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The use of iohexol as a marker to measure glomerular filtration rate in the non-steady state of patients undergoing robot-assisted colorectal surgery

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Foreword

Acute kidney injury is a major risk factor for adverse outcomes following surgery or severe illness. Present criteria for diagnosing acute kidney injury relies on cessation or reduction in urinary output and/or increase of the biomarker creatinine. Serum creatinine is not perfect for this purpose. Several limitations are known, which complicates the interpretation of data particularly in the perioperative setting.

Inspired by a British research group that has studied measuring glomerular filtration rate by continuous infusion of iohexol in patients admitted to the intensive care unit, Professor Bjørn Odvar Eriksen came up with the suggestion that this method should be tested in our patient community. My supervisors, Lars Marius Ytrebø and Stephen Hodges, took on this challenge to test this method at the University Hospital of North Norway. Knowing the multitudes of factors affecting glomerular filtration rate in the severely ill patient, Ytrebø and Hodges decided to perform a pilot study in patients undergoing robot-assisted colorectal cancer surgery. Four patients were included during the spring 2019 and Ole-Martin Fuskevåg carried out the analytical work at Diagnostic Clinic, University Hospital of North Norway.

Professor Ytrebø suggested that I could use this raw data material in a master thesis, because they were neither systematically explored nor presented due to time constraints. Accordingly, my contribution to this work has been to study relevant literature, organize the raw data material, interpret, and present data for my mentors.

Professor Ytrebø suggested the topic for me, and I thank him and my other two supervisors, Stephen Hodges and Ole-Martin Fuskevåg, for including me to an interesting field of research. Ytrebø and Hodges have both been instrumental in advising me during the writing process. Fuskevåg has been very supportive on issues related to the analytical work processes.
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1 Summary

Introduction

Perioperative acute kidney injury (AKI) is associated with increased morbidity and mortality. Estimated glomerular filtration rate (eGFR) is routinely used to monitor renal function. This is an imprecise tool in the non-steady state condition and much kidney function can be lost before it is detectable by the eGFR method. A continuous low-dose infusion of iohexol has been proposed as a potentially valuable method of measuring GFR (mGFR). This novel method was tested in patients undergoing major abdominal cancer surgery. The aim for this thesis was to evaluate the feasibility of using iohexol to measure GFR, and to present data from a clinical feasibility study where the exogenous substance iohexol was applied for measurement of GFR.

Methods

Clinical observational study of a preoperative single iohexol injection and a continuous low-dose infusion of iohexol for 72 hours in four patients undergoing robot-assisted colorectal cancer surgery. Plasma iohexol clearances were measured at timed intervals and compared to eGFR calculated from creatinine and cystatin C levels.

Results

$eGFR_{cystatin\ C}$ demonstrated a different variability from that of $mGFR_{iohexol}$, while $eGFR_{creatinine}$ showed very little variation for the duration of the study. Furthermore, $eGFR_{creatinine}$ and $eGFR_{cystatin\ C}$ underestimated actual measured GFR.

Conclusions

Measurement of renal function in the perioperative setting is feasible with single injection and continuous low-dose infusion of iohexol. Iohexol plasma clearance should be applied when accurate GFR measurements are required.

Key words

Glomerular filtration rate, iohexol, perioperative care

Abbreviations

GFR; glomerular filtration rate, mGFR; measured glomerular filtration rate, eGFR; estimated glomerular filtration rate, AKI; Acute kidney injury, CKD; chronic kidney disease, s-creatinine; serum-creatinine, s-cystatin C; serum-cystatin C, CKD-EPI; Chronic Kidney Disease Epidemiology Collaboration, MDRD; Modification of Diet in Renal Disease, CILDI; continuous infusion of low-dose iohexol, ^{51}Cr-EDTA; chromium-51 ethylenediaminetetraacetic acid, ^{99m}Tc- DTPA; Technetium-99m-diethylenetriaminepentaacetic acid, BSA; body surface area, BMI; body mass index, ICU; intensive care unit, ERAS; Enhanced recovery after surgery, ASA; American Society of Anesthesiologist classification, KDIGO; Kidney Disease: Improving Global Outcomes, REK; Regional Committees for Medical and Health Research Ethics, HPLC-UV; high performance liquid chromatography with ultraviolet detection, LC-MS; liquid chromatography mass spectrometry, LC-MS/MS; liquid chromatography tandem mass spectrometry, CVC; central venous catheter, Cl_P; plasma clearance, CV; coefficient of variation

2 Introduction

Rational and aim for study

Acute kidney injury (AKI) is a source of major mortality and morbidity in the perioperative setting (1). AKI is defined by an abrupt decrease in kidney function that includes, but is not limited to, acute renal failure (1). Perioperative AKI is often underdiagnosed, increases length of hospital stay, and some 30-40% of all AKI cases occur postoperatively (2, 3). The incidence of AKI in surgical patients ranges from 18-47% whereas the incidence in ICU patients ranges from 22% to 57% (3).

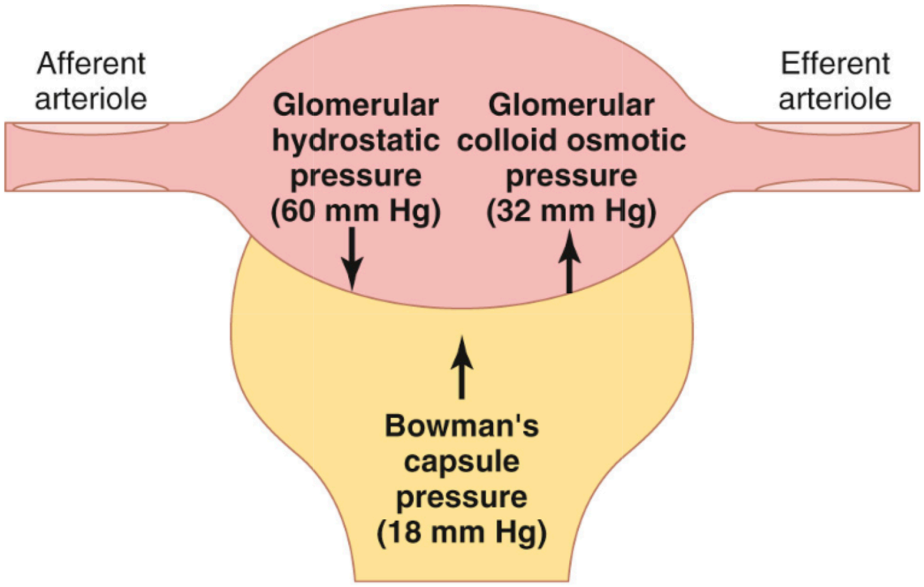
The exact pathogenesis for AKI is not known and there is apparently a large interindividual variation in the renal response to surgery and acute illness (1). However, the single most important patient-related factor regarding the risk for postoperative AKI is preoperative kidney function, with the greatest risk among patients diagnosed with chronic kidney disease (CKD). Delayed treatment of AKI may reduce the success rate of any treatment currently available (4).

Perioperative diagnosis of AKI calls for an accurate biomarker for renal function (3). Estimated glomerular filtration rate (eGFR) based on serum concentration of creatinine has been used as a surrogate for renal function. However, creatinine does not increase before substantial kidney function is lost, making it unreliable in the perioperative non-steady state setting (3, 5). Several exogenous markers for GFR have been considered in order to provide a more accurate monitoring of renal function. Later in the introduction I have briefly summarized the most frequently used endogenous and exogenous markers of GFR. Iohexol is a relatively new exogenous marker that has been suggested as the marker of choice when accurate measurement of GFR is needed (6). Single injection and postoperative continuous low-dose infusion of iohexol for measurement of GFR may provide a more accurate monitoring of kidney function in the non-steady state. The aim for this thesis was to evaluate the feasibility of using iohexol to measure GFR, and to present data from a clinical feasibility study where the exogenous substance iohexol was applied for measurement of GFR.

Glomerular filtration rate

Glomerular filtration rate, GFR, is a measurement of the ability of the glomeruli to produce an ultrafiltrate of blood plasma by means of a pressure-driven filtration across the glomerular capillary basement membrane (7). GFR is presented in mL/min, and it is determined by the net filtration pressure and the glomerular capillary filtration coefficient (8). It is fundamentally the same as the Starling mechanism of any capillary in the body, but in the special case of the kidney the end product is regulation of fluid volume and excretion. It is driven by the hydrostatic pressure in the glomerulus, and inhibited by the plasma oncotic pressure in the glomerulus as well as the hydrostatic pressure of Bowman's capsule (9). The force driving filtration is as such generated by the left ventricle of the heart (9).

Figure 1 - The forces of glomerular filtration[1]

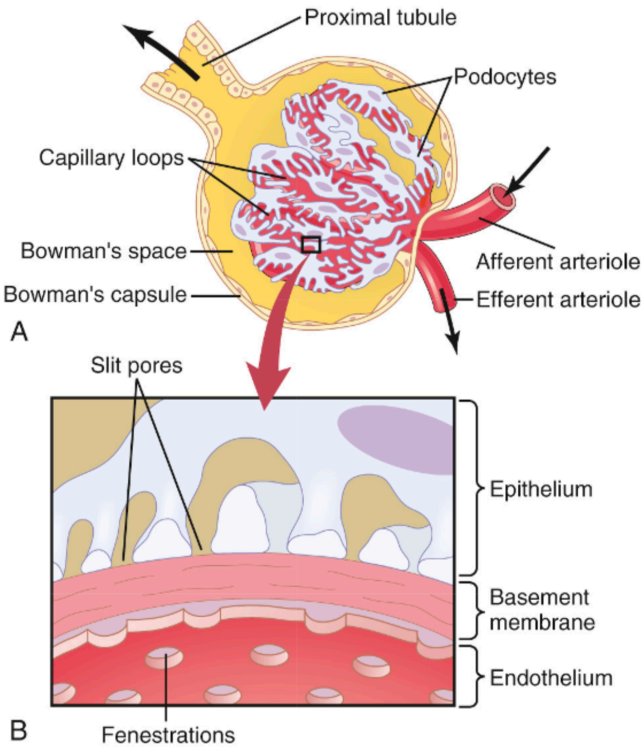


Summary of forces causing filtration by the glomerular capillaries. The values shown are estimates for healthy humans (8).

[1] Reprinted from Pocket Companion to Guyton and Hall Textbook of Medical Physiology, Thirteenth Edition, Thomas H. Adair, John E. Hall, Thomas E. Lohmeier, R. Davis Manning, Glomerular Filtration, Renal Blood Flow, and Their Control, 192-197, Copyright 2016, with permission from Elsevier.

The kidneys receive 20-25% of the cardiac output and 20-35% of the plasma volume is filtered through the glomerular capillaries per unit time (10). The transportation of ultrafiltrate of blood plasma goes through the endothelium and basal membrane of the glomerulus vessels, as well as through epithelial cells lining Bowman's capsule, completing glomerular filtration. The glomerular membrane is fenestrated and lined with a negatively charged basal membrane that hinders proteins and other solutes with high molecular weight from passing over the membrane, favoring smaller, positively charged molecules (8, 10). In a 70 kg person around 170 L is filtrated each day (11).

Figure 2 - The glomerulus and glomerular capillary membrane[2]



A: Basic ultrastructure of the glomerular capillaries. B: Cross section of the glomerular capillary membrane and its major components: capillary endothelium, basement membrane, and epithelium (8).

[2] Reprinted from Pocket Companion to Guyton and Hall Textbook of Medical Physiology, Thirteenth Edition, Thomas H. Adair, John E. Hall, Thomas E. Lohmeier, R. Davis Manning, Glomerular Filtration, Renal Blood Flow, and Their Control, 192-197, Copyright 2016, with permission from Elsevier.

GFR will vary also intra-individually under normal physiological circumstances. Diet, activity and circadian variations all affect GFR throughout the day, in addition to this measurement errors will together make for variations in determining GFR (6). This variation has been shown to lie somewhere between 4.2 to 10% for all markers of mGFR, meaning that an increase or decrease of less than 10% from the previous sample often is considered clinically irrelevant (6).

Secretion and reabsorption

Two other mechanisms separate from filtration are key features in the formation of urine: secretion and reabsorption. Secretion describes the deposition of organic anions and cations into the urine primarily by the proximal tubule, in addition to potassium, ammonium and protons by the distal tubules and collecting ducts (12). Reabsorption is a process where molecules are reabsorbed from the tubules into the systemic circulation (11).

