Clarifying the confusion of GFRs, creatinine, and cystatin C

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Abbreviations

GFR = glomerular filtration rate;
mGFR = measured GFR;
eGFR = estimated GFR;
MDRD = Modification of Diet in Renal Disease;
CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration;
C-G = Cockcroft-Gault;
IDMS = isotope dilution mass spectrometry;
CKD = chronic kidney disease

Summary

Because measurements of GFR are tedious, estimated GFRs based on creatinine or cystatin C are widely used for assessing kidney function. Both measured GFRs and the Staging System for CKD based on GFR have analytical and clinical shortcomings. The advantages of serum creatinine and cystatin C, and eGFR will be discussed.

Abstract

Methods for measuring glomerular filtration rate (GFR) have used either creatinine, inulin, iothalamate, ^{51}Cr-EDTA, or iohexol as markers. Because these methods are all lengthy and expensive, the MDRD estimated GFR (eGFR) calculation based on the creatinine concentration was introduced in 1999 as a more convenient parameter to assess kidney function, with the equation updated in 2009 (CKD-EPI eGFR).

However, all comparisons between eGFR and mGFR have shown wide scatter that appears to be related to the large variability of the mGFR compared to the relatively stable plasma creatinine concentration. Procedures for mGFR often do not agree with each other and have not only wide population variation (as do plasma creatinine and cystatin C), but also much wider within-individual variation than creatinine or cystatin C. Because

the normal range for mGFR overlaps considerably with the Stages 1, 2, and even 3 of chronic kidney disease, mGFR has significant clinical limitations.

Both plasma creatinine and cystatin C are reliable tests for evaluating kidney function, each with some advantages and disadvantages. This report will emphasize the clinical value of serum markers (creatinine and cystatin C), the weaknesses of mGFR measurements, and suggestions for appropriate use of the eGFR parameter.

Introduction

Many methods for assessing kidney function have been developed over the past century, most notably measurements of serum creatinine, glomerular filtration rate (GFR), urea, and cystatin C. Determining the measured GFR (mGFR) require measurement of an endogenous marker such as creatinine [1], or an exogenous marker such as inulin [2], iothalamate, ^{51}Cr-EDTA, or iohexol. However, all techniques for measuring GFR are time-consuming and tedious.

Measurement of serum creatinine and cystatin C are more convenient and rapid tests for evaluating kidney function. Especially for within-individual monitoring, both are reliable kidney function tests for detecting both acute and chronic declines of kidney function [3].

Because of the difficulties of measuring GFR, equations have been developed to calculate an estimated GFR (eGFR) from serum creatinine and cystatin C and demographic factors such as age, gender, race, and weight. However, these eGFR equations:

Cockcroft-Gault (C-G), Modification of Diet in Renal Disease (MDRD), and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) are more correctly regarded as measures of serum creatinine and/or cystatin C that have been adjusted for age, gender, and race, then factored to produce a numerical quantity similar to a measured GFR. While these equations can give average agreement between eGFR and mGFR, the variation is large, with a 30 % difference considered acceptable.

Misconceptions about all these kidney function tests are common, including serum creatinine, mGFR by clearance tests (creatinine, iothalamate, inulin, etc.), the calculated eGFR, the perceived benefit of isotope-dilution mass spectrometry (IDMS) standardization of creatinine methods, and the accurate definition of "clearance".

Serum creatinine

Serum creatinine is a widely available, rapidly measured, relatively inexpensive, and reliable indicator of kidney function that is related to changes in GFR. It is the universal test for diagnosing and monitoring both acute and chronic kidney diseases. Despite this wide use, creatinine is often (incorrectly) regarded as an insensitive marker for early changes in kidney function.

This perception comes from the seemingly wide reference range for creatinine. Regrettably, an early report that serum creatinine remained within normal limits in persons with clearly decreased GFR [4] has contributed to the enduring perception that serum creatinine is an insensitive marker of declining renal function. However, serum creatinine has a smaller within-individual variation than mGFR [5], so that following serial changes of creatinine becomes a more powerful diagnostic tool for detecting relatively early changes in kidney function [3].

