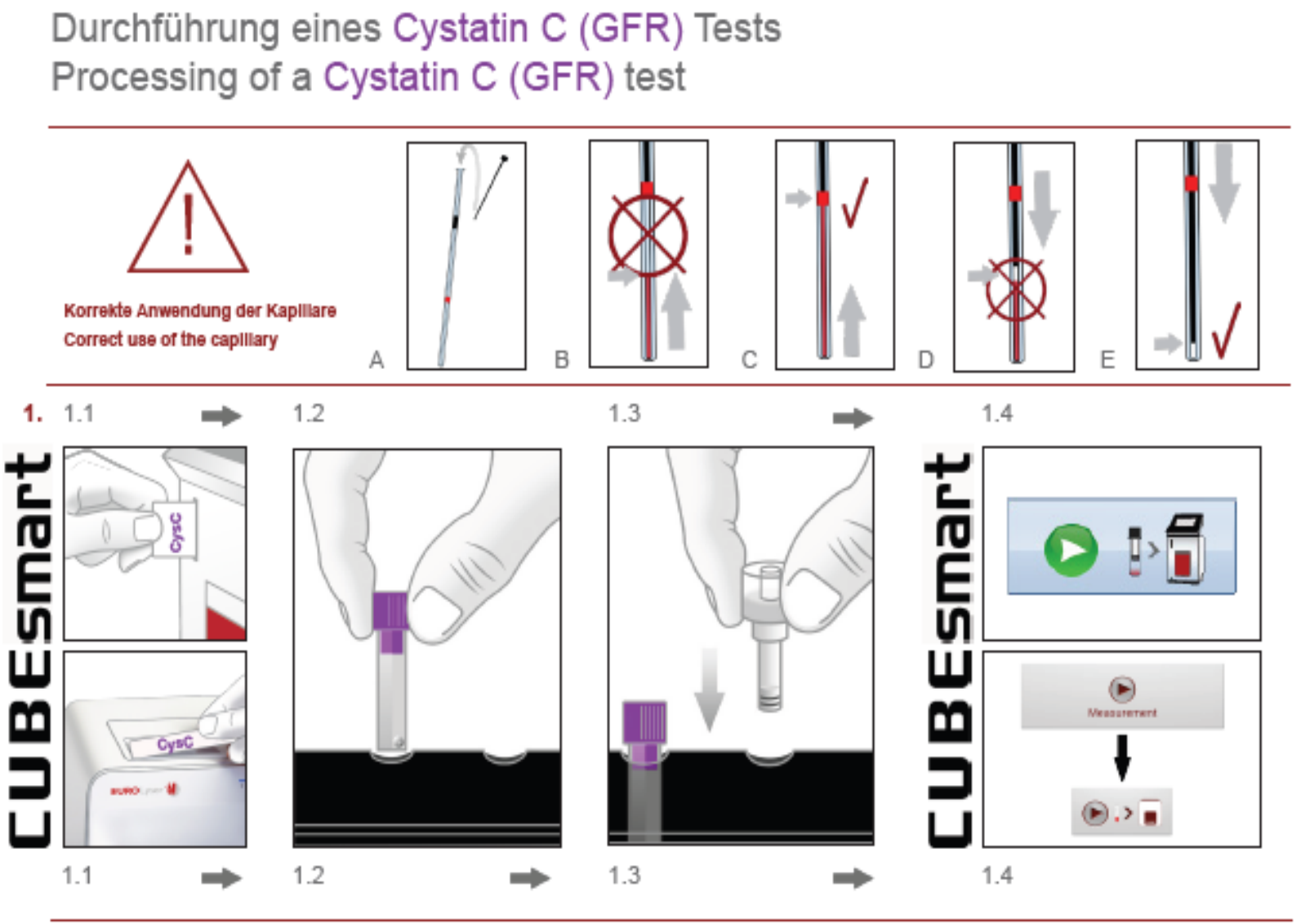
Participant's Signature Date

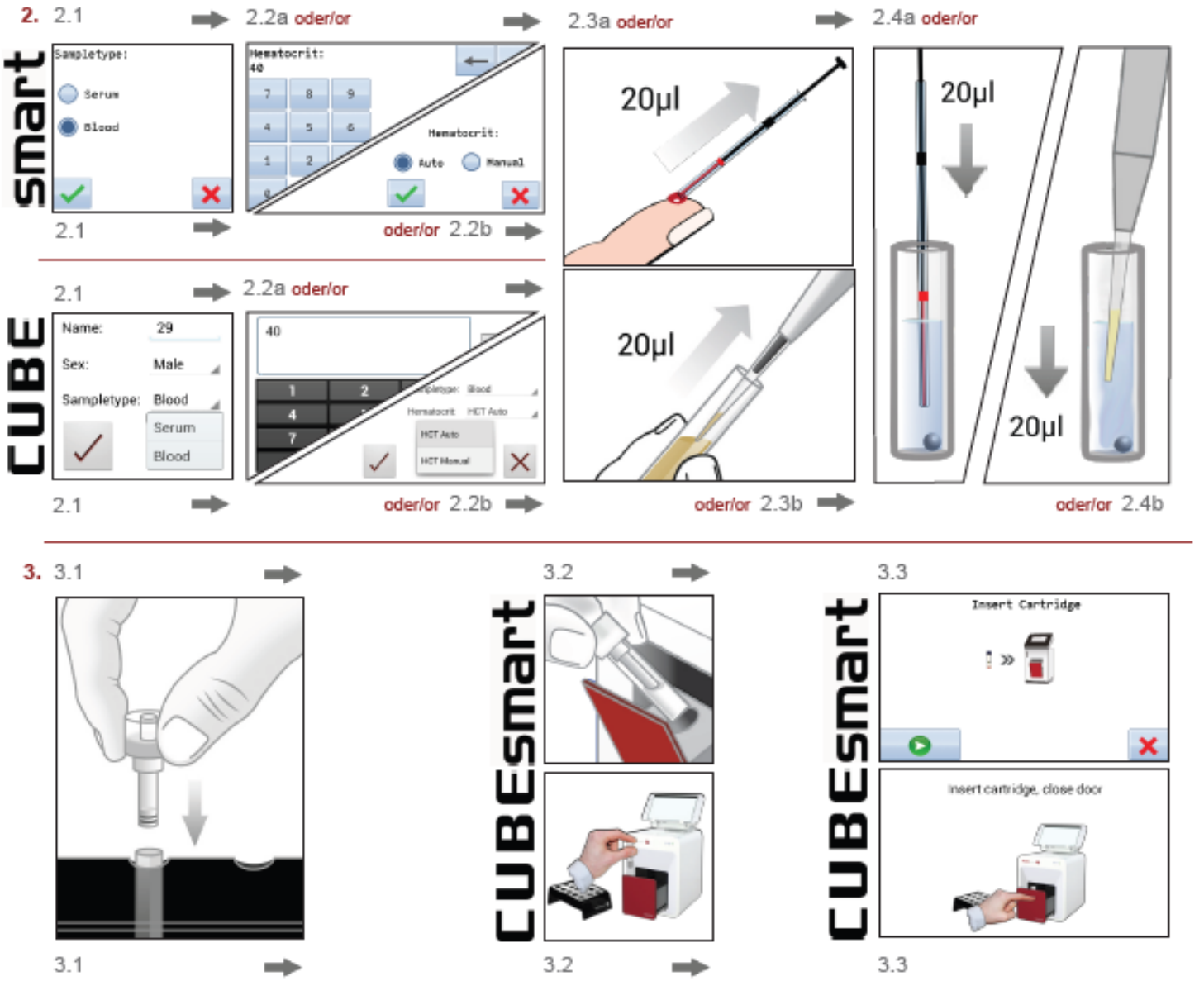
Printed Name of Researcher Designation

Researcher's Signature Date

Biomedical Research Ethics Committee (BMREC)
Research Development
Room 28 C Block New Arts Building
University of the Western Cape
Robert Sobukwe Road
Bellville, 7535
Tel: +2721 9592988
Email: research-ethics@uwc.ac.za

APPENDIX F: EUROLYSER CUBE POC DEVICE STEPS





EUROLYSER CUBE POC DEVICE

The Eurolyser CUBE

Packaging of Cyst C reagents