Indexing and de-indexing GFR

Clinicians are most often presented with an estimation of GFR determined by an equation that includes the serum concentration of an endogenous biomarker, usually creatinine or cystatin C. The equations aim to balance out confounders related to the serum concentration of the given biomarker. GFR and by extent eGFR is dependent upon body size (13). It is therefore most often presented corrected for body surface area (BSA), called indexed eGFR. The BSA normalization is incorporated in the most used equations for calculation of eGFR; the Modification of Diet in Renal Disease (MDRD) study equation and those from the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI$_{creatinine}$, CKD-EPI$_{cystatin\ C}$, CKD-EPI$_{combined}$) (13-15). Indexing GFR for body surface area is done to be able to quickly compare GFR values of individuals of differing body sizes, and for defining normal ranges (16).

The body surface area applied for indexation is not the patient's own, but rather a standardized body surface area of 1.73m^2 (17). This standardization was first applied in a study published in 1928, and the BSA represents the average BSA of a 25 year old American in the 1920's (17). The average BMI of the same individuals were

approximately 22kg/m^2. The average BSA of 25-year-olds has due to increasing average BMI risen greatly since that, and the US average was in 2004 1.92 m^2. Similar number from 2002 in the European Union was 1.86m^2 (17). Adopting the US average from 2004 would give an instant rise of 9% in global eGFR (17).

In the normal weight population, this indexing has negligible impact on the eGFR value (16). Indexing for BSA has a much greater impact on the eGFR of obese patients, resulting in underestimation of absolute GFR. In a group of 81 patients with a BMI >30 examined with the exogenous marker ^{51}Cr-EDTA the mean difference in absolute GFR and indexed eGFR was 18.2+-12.1mL/min, even higher for patients with BMI > 40 (16). A 2015 study with 222 participants found CKD-EPI without normalization to be superior to the CKD-EPI equation in estimating absolute GFR, with the underestimation increasing with increasing BMI (18).

Both the MDRD and CKD-EPI formula index GFR for BSA using 1.73m^2 (15). For renally eliminated drugs the rate of elimination is dependent on the absolute GFR, not the indexed GFR. Using indexed GFR leaves drug elimination underestimated in subjects with a BSA >1.73m^2 and overestimated in subjects with a BSA <1.73m^2 (13, 15). Many drugs are eliminated by the kidneys, and it is as such often advised to reduce the dosage of said drug should the renal function be reduced, thereby reducing risk of toxicity (13). Evaluating de-indexed GFR is especially important when dealing with drugs with a small therapeutic window, such as dabigatran or gentamicin (19). Due to hyperfiltration leading to increased drug elimination, it will hypothetically demand increased dosing to reach the same concentrations at steady state compared to an individual with normofiltration (18). Indeed there are studies implicating that underdosage of chemotherapy with adverse outcomes is not uncommon in obese cancer patients, but the subject is not explored in great detail (18).

It is recommended that when doing dosing adjustments, absolute GFR should be used, in particular when the BSA differs greatly from standardized BSA (15). In a 2013 survey between New Zealand physicians only 39% were aware of BSA being part of the estimated GFR provided from the lab, leading one to question if physicians are using de-indexed GFR when needed for correct dosing (19).

Methods for estimation and measurement of GFR

It is of great clinical interest to evaluate the GFR of the patient, but there is less agreement on the best method of doing so (6). GFR is considered the superior marker of renal function, both in the healthy populations and in states of disease and stress (6, 20-23). Direct measurement of GFR is impossible because the filtration process simultaneously takes place in millions of glomeruli, and filtrate composition and volume change when passing through the kidney (24). Instead, markers are used as a surrogate for GFR. Some markers of GFR are endogenous, meaning they are created by metabolism in the body, others are exogenous, requiring the injection of extrinsic factors into the patient (24). The ideal marker is a small molecule that is kept intravascularly, is not protein bound and is freely filtered across the glomerulus, with no tubular secretion or reabsorption, and no intrarenal metabolization (4). The low molecular weight and low protein binding is necessary to ensure complete filtration (6). Both the endogenous and exogenous markers that are used all have properties that to varying degrees check these boxes.

The most common method in clinical practice is to calculate an estimation of GFR based upon endogenous substances, primarily because of its simplicity and low costs (25). The most reliable and accurate methods for determining GFR involve an exogenous marker (26). The use of exogenous markers is limited, as they are viewed as labor intensive, costly and often involves a potential for harm (20, 27). For a long time, the exogenous substance inulin has been considered the golden standard for measurement of GFR. While inulin clearance is costly and cumbersome, a systematic review on GFR measurement has demonstrated that there are several alternatives to renal inulin clearance for accurate measurement of GFR (24). Today, the most commonly used exogenous GFR markers for clearance measurements is ^{51}Cr-EDTA and iohexol (21).

A variety of endogenous and exogenous substances have been used for the purpose of measuring GFR so far. It is beyond the scope of this thesis to discuss them all, however, the discussion below focusses on the most prominent agents that have been used, their potential and their limitations.

Creatinine

Creatinine, an endogenous breakdown product from protein metabolism, is the most commonly used marker for GFR calculation (28, 29). Muscular creatine and phosphocreatine are converted to creatinine at an almost steady rate at about 2% of total creatine each day, which diffuses out of the cells and into the vascular system. Creatinine is under normal conditions not eliminated by other means than by the kidneys, resulting in s-creatinine only being dependent on production by breakdown of creatine phosphate and on renal elimination (7). Creatinine has many of the traits of a perfect filtration marker, as it is not protein bound and it is freely filtered, not metabolized by the kidney as well as being physiologically inert. (7) A substantial portion of creatinine is, however, secreted in the proximal tubule, giving rise to an overestimation of GFR by 10-40% (7, 28). Even larger overestimations are seen in grave renal insufficiency with lower GFRs, as the secreted portion becomes proportionally larger (7, 28). When compared to the golden standard for GFR measurement, inulin, this overestimation has been demonstrated in a multitude of studies as well as in a systematic review (24).

Creatinine can be used as a marker of GFR in several ways. The serum concentration of creatinine in combination with creatinine based estimated GFR has for many decades been the predominant way of assessing GFR in the clinical setting (7, 28). Current guidelines recommend to always include calculation of eGFR when s-creatinine is measured. For this estimation we use an equation that combines s-creatinine concentration with confounding factors related to muscle mass, such as age, gender and other factors. When GFR is calculated in this way, it is named estimated GFR, eGFR. The different formulas used for calculating eGFR take for granted that the excretion of creatinine is constant and equal to production (7). The two equations seeing the most use today is the Modification of Diet in Renal Disease (MDRD) and the newer CKD Epidemiology Collaboration (CKD-EPI) equation (14).

The CKD-EPI equation is recognized as superior for most situations, as it results in a lower prevalence of decreased eGFR, while improving risk stratification, more accurately reflecting the mortality and risk for end stage renal disease (30). The MDRD and the CKD-EPI equation both use age, sex, race and serum creatinine as their four included variables (31). The CKD-EPI equation did, however, try to improve

on the MDRD equation by developing the equation using a population with a better kidney function than that of the population in the MDRD studies (31). The goal of the CKD-EPI consortium in designing this equation was to not systematically underestimate GFR in the setting of a high GFR, a problem seen with use of the MDRD formula (28). The MDRD equation provides reasonably unbiased results in patients with an GFR under 60 mL/min/1.73m^2, but is plagued by greater bias when the GFR is over 60 mL/min/1.73m^2 (32). The MDRD formula was originally validated in CKD patients, therefore the use of the formula in healthy individuals is unclear (33). CKD-EPI had a smaller median difference between eGFR and mGFR compared to that of MDRD, with the best improvements seen in individuals with a GFR greater than 60 mL/min/1.73m^2 (28). However, both the MDRD and CKD-EPI equations are demonstrated to perform reasonably well in the general population, correlating positively with the measured GFR by the exogenous substance iohexol (25).

The Cockcroft-Gault equation is the third most often used equation, though much more commonly used before the coming of the MDRD and CKD-EPI equations (28). One of the strengths of the Cockcroft-Gault equation was its ease of calculation, making it prime in the pre-smartphone age (28). It still sees some use, especially for drug-dosing (28). Cockcroft-Gaults estimates creatinine clearance without indexation for body surface area, and is presented in mL/min, whereas eGFR from the MDRD and CKD-EPI equations are presented in mL/min/1.73m^2 (13).

While the formulas attempt to balance out the variables affecting s-creatinine concentration, they do not account for everything. Dietary intake, certain medications, nutritional supplements and muscle mass are factors greatly affecting s-creatinine concentration and are not fully accounted for in the most used formulas for determining creatinine based eGFR (6, 20, 28, 34). Especially low muscle mass in the elderly is a major problem determining GFR using creatinine based methods (35). To a certain degree the covariates “age”, “ethnicity” and “gender” are used to correct for muscle mass (28). In critically ill patients especially the potential immobilization and malnutrition can affect creatinine values, and increases in total body water as a consequence of fluid treatment increases the distribution volume of creatinine, reducing serum creatinine concentration, potentially masking the severity of AKI (36, 37).

Several studies has underlined the imprecise eGFR values found in the normal range no matter the equation used (25). As creatinine rises eGFR falls exponentially, so that a smaller rise in creatinine creates a relatively larger decrease in GFR (28). Altering serum creatinine concentration at high GFR demands a large change in GFR, and will only result in a small change of s-creatinine (4). Furthermore, creatinine based measurements of GFR is a late marker of AKI (2). The GFR can be reduced from 100 to 5 mL/min relatively quickly, but s-creatinine will only rise by 1-2 mg/dL/day (4). This will hide an abrupt decline in GFR, for potentially several days, until the s-creatinine concentration starts rising (4). In a setting of AKI it is therefore important to understand that even in anuria, time is needed before s-creatinine reaches a steady-state concentration associated with severe AKI (7).

Other creatinine-based measurements are a simple measurement of urinary creatinine, called renal creatinine clearance. Renal creatinine clearance is considered superior to $eGFR_{creatinine}$ in its accuracy (36). Renal creatinine clearance requires urine sampling for measurement of urine-creatinine in addition to blood sampling (36). It is usually done by 24-hour urine sampling. Hemodynamic changes can alter renal function at several times during the 24-hour sampling period, giving the test less reliance for the ICU population (36). It also represents inconvenience for the patients and staff (27, 36). Problems like uncomplete emptying and failure to collect the entire sample makes the exercise harder (7). Because of these difficulties it sees far less use than $eGFR_{creatinine}$ in common clinical practice.

Cystatin C

Cystatin C is a small protein at 13-kDa that is produced by all nucleated cells at a constant rate throughout most of life (28, 33, 35). Cystatin C is practically freely filtered at the glomerulus, completely absorbed in the proximal tubule and subsequently fully catabolized there in the matter of minutes (27, 28). For this reason is not eligible for calculation of renal clearance, however, as the formation rate of cystatin C is generally considered to be rather constant the plasma and serum levels of cystatin C nonetheless makes for a useful marker for GFR (33). A meta-analysis from 2002 concluded that s-cystatin C is superior to s-creatinine as a marker of GFR

(38). This was further supported in a meta-analysis from 2007, with data indicating a better diagnostic accuracy when using cystatin C for estimation of GFR, compared to creatinine (39). There is, however, sparse evidence implicating better decision making and outcomes in clinical practice by using $eGFR_{cystatin\ C}$ instead of $eGFR_{creatinine}$ (33). A large, more recent study in the general population did not find evidence that supports equations based on cystatin C alone or in combination with creatinine providing better GFR estimates than the commonly used MDRD and CKD-EPI equations (25).

Cystatin C is not affected by gender, muscle mass, ethnicity or malignancy and, generally, has a constant production rate, with the main determining factor for its plasma concentration being GFR (33, 35, 40). Some studies do, however, dispute cystatin C's independence from muscle mass, demonstrating total lean mass to affect cystatin C levels, though still at a much lesser degree than that of creatinine (28, 34). While ethnicity is a major component of the eGFR formulas based on creatinine, it is not for cystatin C as it is far less affected by ethnic variation compared to s-creatinine (28). This is an advantage, especially due to the laboratory often not knowing the race of the test subject (41). However, Cystatin C levels is affected by obesity, thyroid function and cardiovascular risk factors (22).

The CKD-EPI consortium has developed equations for estimated GFR for use with cystatin C alone, and for cystatin C combined with s-creatinine, with the latter aptly named $CKD\text{-}EPI_{combined}$ (28, 41). While the $CKD\text{-}EPI_{cystatin\ C}$ performed equally well to the $CKD\text{-}EPI_{creatinine}$ equation, the $CKD\text{-}EPI_{combined}$ was demonstrated to perform significantly better compared to the $CKD\text{-}EPI_{creatinine}$ equation, owing to a gain of precision (28, 41). Cystatin C based equations have been demonstrated to be more accurate than the $CKD\text{-}EPI_{creatinine}$ equation in hospitalized patients, especially as they are less dependent on the nutritional status or the muscle mass of the patient (42). It also reacts to smaller reductions in GFR than serum creatinine does (35, 40).

It is interesting that $eGFR_{cystatin\ C}$ is shown to be a better predictor of mortality than creatinine based eGFR (14, 41). Several studies have underlined cystatin C as better at predicting cardiovascular disease than creatinine based eGFR (43, 44). It is, however, debated if this reflects cystatin C being more accurate at determining GFR,

or if it is a result of cystatin C being influenced by factors influencing cardiovascular disease (25).

Whereas creatinine based eGFR is notoriously inaccurate in children and adolescents, cystatin C provides a good substitute in the age 2 to 18 years old (28). Several equations for estimation of GFR by cystatin C in children has been developed, some also in combination with creatinine (28).

Urea

Urea lacks both specificity and sensitivity for change in GFR (45). GFR must be reduced by some 50% before serum urea reaches pathological levels (45). Urea can also demonstrate increased serum levels for other reasons than reduced GFR (45). For these reasons urea seldom sees use as a standalone marker for GFR. An average of urinary clearance of creatinine and urea has been proposed, and in a study with 12 peritoneal dialysis patients shown to correlate better with ^{51}Cr-EDTA based mGFR than $eGFR_{cystatin\ C}$ (46).

Inulin

Inulin is an exogenous fructose polymer, freely filtered at the glomerulus, not reabsorbed, secreted, or metabolized by the renal tubule (47). Neither is it degraded or synthesized in the body, bound to plasma proteins, it is also nontoxic and it is physiologically inert (47). Inulin clearance is by definition equal to GFR, and it is independent of its concentration in the blood and of the infusion rate (7). This made renal clearance of inulin the golden standard for measurement of GFR for many years, since its introduction in the 1930s (21, 23, 24, 47).

Due to its inconvenience in use and its rising costs, it has lately been contested by the non-steady state single-bolus plasma clearance rate of ^{51}Cr-EDTA, iohexol and iothalamate (6, 48). As the rate of its removal from plasma must equal the rate of its excretion by urine, we can by knowing the urine and plasma concentration of inulin, as well as the urine flow rate, calculate its clearance (7). It requires continuous IV

infusion, as well as urine collection, sometimes demanding bladder catheterization as it is important that the collection starts and ends with an empty bladder (47). This is expensive and resource demanding, time consuming and demands precision in urine collection for accurate results, making for little use in clinical practice (6, 21, 24, 47).

Plasma clearance of inulin has also been demonstrated for assessment of GFR. In a systematic review these have been demonstrated to be sufficiently accurate to measure GFR, when compared to renal inulin clearance (24). This method sees very little use, it is hindered by the same problems as renal clearance of inulin, without having the benefit of being the “golden standard”. Measurement of GFR by plasma clearance, irrespective of the marker being used, is limited in the setting of massive infusions, fluid loss or sequestration in different compartments (6). This alters the distribution volume of the marker greatly, both intra- and inter-individually, impacting the measurement of GFR (6).

^{51}Cr-EDTA

^{51}Cr-EDTA is considered a reliable marker for measuring GFR (49). A systematic review found that both renal and plasma clearance of ^{51}Cr-EDTA give accurate GFR measurements, when compared to inulin clearance (24). ^{51}Cr-EDTA has been the most used clearance based marker for GFR in Europe for decades, but sees little use in the US owing to lack of equipment for measurement and production (21). The measurement is considered labor-intensive and expensive compared to estimation of GFR through creatinine, though simple and rapid compared to inulin clearance (26, 47). ^{51}Cr-EDTA can only be produced at nuclear medicine units, and because of its radioactivity the use of the marker gets complicated by regulations with strict handling, storage and disposal routines, and frequently requiring special licensing (47). It also makes transport challenging, and makes the marker unsuitable for use in pregnant women (47).

Renal ^{51}Cr-EDTA is limited by the risk of incomplete urine collection (26). Precision is also challenged if the patient is not able to fully empty their bladder at the start and end of each clearance period, alternatively requiring catheterization (26).

^{99m}Tc- DTPA

The majority of studies comparing ^{99m}Tc-DTPA to iohexol demonstrates questionable methodologies, but a study of 21 diabetic patients demonstrated good correlation with iohexol (6). A systematic review found that renal clearance has sufficient accuracy compared to inulin, plasma clearance was, however, insufficient (24). These findings has been refuted by another group, claiming there are several studies demonstrating that ^{99m}Tc-DTPA shows good precision and minimal bias when compared to ^{51}Cr-EDTA (50). In any case, ^{99m}Tc-DTPA is an isotopic marker, presenting many of the same practical problems seen with ^{51}Cr-EDTA (6).

Iothalamate

Iothalamate is a non-isotopic contrast medium, with properties and excretion similar to that of inulin (6, 23). Whereas ^{51}Cr-EDTA is the most used marker for measured GFR in Europe, iothalamate is the most used marker in the USA (6). The plasma clearance of iothalamate has been demonstrated to correlate perfectly with renal clearance of inulin, but there is limited scientific evidence to assess the accuracy of plasma iothalamate clearance (23, 24). Several other studies have been conducted comparing iohexol and iothalamate clearance, with most studies finding a systematic overestimation of GFR when using iothalamate (6). Iothalamate is, unlike iohexol, ionic, and tubular secretion of iothalamate is demonstrated (6). Iothalamate and iohexol have a similar kinetic profile, iothalamate is, however, more allergenic. (47) For these reasons, iohexol is considered superior to iothalamate for measurement of GFR.

It is also possible to measure using urine clearance, and a systematic review found that renal iothalamate clearance had sufficient accuracy to measure GFR when compared to inulin (24). Like other renal clearance methods, it is cumbersome, resource demanding and prone to errors in sampling (23). 125I-iothalamate is have also seen use as a marker for measurement of GFR. This is, like ^{51}Cr-EDTA, a radiolabeled marker, and is as such plagued by the same practical problems and sees little use (6).

Iohexol

Iohexol is a more recently introduced marker of GFR compared to ^{51}Cr-EDTA, first described used in humans in 1980 (51). Despite its late introduction, iohexol plasma clearance has been proposed as the best candidate for accurate GFR determination (6, 21). Iohexol demonstrates the important requirements for an ideal GFR markers, with extra-renal elimination deemed negligible even at extremely low GFR values, with a low molecular weight of 821 Daltons, low protein binding and in being neither secreted nor reabsorbed by the kidney (6, 47). Iohexol is nonradioactive, non-toxic, inexpensive and is also safe to administer to patients with severe renal insufficiency and to patients in general (6, 20). As it is most often measured using plasma clearance, it is easier in use than other exogenous markers, many relying on urine sampling (20, 47). Iohexol clearance is shown to correlate closely with ^{51}Cr-EDTA (21). Iohexol is also demonstrated to have an identical clearance to that of inulin, while being less cumbersome in use (6, 47). This implies that the total plasma clearance of iohexol equals actual GFR, necessitating complete excretion through glomerular filtration, with negligible to none tubular secretion and reabsorption (47). A systematic review found both plasma and renal iohexol clearance to have sufficient accuracy to measure GFR when compared to inulin clearance (24).

While being a contrast media, it is injected in small doses compared to those of contrast X-ray examinations, and it has not been shown to be nephrotoxic even in patients with minimal renal function (6, 21). Over 25 000 iohexol clearances has been performed in Italy, with no anaphylactic reaction occurring (6). It is also confirmed that iohexol infusion does not itself affect GFR (51). A 2016 study on hemodynamically unstable patients in the ICU concluded that both renal and plasma iohexol clearance can be used for measuring GFR even in the setting of massive transfusion, as opposed to creatinine and cystatin C, that could be misleading in the same situation (52).

Single sample plasma iohexol clearance has been demonstrated to be as reliable as multi-sample plasma iohexol clearance. They were validated against ^{51}Cr-EDTA clearance, with both methods demonstrating a very high correlation (48). Capillary sampling for determination of GFR has also been introduced, this does, however, still have an unsatisfactory precision and needs further development (6).

The most common protocols involve bolus infusions of iohexol, followed by sampling of blood plasma for measurement of GFR. GFR can be calculated with single samples, with two or with multiple samples. Various formulas have been developed for the different methods, making also single and double sample methods accurate methods (6, 47). When relying on fewer samples one should not sample before 2 hours has passed since the injection, as iohexol has yet to distribute completely in the extracellular volume (6). A prerequisite for GFR determination through single-sample iohexol clearance is that there is knowledge of the distribution volume of the GFR marker and that the plasma sample is collected at the time-point when the size of the distribution volume has a minimal influence on the mathematical calculation of the GFR (53). This time-point must be delayed as renal function declines. While single-sample protocols dominate in use, in a setting of low GFR multi-sample protocols with late samples is a better option for enhancing accuracy (6). Single-sample GFR demand a GFR over 60 mL/min/1.73m^2, unless sampled after 4 hours, for reliable measurement (48). However, waiting too long before sampling the final sample risks complete clearance of iohexol (6). For the single sample method, it is useful to have an estimated GFR first, to evaluate when to take the plasma sample for iohexol measurement. The same position applies to other exogenous markers such as ^{51}Cr-EDTA, ^{99m}Tc – DTPA and iothalamate (6).

Lately, continuous infusion of low dose iohexol (CILDI) has been proposed as an option for measuring GFR in critically ill patients (54). Multi-sample iohexol is plagued by increased risk of toxicity due to higher concentrations compared to CILDI, and these protocols demand a stable GFR for reliable measurement, a problem in this setting due to the high risk of developing AKI (3, 55). For these reasons CILDI has been launched as a viable option in the non-steady state.

mGFR can also be calculated from the renal clearance of iohexol. Renal clearance of iohexol has been demonstrated to correlate with renal clearance of inulin (56). A systematic review found the evidence for the use of renal clearance iohexol as a measurement of GFR to be limited (24). Renal clearance is resource demanding and, as mentioned previously, is prone to human errors in measurement of urinary flows, making some to consider plasma clearance the superior alternative for clinical use (6). In patients with significant oedema or ascites, urinary clearance protocols are

more accurate at estimating GFR, this because the distribution of the administered iohexol takes many days (6).

Iohexol can be analyzed in a variety of ways, with the most common being high performance liquid chromatography with ultraviolet detection (HPLC-UV). This is a sensitive, specific and reproducible method (6). LC-MS/MS is a newer method of measurement, and theoretically a more sensitive and specific method compared with HPLC-UV, though at a higher cost (6). Additionally, there is an external quality program for iohexol analysis by both HPLC-UV and LC-MS/MS, with high agreement between the participating laboratories as of 2015 (6).

Iohexol has a major benefit over ^{51}Cr-EDTA in its transportability. As it is non-radioactive it does not need special safety requirements, nor will the time in transport affect the results. It is stable between -20 °C and -80 °C, making for ease of transport to an analyzing unit (6). The cost of iohexol is estimated to be around 5-10% the cost of inulin, and the cost of measuring GFR using iohexol 15% the cost of measuring using inulin (54).

The rational for using iohexol plasma clearance

Investigation into the pathobiology of renal dysfunction requires an accurate determination of GFR, which is not biased by the potential confounders associated with the use of eGFR (22). Iothalamate is considered inferior to iohexol due to its allergenic potential and the fact that it tends to overestimate GFR as a result of tubular secretion (6). ^{51}Cr-EDTA is hindered by a multitude of practical problems (produced only at nuclear medicine units, safety issues related to its radioactivity, and challenges related to its transportability) (6). Use of inulin is limited by its high cost and by the practical challenges related to the collection of accurate urine volumes (6). A group of leading researchers have therefore recommended iohexol plasma clearance as the new gold standard for measurement of GFR. Main benefits are low cost, excellent accuracy and precision, safety, availability, and ease of administration to patients (6).

3 Material and methods

The practical part of the pilot study has been performed by Lars Marius Ytrebø and Stephen Hodges back in 2019. Ole-Martin Fuskevåg has provided written information on the relevant laboratory procedures for the thesis. My contribution has been to retrieve all raw data, systematically organize and perform the calculations. Processing and presentation of data have also been performed by me under supervision from my mentors.

Study registration and ethical approval

Ethical approval was obtained from Regional Ethics Committee, REK Nord, 9037 Tromsø, Norway (2018/1934-7/REK). The principles of the Declaration of Helsinki (2008) were adhered to throughout the study. Approval by the Institutional Board at the University Hospital of North Norway was obtained (Project nr: 02139/2019) and the study was registered at www.clinicaltrials.gov (ClinicalTrials.gov ID NCT03881332). Informed, signed consent was obtained from the patients before inclusion in the study.

Patients

Inclusion criteria

Patients >60 years scheduled for robot-assisted laparoscopic colorectal cancer surgery at the University Hospital of North Norway, Tromsø, treated in accordance with the Enhanced Recovery After Surgery (ERAS) recommendations (57).

Exclusion criteria

Inability to provide informed consent prior to elective surgery. Diabetes. A radiological examination using contrast within a week before. Allergy to radio-contrast media. GFR<45 mL/min. Patients taking drugs which could potentially interact with iohexol (metformin if s-creatinine > 150mmol/L, phenothiazines, mono-amine oxidase inhibitors, levo-thyroxine, amiodarone, interleukin-2 agents, Tc99m-MDP). Disorders

in which iohexol may potentially interfere with monitoring (thyroid disease, myasthenia gravis, phaeochromocytoma). Hyperviscosity disorders (sickle cell disease, homocystinuria, multiple myeloma). Pregnancy or breast-feeding.

Study protocol

Preoperative single injection iohexol administration and sampling

Baseline GFR was determined one day before surgery and was measured using single-sample plasma clearance of iohexol as previously described (25, 58). Briefly, patients were instructed to avoid large meals with meat and non-steroid anti-inflammatory drugs two days before the investigation, which was performed after overnight fasting, including abstinence from nicotine. The subjects were reminded to not restrict the intake of water. On the morning of measurement on day 1 a 3-lumen central venous catheter (CVC) was inserted, and baseline venous blood samples were collected. A total of 5 mL of iohexol (Omnipaque, 300 mg/mL; Amersham Health, London, UK) was then subsequently injected intravenously and the catheter flushed with 20 mL isotonic saline. The subjects were monitored for allergic reactions for 30 min and then allowed to walk about freely and eat a light breakfast, but intake of meat or smoking were restricted. To ensure complete distribution of iohexol in the extracellular fluid volume, the shortest sampling time was set at 180 min. The exact time from injection to sampling was measured in minutes using a separate stopwatch for each patient. Omnipaque from one batch purchased from Amersham was used.

Continuous iohexol administration and sampling

Iohexol was administered via a dedicated line of the CVC catheter. The giving set and syringe containing iohexol was covered in a light-impermeable sheath. A loading dose (LD) of 2 mL iohexol was administrated within 1 hour after the patient was admitted to the post-anesthesia care facility. Thereafter, patients received a continuous infusion of iohexol (Omnipaque 300®) at 0.5mL/h (343.5mg/h) for up to 72h via a syringe pump as described by Dixon et al. (54). Volumetric mean accuracy in these pumps were +/- 2% according to the manufacturer.

Plasma samples were taken for plasma clearance measurements (Cl_P) at 30 mins, 1h, 2h and 4h on day 2, and at 08:00h, 10:00h, 18:00h and 20:00h on postoperative days up to 72 hrs. Sampling was performed from a separate line of the CVC catheter after the syringe pump had been stopped for 1 minute. Time zero was considered to be the time of commencing the infusion of iohexol. Cl_P will be calculated by the formula: Cl_P (mL/min) = [Iohexol infusion rate (µmol/min)] / [plasma Iohexol concentration (µmol/mL)].

Creatinine and cystatin C sampling

Samples for analysis of creatinine and cystatin C was collected at the same time as plasma samples for plasma clearance measurements of iohexol. The samples were stored at -70°C from sampling until early 2021, when all available samples were analyzed in one run.

Laboratory Procedures

Chemicals and solutions

Iohexol and Iohexol-d5 was obtained from Toronto Research Chemicals Inc. (Ontario, Canada) and iohexol for quality controls (QCs) was purchased from TCI Chemicals (Tokyo, Japan). LC-MS grade methanol was purchased from Honeywell™ Riedel-de Häen™ (Seelze, Germany). LC-MS grade formic acid was obtained from Fluka (Sigma-Aldrich, St. Louis, MO). Ultrapure water (18.2 MΩ) was obtained from a Millipore Advantage Milli-Q system (Millipore SAS, Molsheim, France).

Determination of iohexol in human serum

Two stock solutions of iohexol were prepared in methanol and stored at -30 °C. A 6-point calibration curve and two QCs for iohexol was constructed in drug-free serum (1-240 mg/l). A Tecan Freedom Evo 200 (Männedorf, Switzerland) liquid handling workstation was used for sample preparation. Calibrators, QCs and samples (50 µL) were prepared by adding 50 µl internal standard (aqueous iohexol-d5, 3.3mg/L) in a 96-well MegaBlock® 1.2 mL, PP, (Sarstedt, Germany). To each of the wells 0.5 mL ice-cold methanol was added. The plate was mixed on a Bioshake (Quantifoil Instruments, Jena, Germany) at 1500 rpm for 3 min and centrifuged at 240 x g for 8

min (Hettich Rotina 320R, Tuttlingen, Germany). 100 µl of the supernatant was transferred to a 96-well collection plate (Waters, Milford, MA). After sealing of the plate, 0.1 µl of the supernatant was injected to the LC-MS/MS system and analyzed by LC-MS/MS using a Waters Acquity UPLC I-Class FTN system with an autosampler and a binary solvent delivery system (Waters, Milford, MA) interfaced to Waters Xevo TQ-S benchtop tandem quadrupole mass spectrometer (Waters, Manchester, UK). The chromatography was performed on a 2.1 x 100 mm Waters Acquity Cortecs® T3, 1.6 µm column. Eluent A consisted of 0.1% formic acid in water; eluent B consisted of 0.1% formic acid in methanol. Gradient elution was performed with 2% B at start and had a linear increase to 60% B until 0.6 min, a linear increase to 98% B until 1.5 min, and re-equilibration until 2.7 min with 1% B. The flow rate was 0.3 mL/min and the column temperature was maintained at 50 °C. The mass spectrometer was operated in positive electrospray ion mode and spray voltage was set to 0.9 kV. The system was controlled by MassLynx version 4.1 software. Desolvation gas temperature was 500 °C; source temperature was 150 °C; desolvation gas flow was 1000 L/h; cone gas flow was 150 L/h; collision gas pressure was 4 x 10-3 mBar. For quantitative analysis of iohexol the following multiple reaction monitoring (MRM) transitions were used (bold transitions are qualifiers): m/z 821.9->803.8/**602.4** and 826.9->808.8/**607.5** (iohexol and iohexol-d5).

Precision and accuracy

The method was validated and was found to be linear from 1.5 to at least 240 mg/L ($r^2 > 0.999$). Lower limit of quantification was found to be 0.5 mg/L (0.1 µl injection volume). Between-day coefficient of variation (CV) for iohexol was < 6% on four consecutive days. CV for intraday precision value was < 3.6 % and was calculated by assaying three samples (low, medium and high concentration) six times on the same day. Accuracy for recovery test was 91.1-107.9 % (9 levels, n = 3 for each). Additionally, the quality is assured through the Equalis external quality assessment program for iohexol four times a year. Single-sample iohexol clearance was calculated as described by Eriksen et al. (59).

Determination of creatinine and cystatin C in human serum

Serum creatinine was measured using an enzymatic assay standardized to the isotope dilution mass spectrometry method (CREA Plus, Roche Diagnostics). Cystatin C was analyzed with a particle enhanced turbidimetric immunoassay with reagents from Gentian (Gentian, Moss, Norway) and a Modular E analyzer (Roche Diagnostics). The cystatin C measurements were then recalibrated to the international reference standard using a Cobas 8000 (Roche Diagnostics). CKD-EPI equations were applied to estimate GFR (31, 41).

Statistics

Due to only four included patients we decided to apply descriptive methods only. Figures were made in SPSS and Microsoft Excel. All calculations were performed with Microsoft Excel.

As the CKD-EPI calculations are not validated for use under non-steady state conditions, relative changes in concentration of iohexol, creatinine and cystatin C are also presented. They were calculated as (actual measure – 2 hrs postoperative measure)/2 hrs postoperative measure.

4 Results

Four patients scheduled for robot-assisted laparoscopic colorectal cancer surgery at the University Hospital of North Norway were studied, all treated in accordance with the study protocol. None were excluded and all available data were included in the final data analyses. Demographic data are shown in table 1. Indexed perioperative $eGFR_{creatinine}$, $eGFR_{cystatin\ C}$ and $mGFR_{iohexol}$ are presented in separate graphs for each patient. In addition, a graph for each patient demonstrating the individual changes in concentration of creatinine, cystatin C and iohexol relative to the 2 hrs post-surgery sample is shown.

Patient demographics

Table 1 – patient demographics

	Patient 1	Patient 2	Patient 3	Patient 4
Sex (Male/female)	F	M	M	M
Age (years)	60	81	66	63
Height (cm)	176	172	183	173
Weight preoperative (kg)	90	91,1	86	80
Weight gain (kg)	3,6	-	-	1,6
BMI preoperative (kg/m^2)	29,1	30,8	25,7	26,7
BSA preoperative (m^2)	2,07	2,04	2,08	1,94
ASA	2	3	2	2
Intraoperative IV fluids (mL)	1300	1300	1100	1000
Intraoperative bleed (mL)	100+	150	100	<100
Length of surgery (min)	270	255	350	205

BMI; Body mass index, BSA; Body surface area, ASA; American Society of Anesthesiologist classification, IV; intravenous

The four included patients represented three male and one female. As determined by ASA, patients 1, 3 and 4 were evaluated to have mild comorbidity, whereas patient 2 was evaluated to have a severe comorbidity. Patients 1, 3 and 4 were overweight, patient 2 was moderately obese. All patients had an BSA higher than that of the standardized BSA of 1.73m^2. Only small amounts of intraoperative bleeding occurred in all four patients.

Patient 1

Indexed GFR

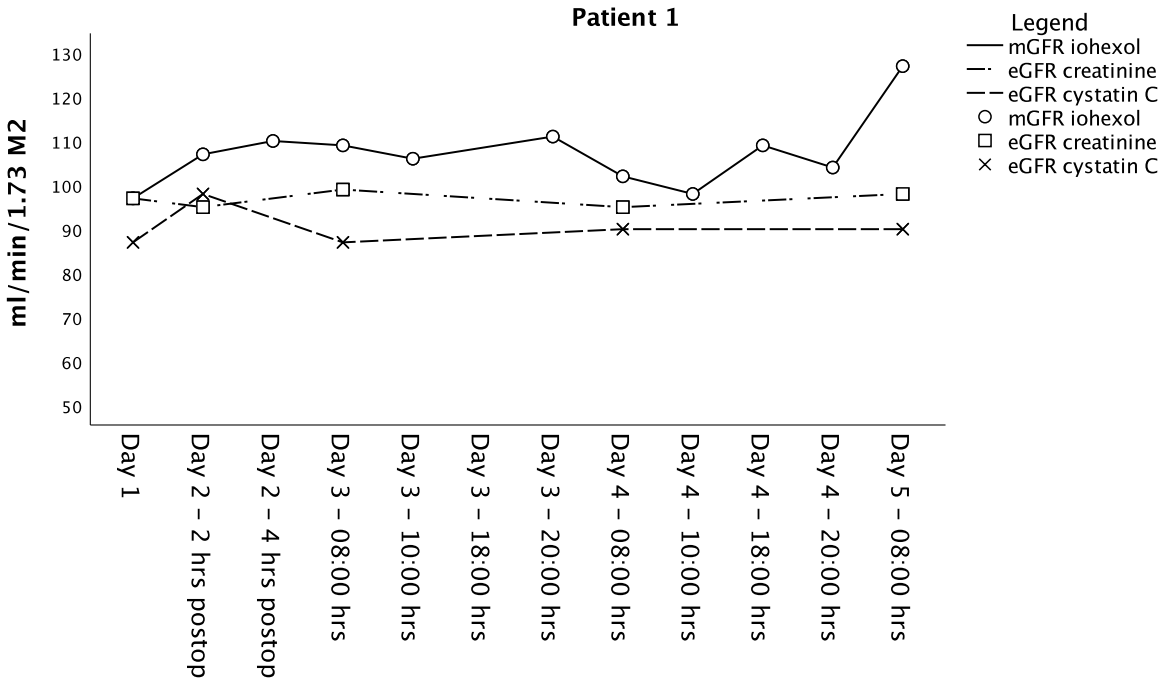


Figure 3 – patient 1 indexed GFR

$eGFR_{creatinine}$ and $mGFR_{iohexol}$ presented the exact same GFR values at baseline, being 11,5% higher than $eGFR_{cystatin\ C}$ at baseline. $mGFR_{iohexol}$ increased slightly compared to baseline, but remained fairly stable from baseline until day 5, when $mGFR_{iohexol}$ peaked at 127 mL/min/1.73m^2, a 31% increase from baseline. This was not reflected by $eGFR_{creatinine}$ or $eGFR_{cystatin\ C}$. For the duration of the sampling $eGFR_{cystatin\ C}$ trended noticeably lower compared to the other two, and $mGFR_{iohexol}$ demonstrated at all times the highest GFR. All measurements of $mGFR_{iohexol}$ in the postoperative period were higher than baseline. Due to some technical challenges this patient had fewer repeated measurements compared to patients 2-4.

Relative change of marker

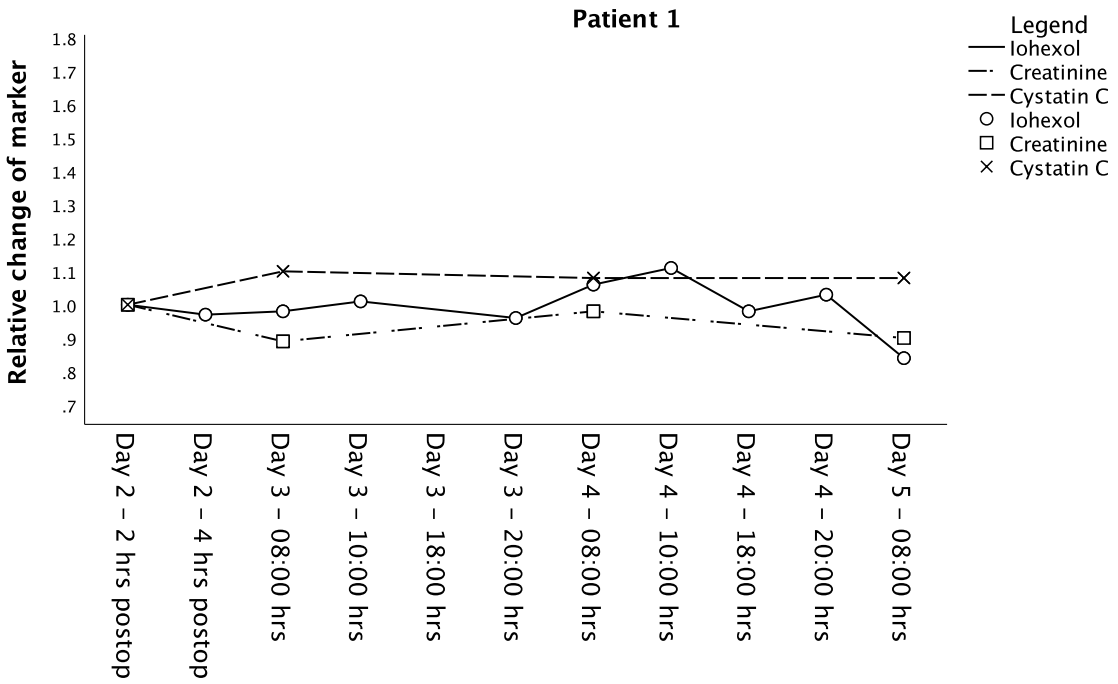


Figure 4 – patient 1 relative change of marker

Creatinine and cystatin C were stable for the duration of the sampling, with only minor alterations in plasma levels. Iohexol levels were more volatile, peaking on day 4, subsequently falling to its lowest on day 5.

Patient 2

Indexed GFR

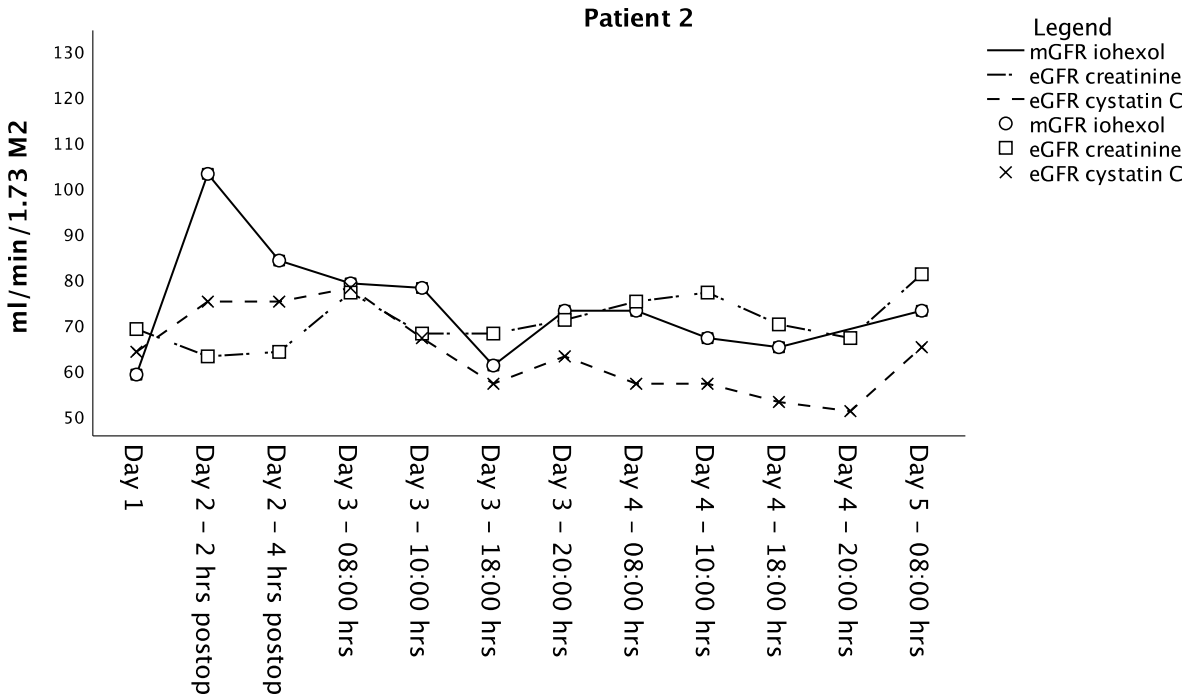


Figure 5 – patient 2 indexed GFR

With a baseline $mGFR_{iohexol}$ of 59 mL/min/1.73m^2, $mGFR_{iohexol}$ was lower than the eGFR methods, with $eGFR_{creatinine}$ being 17% higher than $mGFR_{iohexol}$. With exception of baseline, $eGFR_{cystatin\ C}$ was lower than $eGFR_{creatinine}$ and $mGFR_{iohexol}$ at all times.

All measurements of $mGFR_{iohexol}$ in the postoperative period were increased from baseline. At two hours postoperatively $mGFR_{iohexol}$ peaked, demonstrating a 74% increase over baseline. $mGFR_{iohexol}$ was 24% higher on day 5 when compared to baseline. Neither $eGFR_{creatinine}$ nor $eGFR_{cystatin\ C}$ demonstrate the same pattern, although $eGFR_{cystatin\ C}$ seemingly had a more similar pattern to $mGFR_{iohexol}$ than $eGFR_{creatinine}$ did.

Relative change of marker

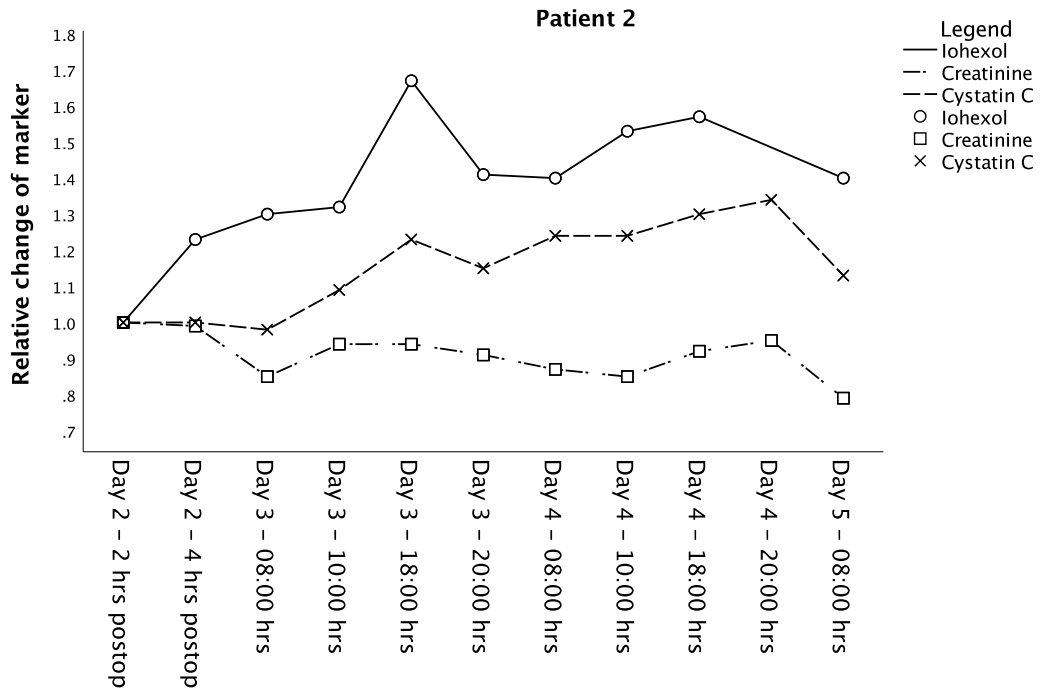


Figure 6 – patient 2 relative change of marker

Iohexol levels increased continuously from t=2 hrs, and peaked on day 3. Cystatin C followed a similar trajectory, but with less variation in relative concentration changes. Creatinine levels fell on day 3, before it remained fairly stable up until day 5, when it saw a drop in concentration.

Patient 3

Indexed GFR

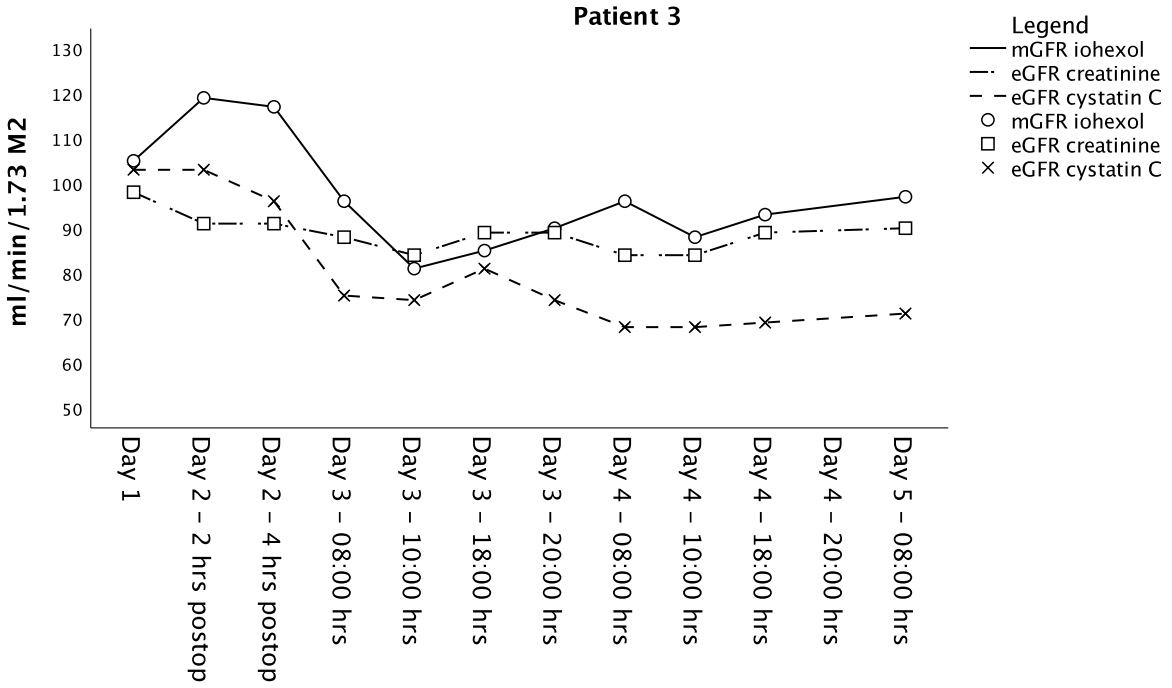


Figure 7 – patient 3 indexed GFR

The different methods demonstrated fairly similar GFR values at baseline, with $eGFR_{creatinine}$ 6.7% lower than $mGFR_{iohexol}$ and $eGFR_{cystatin\ C}$ 1.9% lower than $mGFR_{iohexol}$. $eGFR_{cystatin\ C}$ was at all times lower than $mGFR_{iohexol}$ and was only higher than $eGFR_{creatinine}$ at baseline and on day 2. $eGFR_{cystatin\ C}$ was down 27% at discharge when compared to baseline.

$mGFR_{iohexol}$ peaked at 2 hours postoperatively at 119 mL/min/1.73m^2, a 14% rise from baseline. $mGFR_{iohexol}$ subsequently decreased to the lowest level on day 3 at 81 mL/min/1.73m^2, down 23% from baseline, before it slightly increased towards day 5, never reaching baseline levels. $mGFR_{iohexol}$ at day 5 was down 7% from baseline. $eGFR_{creatinine}$ did not respond to the variability seen throughout, but $eGFR_{cystatin\ C}$ seemingly follows a similar trajectory to that of $mGFR_{iohexol}$ at times, albeit at a far lower GFR than that of $mGFR_{iohexol}$.

Relative change of marker

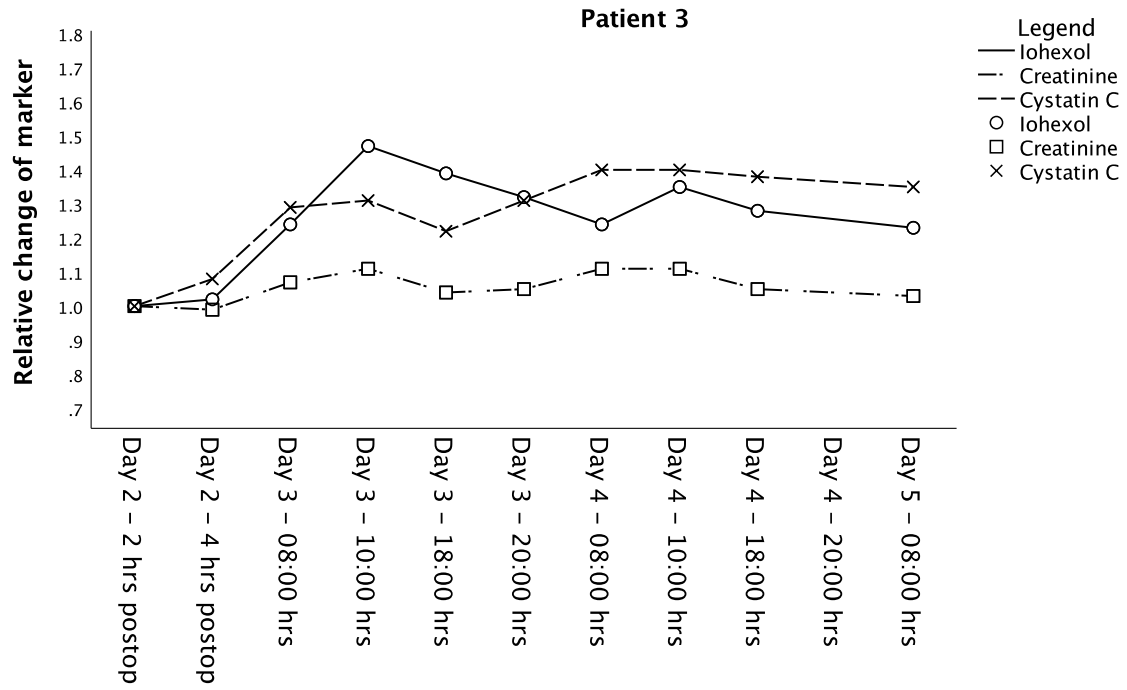


Figure 8 – patient 3 relative change of marker

Iohexol increased sharply on day 3, before it subsequently saw a decline towards day 5. Cystatin C seemed to mirror this. Creatinine remained fairly unchanged compared to the postoperative 2 hrs measurement for the duration of the study.

Patient 4

Indexed GFR

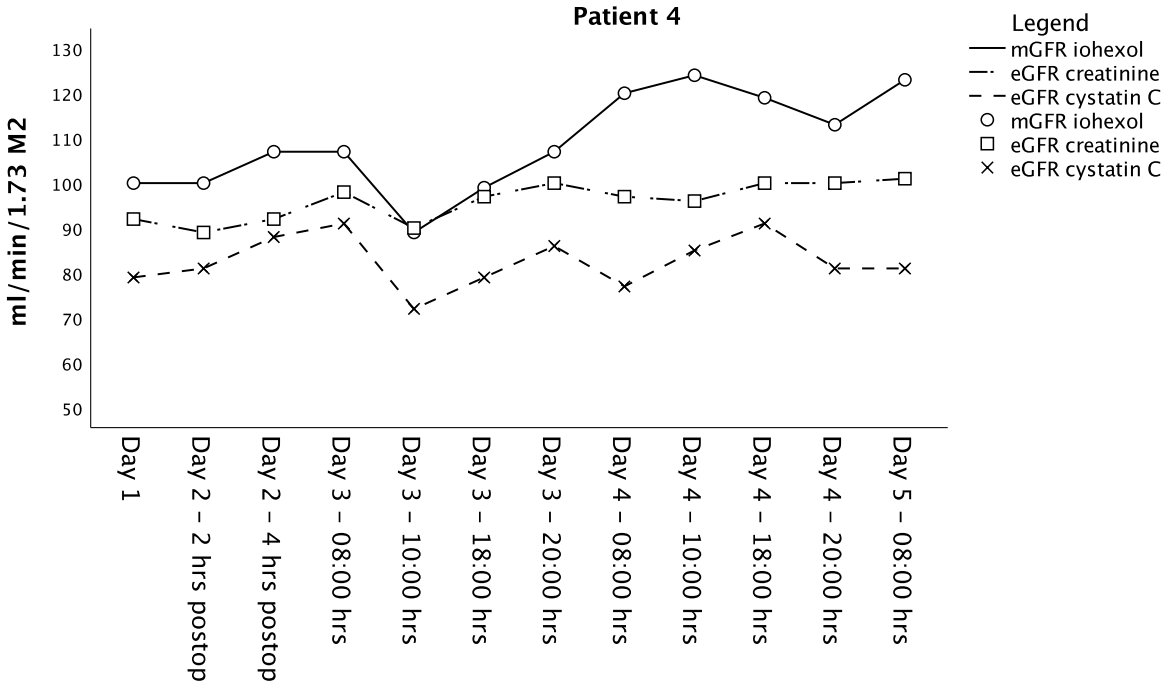


Figure 9 – patient 4 indexed GFR

$mGFR_{iohexol}$ was higher than both methods of estimation at baseline, with it being 8.7% higher than $eGFR_{creatinine}$ and 26,6% higher than $eGFR_{cystatin\ C}$ at baseline. For the duration of the sampling $eGFR_{cystatin\ C}$ was lowest at all times, and $mGFR_{iohexol}$ highest at all times.

$mGFR_{iohexol}$ remained unchanged until day 3, when initially decreased to 89 mL/min/1.73m^2, down 11% from baseline. Thereafter it steadily increased towards day 5 to 123 mL/min/1.73m^2, up 23% from baseline. $eGFR_{cystatin\ C}$ followed a similar trajectory with few exceptions, while $eGFR_{creatinine}$ seemed unaffected compared to $mGFR_{iohexol}$.

Relative change of marker

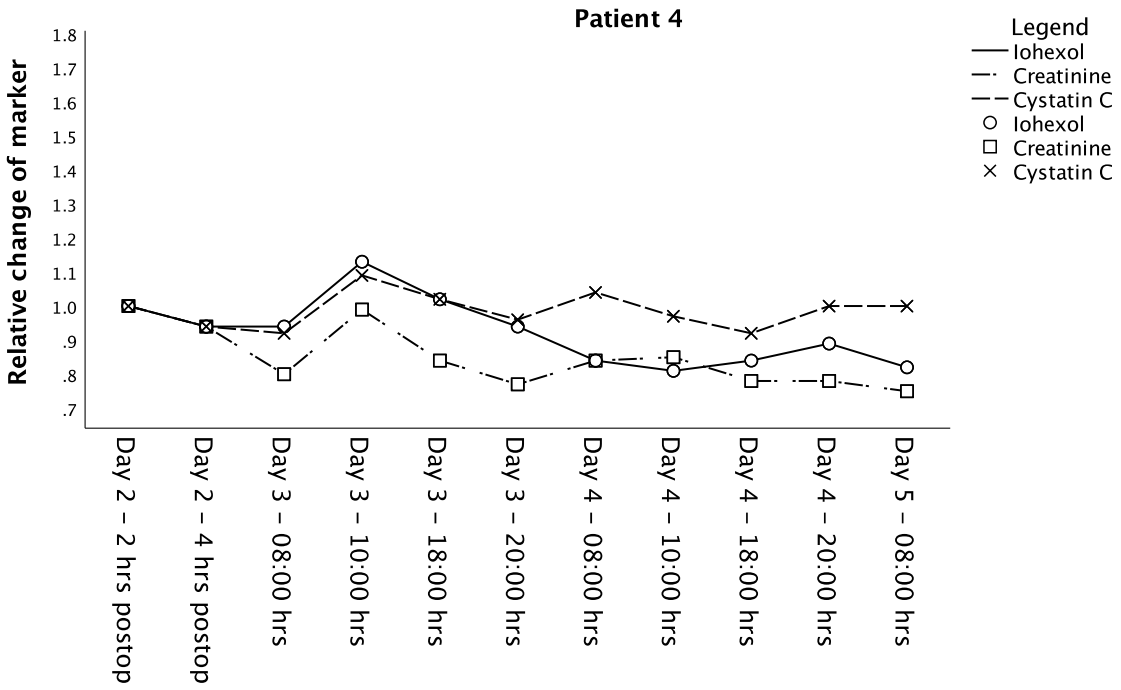


Figure 10 – patient 4 relative change of marker

Iohexol increased at day 3 but decreased thereafter and remained lower compared to baseline. Cystatin C seemed to follow a similar trajectory to that of iohexol at first but demonstrated less change from baseline on day 4 and 5 when compared to iohexol. Creatinine demonstrated variability on day 3 but after dropping it remains low up to day 5, where the lowest level was measured.

5 Discussion

The current pilot study demonstrated the feasibility of the single injection iohexol method in combination with CILDI for measuring GFR in the perioperative period. Furthermore, $mGFR_{iohexol}$ seemed to demonstrate a greater variability than what is detectable using eGFR. Lastly, $eGFR_{creatinine}$ and, in particular, $eGFR_{cystatin\ C}$ underestimated GFR in this pilot study.

It has long been held that measurement of exact GFR by means of exogenous substances is far too cumbersome, costly, and time consuming to be implemented in clinical practice. This belief has also restricted research on dynamic health states. The estimation of GFR by in particular the endogenous substance creatinine is much more common, being cheaper and more available than mGFR by CILDI. But it is of limited use in this non-steady state, as it struggles detecting the more subtle changes in GFR. Creatinine is a late marker of AKI as it does not rise before considerable kidney function is lost, and it tends to underdiagnose AKI in the non-steady state when compared to $mGFR_{iohexol}$ (2, 3, 5, 54).

Iohexol looks to be the ultimate exogenous substance for measurement of GFR (52). The chemical properties are excellent, as it has minimal protein binding, negligible tubular reabsorption and secretion, and is filtered freely at the glomerulus (54). As a contrast agent, it is not costly, and it is readily available. It is non radiolabeled, meaning it is not hindered by the durability issues, production and transportation issues, or concerns related to safety seen when using radiolabeled substances like ^{51}Cr-EDTA (6). The dosages used for monitoring GFR do not cause nephropathy even in patients with minimal renal function, and the procedure is not known to be associated with anaphylactic shock (6).

In the current study blood samples were collected from a central venous catheter, making repeated samples convenient. Processing and analyses of samples were performed at the local hospital laboratory, and all samples were analyzed in one run after being kept at -70°C. The measurement of GFR did not delay or hinder other clinical interventions or investigations, but from a patient perspective connection to the syringe pump for 72 hours may have been cumbersome, although none of the patients complained about it. In total, the method performed is feasible for use both in

research and in a clinical setting. However, analyses of iohexol requires access to advanced laboratory facilities, which is not usually available at smaller hospitals. The ease of transportation and stability of the compound does, however, make it possible to perform CILDI even at a smaller hospital and subsequently send the blood samples to a central, better equipped laboratory for analysis.

The CKD-EPI equations are not validated for use in the non-steady state such as during the perioperative period. It therefore makes little sense to compare CKD-EPI to the accurate $mGFR_{iohexol}$. However, clinicians routinely use eGFR to monitor kidney function in this setting. The present data documented that eGFR differs vastly from actual GFR as measured by $mGFR_{iohexol}$. This discrepancy is clinically relevant and therefore of interest to discuss more closely.

In patient 1 $mGFR_{iohexol}$ was higher compared to eGFR during the entire postoperative period. For patient 2 $eGFR_{creatinine}$ levels followed $mGFR_{iohexol}$ closer than what we saw in patient 1, but did not show the great variability in GFR that was demonstrated by $mGFR_{iohexol}$. $eGFR_{creatinine}$ in patient 3 and 4 demonstrated very little variability compared to $mGFR_{iohexol}$. These dynamic changes of kidney function would have remained unknown for the clinician if only traditional endogenous markers are applied. The low variability observed could be due to the considerable renal secretion of creatinine, neutralizing the small changes in s-creatinine caused by a variating GFR (28). Furthermore, as creatinine concentration increase exponentially as GFR decreases, current formulas for calculating eGFR will only demonstrate minor to negligible changes in eGFR as long as the variations in s-creatinine are in the normal ranges (4). There is also a delay in increased plasma concentrations of creatinine when GFR declines, as it is only produced at a rate of 1-2mg/dL/day (4). In total, this could explain the low variation in $eGFR_{creatinine}$ compared to $mGFR_{iohexol}$.

In patient 3 and 4 $eGFR_{cystatin\ C}$ demonstrated trajectories similar to that of $mGFR_{iohexol}$, but with some variation for the duration of the study period. $eGFR_{cystatin\ C}$ is known to respond to smaller changes in absolute GFR than $eGFR_{creatinine}$ does (35, 40). Cystatin C is, contrary to creatinine, not biased by considerable renal secretion (60), which makes it more suitable to monitor variations in actual GFR more closely. This could explain why $eGFR_{cystatin\ C}$ seemed to mirror the trajectory of $mGFR_{iohexol}$ more closely compared to $eGFR_{creatinine}$.

In all four patients, $eGFR_{creatinine}$ and $eGFR_{cystatin\ C}$ underestimated actual measured GFR. On the contrary, most studies have found that $eGFR_{creatinine}$ overestimate GFR, especially at lower levels (29). This is supported by well-known physiology, as creatinine in addition to glomerular filtration is eliminated by secretion in the renal tubules (29). A possible explanation for this underestimation could be that GFR was relatively high in all study patients. A relatively large change in actual GFR is required in order to cause only a small change in plasma concentration of creatinine or cystatin C at high GFR ranges (4). Several studies on kidney function in the setting of acute heart failure have previously found $eGFR_{cystatin\ C}$ to be consistently lower than $eGFR_{creatinine}$ (14, 61-63), but this finding could not be confirmed in a large population study (25).

The single injection iohexol method performed may prove useful for preoperative risk assessment. Baseline $eGFR_{creatinine}$ for patient 2 overestimated $mGFR_{iohexol}$ by almost 20%. In this instance the level of renal function as measured by iohexol would over time qualify for stage 3a of chronic kidney disease (CKD), while $eGFR_{creatinine}$ using the $CKD\text{-}EPI_{creatinine}$ formula would qualify for stage 2 (table 3) (64). Knowing that the most important patient-related factor for developing postoperative AKI is preoperative reduced kidney function (3), the single injection iohexol method may provide clinician with a more reliable tool for preoperative risk assessment of patients. In this instance the different GFR measured and estimated would affect the risk as determined by following ERAS guidelines for preoperative risk assessment (57). Iohexol may also be beneficial in stratification of patients into clinical studies.

Accurate measurement of GFR is very important at discharge from hospital as patients at risk should be followed up more closely. In patient 1 $mGFR_{iohexol}$ increased by 22% between day 4 and 5, while this increase was not detectable by eGFR. In fact, $mGFR_{iohexol}$ was 29.6% higher than $eGFR_{creatinine}$, and 37,8% higher than $eGFR_{cystatin\ C}$ at discharge. These numbers, if known by the clinician, could qualify for a closer follow up. Patient 2 and 3 demonstrated a marked increase in $mGFR_{iohexol}$ during the early postoperative phase. Unfortunately, patients 2 and 3 were not weighed postoperatively. Significant change in fluid balance could have explained changes in GFR. However, neither of the four patients were subjected to large losses of fluids during and after surgery, nor were they subject to large transfusions. Rapid

alterations in distribution volume of iohexol is therefore unlikely to have influenced these changes in iohexol concentration and ultimately $mGFR_{iohexol}$.

The sharp increase in postoperative $mGFR_{iohexol}$ levels may indicate some degree of renal stress. The relevance of this observation is unclear, as the importance of renal hyperfiltration is a subject of ongoing discussion (65). While there is no consensus on the definition of hyperfiltration, some studies have been operating with thresholds as low as 125 mL/min/1.73m^2 (65). In the present study mGFR was higher than 125 mL/min/1.73m^2 in only one patient. However, GFR does not account for the age-related reduced GFR, furthermore there is a possibility of single nephron hyperfiltration in a setting of globally reduced GFR (65). For these reasons, some use filtration fractions by monitoring renal blood flow as a means of determining hyperfiltration (65).

The diagnose of AKI represents a major risk factor for adverse outcomes following surgery. Even a small acute increase in creatinine concentration increases the risk for complications following surgery (3). Complications include chronic kidney disease, end-stage renal disease, cardiovascular disease, infection, bleeding and death (3). In a study of patients undergoing cardiac surgery, stage-1 AKI was associated with an almost five-fold increase in the odds for intrahospital death compared with those who did not develop AKI (66). Odds ratio for stage-3 AKI compared to no AKI were 81.2 for intrahospital death (66). There was also a steep increase in other complications associated with AKI (66). A cohort-study from 2009 looking at patients undergoing major surgery demonstrated a 30-day mortality of 1.9% in patients without AKI compared to 31% in patients with AKI. Even smaller changes in serum creatinine was associated with an independent long-term risk of death (67). Regardless of cause and stage, patients with AKI were found to have an increased risk of developing chronic kidney disease (hazard ratio of 8.8) (67). The measures taken to hinder the development of postoperative AKI is still very much undeveloped, and treatment options are scarce, calling for further research on the subjects (3).

For many years there was no consensus on the definition of AKI, resulting in vastly different rates of reported incidence and mortality (3). This lead to the creation of the current KDIGO criteria, that define AKI by cessation or reduction in urinary output and/or increase of the biomarker creatinine (table 2) (3). mGFR has not been

included in the current AKI guidelines. As serum creatinine does not rise before kidney function is significantly reduced, as well as *have been* reduced for sufficient period of time to increase plasma concentrations, it is necessarily an unreliable parameter for monitoring of perioperative renal function (4, 22). The delay in the diagnostic process may be a source of harm to the patients, due to lack of preventive measures and inadequate clinical interventions. Dixon et al. found the mean intra individual variation of $mGFR_{iohexol}$ with CILDI to be 10.3%, hypothesizing that changes in GFR >10,3% represents evolving AKI in critically ill patients (68). The group applied CILDI in patients with AKI and patients undergoing nephrectomy, vascular surgery and established AKI, confirming excellent correlation with expected 50% drop in GFR in the nephrectomized patients (54). When looking at 21 patients undergoing vascular surgery or nephrectomy they also discovered 9 cases of AKI that would not be registered using KDIGO criteria (55). Furthermore, two cases deemed to be AKI by the KDIGO criteria was identified as incorrect using CILDI. The group suggest that AKI diagnosis by mGFR determination via CILDI might be a viable way forward for the KDIGO criteria (54). The implementation of a more accurate method to monitor GFR has also a great potential for research on renal pathobiology.

In this thesis I have focused mostly on indexed GFR as this is what clinicians are most familiar with. It is interesting to note that BSA was > $1.73m^2$ in all four patients, and as such they have a higher absolute GFR than indexed GFR. As discussed previously, this has implications for drug dosage. Failure to calculate de-indexed GFR could lead to underestimation of drug dosage and a subsequent therapy failure of renally eliminated drugs. This is highly relevant in the cases where potential nephrotoxic drugs are used, as their narrow therapeutic window leads to insufficient plasma concentrations of the administered drug when the clinician underestimate absolute GFR. The fact that GFR as measured by iohexol is even higher than the estimated values as calculated from creatinine and cystatin C, increases the risk of underestimating drug dosage. As an example, for patient 2's two-hour postoperative sample the indexed $eGFR_{creatinine}$ was 63 mL/min/$1.73m^2$, whereas the corresponding de-indexed $mGFR_{iohexol}$ was 121 mL/min, nearly twice as high. A renally eliminated drug will in theory in this particular situation be eliminated twice as fast as you would expect if you were to only look at the indexed $eGFR_{creatinine}$.

This pilot study is the second to confirm the viability of obtaining the accurate measurement of GFR in a dynamic clinical setting following Dixon et al. (54). While it seems unlikely that CILDI will become a routine methodology in the clinic, it may prove useful in the evaluation of renal function in patients included in clinical trials.

Strengths

GFR varies intra-individually. Diet, activity, circadian variations and measurement errors may create significant variations in GFR (6). The design of this pilot study focused on limiting confounding factors. The study examined four patients undergoing major abdominal cancer surgery, with a highly standardized laparoscopic robot-assisted technique, which presumably would reduce interindividual differences in trauma and inflammation caused by the surgery, while still representing a physiological renal stress. This assumption is supported by all patients having similar small amounts of blood loss during surgery, standardized intravenous fluid infused, and length of surgery was similar in three of the four cases. Importantly, all patients were treated in accordance with the ERAS recommendations, which further contribute to standardization of the perioperative treatment protocols. Sampling was performed at the same time intervals in the immediate postoperative period, as well as at the same points each day for three consecutive postoperative days.

Limitations

The study is limited by the low number of included patients. It is also limited by the lack of controls. The lack of urine sampling for measurement of renal iohexol clearance can be noted as a limitation, this was not performed due to the practical problems with accurately collecting urine for 120 hours from otherwise ambulatory patients. Furthermore, a 2014 review found that there is less evidence for the accuracy of urine iohexol clearance than there is for plasma iohexol clearance, and there is substantial evidence for plasma clearance of iohexol as a perfectly accurate and practically easy method for measurement of GFR (6, 24). This relation was also found by Dixon et al., who reported good agreement between plasma and urinary iohexol clearance (54).

Challenges in obtaining vascular access led to fewer samples being collected from patient 1. We also missed a few blood samples from patients 2 and 3. Patient 2 and 3 was not weighed at discharge, but according to the ERAS protocol it is not expected any major shift in fluid balance.

Iohexol levels were analyzed right after the studies were terminated in 2019, while analyses of creatinine and cystatin C were performed early 2021. This should be of no matter to the reliability of data, as the blood samples were stored at -70 °C for the entire time, and all samples were analyzed in one run at a certified laboratory.

A discontinuation or change of rate of the administration of iohexol could be a limitation of the study, but there is no evidence that this was an issue. Nursing staff were very supportive and secured an uneventful infusion over 72 hours in each patient. According to the manufacturer of the syringe pumps infusion rate vary by only +/- 2%, which should not represent any systematic bias or limitation in the interpretation of data.

Perspectives

The measurement of accurate GFR using exogenous substances in the non-steady state is still very much a novel area. Our study examined patients who received a highly standardized surgical procedure. Other surgical procedures may cause different alterations in renal function and should thus be examined. Studies of patients in need for intermediate level and ICU level after surgery are of outmost interest as these patients are at a much higher risk for development of AKI.

In future studies it would be interesting to combine the measurement of accurate GFR with measurement of systemic and regional (renal) hemodynamics. Metabolic changes in high-risk populations seems relevant in this context.

6 Conclusion

Single iohexol injection in combination with continuous low-dose infusion of iohexol is a feasible method for measuring GFR in the perioperative non-steady state. $eGFR_{cystatin\ C}$ demonstrated a variability different from that of $mGFR_{iohexol}$, while only little variation could be detected for $eGFR_{creatinine}$. eGFR, as calculated by the CKD-EPI formula for creatinine and cystatin C, underestimates $mGFR_{iohexol}$. Iohexol plasma clearance should be applied when accurate GFR measurement are required.

7 References

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8 Appendix

Table 2 - KDIGO staging of AKI (1)

Stage	Serum creatinine	Urine output
1	1.5-1.9 times baseline OR ≥0.3 mg/dL (≥26.5 μmol/L) increase within 48 hours	<0.5mL/kg/hour for 6-12 hours
2	2.0-2.9 times baseline	<0.5mL/kg/hour for ≥12 hours
3	3.0 times baseline OR Increase in serum creatinine to ≥4.0 mg/dL (≥353,6 μmol/L) OR Initiation of renal replacement therapy OR in patients <18 years, decrease in eGFR to <35mL/min/1.73m^2	<0.3mL/kg/hour for ≥24 hours or anuria for ≥12 hours

Table 3 - KDIGO GFR categories of CKD (64)

Stage	GFR mL/min per 1.73m^2	Description
G1	≥90	Normal or high
G2	60-89	Mildly decreased
G3a	45-59	Mildly to moderately decreased
G3b	30-44	Moderately to severely decreased
G4	15-29	Severely decreased
G5	<15	Kidney failure

9 Summary of knowledge evaluated key articles

Reference:	Design: Diagnostic test study
Gaspari F, Perico N, Ruggenenti P, Mosconi L, Amuchastegui CS, Guerini E, et al. Plasma clearance of nonradioactive iohexol as a measure of glomerular filtration rate. J Am Soc Nephrol. 1995;6(2):257-63	Quality of evidence: Low GRADE: ⊕⊕⊕1

Aim	Materials and methods	Results	Discussion/comments
To find a reliable alternative to inulin clearance that would allow one to avoid the use of radioactivity and problems related to the continuous infusion of the marker. **Conclusion** ***Measuring GFR by the plasma clearance of unlabelled iohexol is a good alternative to the inulin clearance technique*** **Country** **Italy** **Year of data collection** **Unknown, published 1995**	***Population: 41 patients 20-62y with a wide range of renal function.*** ***All were simultaneously measured for renal clearance of inulin and plasma clearance of iohexol. In a subgroup of 29 patients plasma clearance of iohexol was examined simultaneously with plasma clearance of iopromide.*** ***Statistical methods: the results were analysed using a t-test and linear regression. P values under 0.05 was considered significant.***	**Main findings:** **Highly significant correlation between the plasma clearance of iohexol and the renal clearance of inulin over a wide range of GFR values.** **Secondary findings:** **The agreement between plasma clearance of iohexol and renal clearance of inulin is acceptable for clinical purposes.** **Highly significant correlation between the plasma clearance of iohexol and iopromide over a wide range of GFR values.**	**Checklist:** • **Is the aim of the study clearly formulated? Yes** • **Is the reference test appropriate? Yes** • **Is the health status for the patient population well described? Yes** • **Were all participants examined with all tests? Yes** • **Were the tests done separately/blinded so that the test result remained unknown? No, not possible** • **Were the tests performed according to protocol? Yes** • **Is the gold standard validated? Yes** • **Results – Plasma clearance iohexol is suitable for measuring GFR** • **Can the results be translated to practice? Yes. There is a strong correlation and the population is relevant for the use.** • **Will the knowledge from the test result improve the comfort or management of the patient or the prognosis? Yes, it is simpler in use for measurement of exact GFR than inulin.** **What is discussed as** **Strengths None** **Limitations: data with high correlation may be in poor agreement, but the agreement is deemed acceptable.** **Do the writers refer to other literature hat strengthens or weakens the results?** **1: strong association**

Reference:	Design: Diagnostic test study
Stevens LA, Coresh J, Feldman HI, Greene T, Lash JP, Nelson RG, et al. Evaluation of the modification of diet in renal disease study equation in a large diverse population. J Am Soc Nephrol. 2007;18(10):2749-57.	Quality of evidence: Low GRADE: ⊕⊕⊕1

Aim	Materials and methods	Results	Discussion/comments
To describe the performance of the MDRD Study equation, with attention to the level of GFR and participant clinical characteristics. **Conclusion** ***The MDRD Study equation provides unbiased and reasonably accurate estimates for eGFR <60mL/min/1.73m² to be used in clinical practice.*** **Country** **United States of America** **Year of data collection** Unknown	***Population:*** ***5504 participants from 10 studies, that were measured by standardized serum creatinine and urinary clearance of iothalamate. With and without kidney disease, and a wide range of GFR. Pooling of data from different sources is justified because of the similarity of GFR measurement methods and ability to calibrate serum creatinine assays.*** ***Statistical analyses*** ***The population was stratified by subgroups defined by clinical characteristics and level of eGFR. Comparison between mGFR and eGFR was determined graphically by plotting mGFR and the difference (mGFR - eGFR) against eGFR. Bias was measured as the difference (mGFR - eGFR) and percentage difference [(mGFR - eGFR)/mGFR x 100]) between mGFR and eGFR, with positive values indicating lower eGFR than mGFR (underestimation). Precision was measured as IQR for the differences. Accuracy was measured as P30, which takes into account higher errors at higher values. Analyses were computed using R and SAS software.***	**Main findings: Compared to mGFR, the MDRD Study equation had a median difference of 2.7 ml/min per 1.73m², median percentage difference of 5.8%, and a percentage of estimates within 30% of mGFR of 83%.** **Secondary findings: At eGFR <60 ml/min per 1.73 m2, the median (IQR) difference was 0.9 (9.6) ml/min per 1.73 m2, P30 was 82%, and differences in performance among subgroups were small. At eGFR >60 ml/min per 1.73 m2, the median (IQR) difference was 8.3 (26.6) ml/min per 1.73 m2, the P30 was 84%, and there was substantial variation in performance among subgroups.**	**Checklist:** • **Is the aim of the study clearly formulated? Yes** • **Is the reference test appropriate? Yes** • **Is the health status for the patient population well described? Yes** • **Were all participants examined with all tests? Yes** • **Were the tests done separately/blinded so that the test result remained unknown? No, not possible.** • **Were the tests performed according to protocol? Yes** • **Is the gold standard validated? Yes** • **Results – $eGFR_{MDRD}$ correlates well with mGFR at <60mL/min/1.73m²** • **Can the results be translated to practice? Yes. The population is large and diverse.** • **Will the knowledge from the test result improve the comfort or management of the patient or the prognosis? Yes, it solidifies the usefulness of the MDRD study equation at eGFR <60mL/min per 1.73m²** **Strengths: A large and diverse population and use of similar GFR measurement protocols and calibrated serum creatinine in all studies.** **Limitations: The pooled studies differ from one another in their populations.** **Do the writers refer to other literature that strengthens or weakens the results? Yes** **1: Upgraded due to strong association**

Reference:	Design: Diagnostic test study
Eriksen BO, Mathisen UD, Melsom T, Ingebretsen OC, Jenssen TG, Njolstad I, et al. Cystatin C is not a better estimator of GFR than plasma creatinine in the general population. Kidney Int. 2010;78(12):1305-11.	Quality of evidence: Low GRADE: ⊕⊕⊕1

Aim	Materials and methods	Results	Discussion/comments
Compare cystatin C-based equations for eGFR with the most commonly used creatinine-based equations and to validate both against iohexol clearance **Conclusion** ***eGFRcystatin C were not superior to eGFRcreatinine in the general population*** **Country** **Norway** **Year of data collection** 2007-2008	***Population: 1627 middle-aged (50-62 years) individuals from the general population. Coronary heart or kidney disease, stroke or diabetes mellitus excluded.*** ***Statistical methods*** ***Bias and precision were calculated as the median and interquartile range of eGFR–mGFR, respectively; percentage bias and precision as the median and interquartile range of 100 × (eGFR-mGFR)/mGFR, respectively; and accuracy as the percentage of subjects in which absolute percentage bias was <30% (P30). Pearson's correlation coefficient with mGFR was estimated for each equation. The sensitivity and specificity for detecting a GFR <60 and <80 ml/min per 1.73 m^2 were calculated for each equation. The area under the receiver operating characteristic curve for each of the cut-offs was calculated by estimating the c-statistic for a logistic model with the GFR estimate in question as the only independent variable.***	**Main findings:** **The correlation coefficients between eGFRcys and mGFR were between 0.50 and 0.58. The coefficients for the MDRD and CKD-EPI equations were 0.56 and 0.55, respectively.** **Secondary findings:** **The correlation coefficient for Stevens' eGFRcombined equations reached 0.65, which was significantly higher than all of the other equations. No evidence of cystatin C-based equations being superior for either men or women.**	**Checklist:** • **Is the aim of the study clearly formulated? Yes** • **Is the reference test appropriate? Yes** • **Is the health status for the patient population well described? Yes** • **Were all participants examined with all tests? Yes** • **Were the tests done separately/blinded so that the test result remained unknown? No, not possible** • **Were the tests performed according to protocol? Yes** • **Is the gold standard validated? Yes** • **Results – Equations based upon cystatin C alone or in combination with creatinine does not provide better GFR estimates in the examined population** • **Can the results be translated to practice? Yes. The population is large and diverse.** • **Will the knowledge from the test result improve the comfort or management of the patient or the prognosis? Yes** • **Strengths – not confounded by persons with self-reported cardiovascular disease or diabetes mellitus.** **Limitations – restricted to persons between 50 and 62 years. Did not study the effect of ethnicity.** **Do the writers refer to other literature that strengthens or weakens the results? Yes** **1: Strong association**

Reference:	Design: Diagnostic test study
Bird NJ, Peters C, Michell AR, Peters AM. Comparison of GFR measurements assessed from single versus multiple samples. Am J Kidney Dis. 2009;54(2):278-88	Quality of evidence: Low GRADE: ⊕⊕

Aim	Materials and methods	Results	Discussion/comments
Compare single-sample GFR with a multisample technique. **Conclusion** ***Assessment of single-sample GFR against an independent multisample measurement of GFR shows it to be at least as reliable as multisample GFR and suitable as a simple method for use in clinical investigations and clinical trials, as well as routine clinical work.*** **Country** **United Kingdom** **Year of data collection** Unknown	***Population:*** ***95 analysed. 60 patients and 20 healthy individuals participated for sampling. 36 of the patients had diabetes, 10 had cancer and 13 had skin disease. All 20 healthy individuals were studied twice. 5 were omitted due to iohexol clearance not being resolved.*** ***GFR measurements were compared by using the same indicator and also by using different indicators. When the indicators were different, the reference measurement for single-sample GFR measured by using 51Cr-EDTA was iohexol multisample GFR, whereas the reference measurement for single-sample GFR measured with iohexol was 51Cr-EDTA multisample GFR.***	**Main findings:** **Correlations between iohexol and 51Cr-EDTA with respect to single-sample GFR were similar to that recorded for multisample GFR and were very high There were very close correlations and narrow limits of agreement between multisample GFR and single-sample GFR when they were measured by using the same indicator. Correlation coefficients generally were less and limits of agreement were wider between multisample GFR and single-sample GFR when they were measured by using different indicators as opposed to the same indicator** **Secondary findings:** **Single-sample GFR was equally repeatable and sensitive to effects of a light meal as multisample GFR.**	**Checklist:** • **Is the aim of the study clearly formulated? Yes** • **Is the reference test appropriate? Yes** • **Is the health status for the patient population well described? Yes** • **Were all participants examined with all tests? Yes** • **Were the tests done separately/blinded so that the test result remained unknown? No, not possible** • **Were the tests performed according to protocol? Yes** • **Is the gold standard validated? Yes** • **Results – Single-sample is as least as reliable as multisample, and feasible.** • **Can the results be translated to practice? Yes. Single-sample is more feasible than multisample for use in research.** • **Will the knowledge from the test result improve the comfort or management of the patient or the prognosis? Yes** **Strengths: Direct comparison between multisample and single sample GFR.** **Limitations: No separate gold standard** **Do the writers refer to other literature that strengthens or weakens the results? Yes**

Reference: Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.	Design: Diagnostic test study
	Quality of evidence: Low
	GRADE: ⊕⊕⊕[1]

Aim	Materials and methods	Results	Discussion/comments
Develop and validate a new estimating equation for GFR based on serum creatinine that would be as accurate as the MDRD Study equation at GFR less than 60 mL/min/1.73m^2, and more accurate at higher GFR. **Conclusion** The CKD-EPI creatinine equation is more accurate than the MDRD Study equation across a wide variety of populations and clinical conditions. **Country** **United States** **Year of data collection** Unknown	**A cross-sectional analysis using separate pooled databases for equation development (10 studies) and validation (16 studies).** **Inclusion criteria for studies: Studies measuring GFR using exogenous filtration markers and having the ability to calibrate serum creatinine assay. Studies for development and internal validation of equations were restricted to those using urinary clearance of iothalamate. Studies for external validation included iothalamate and other filtration markers.** **Population: 8254 people in equation development, 3896 people for external validation. Prevalence based on 16032 people. All populations having diverse clinical characteristics, with and without renal disease and across a wide range of mGFR. Lower amount of included of black origin in the external validation group than in the equation development group.**	**Main findings: Median difference (bias), IQR, P30 and root mean square error, were all improved with the CKD-EPI equation (p<0.001 for all) The CKD-EPI equation was as accurate as the MDRD Study equation in the subgroup with estimated GFR less than 60 ml/min/1.73 m2 and substantially more accurate in the subgroup with estimated GFR greater than 60 ml/min/1.73m2.** **Secondary findings: Results were consistent across studies and subgroups defined by age, sex, race, diabetes, transplant status, and body mass index (BMI). The CKD-EPI equation leads to a lower estimated prevalence of CKD compared to the MDRD Study equation.**	**Checklist:** • **Is the aim of the study clearly formulated? Yes** • **Is the reference test appropriate? Yes** • **Is the health status for the patient population well described? Yes** • **Were all participants examined with all tests? Yes** • **Were the tests done separately/blinded so that the test result remained unknown? Yes** • **Were the tests performed according to protocol? Yes** • **Is the gold standard validated? Yes** • **Results – CKD-EPI was an improvement upon the MDRD study equation** • **Can the results be translated to practice? Yes. The population is large and diverse.** • **Will the knowledge from the test result improve the comfort or management of the patient or the prognosis? Yes** **Strengths: separate large databases for development and validation. Limitations: Limited number of elderly and racial and ethnic minorities with measured GFR. Unlikely that a single equation will work well in all populations.** **Do the writers refer to other literature that strengthens or weakens the results? Yes** **1: Upgraded due to a strong association for improvements by use of CKD-EPI equation.**