The belief that serum creatinine is insensitive to early changes in kidney function is also based on a commonly published plot of serum creatinine (Y axis) vs diminishing GFR (X axis). As shown in Fig. 1, this plot gives the visual illusion that serum creatinine changes minimally as GFR clearly declines from around 120 mL/min to around 60 mL/min [6].
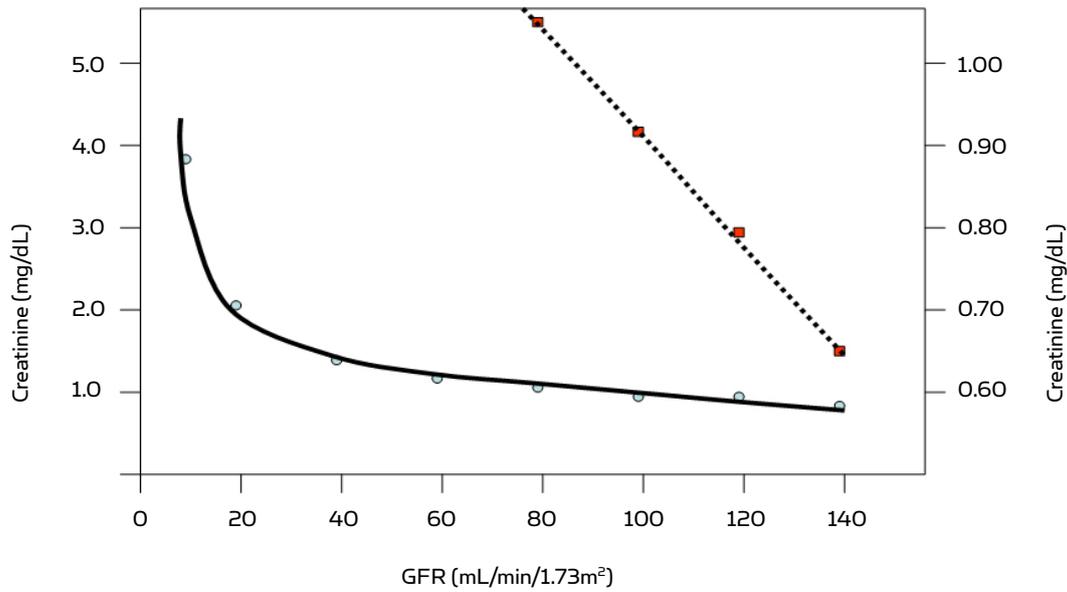
FIG. 1: Best-fit line plots of serum creatinine vs inulin GFR. Note both the apparently small increase in serum creatinine when plotted on a scale of 1.0 mg/dL, versus the apparently large increase in serum creatinine when plotted on a scale of 0.1 mg/dL. Creatinine is plotted on scales of both 1.0 mg/dL (solid line with open circles) and of 0.1 mg/dL (dotted line with squares).
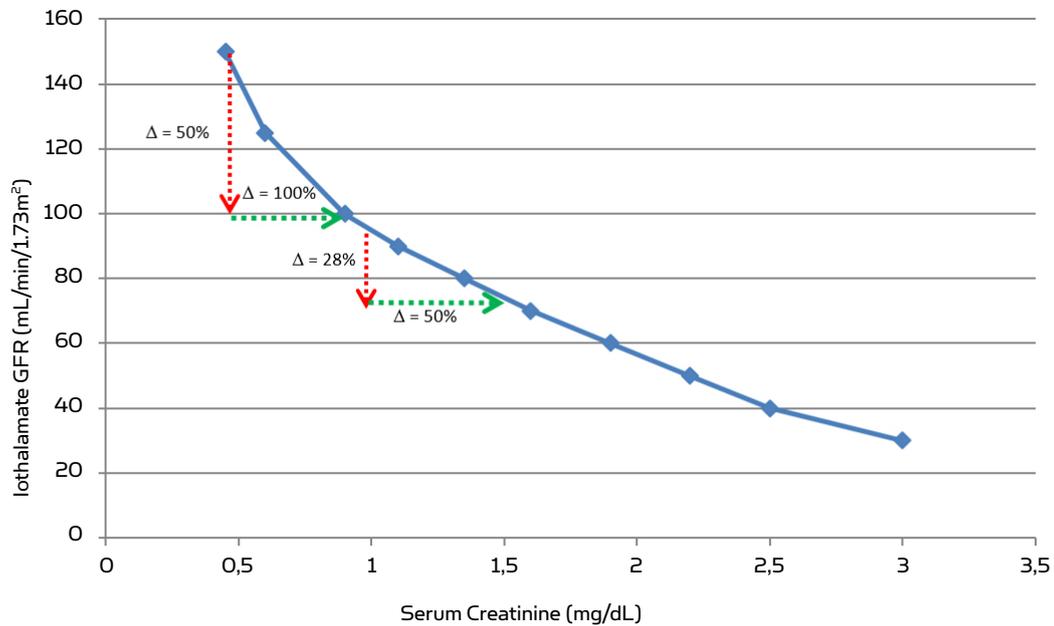

FIG. 2: Plot of iothalamate GFR vs serum creatinine, showing that the average percent change in creatinine is larger than the average percent change in GFR. Examples: For a GFR change of 150 to 100 (50 % change), the creatinine increases from about 0.45 to 0.90 mg/dL (100 % change). For a GFR decrease from 95 to 74 (28 % change), the creatinine increased from 1.0 to 1.5 mg/dL (50 % change). Plotted from data in J Am Soc Nephrol 2006; 17: 854-62.

However, such plots use a compacted y-axis scale of serum creatinine in increments of 1.0 mg/dL (88 µmol/L), which is well above the detection limit of modern automated methods for serum creatinine of about 0.05 mg/dL (5 µmol/L). If this best-fit line is plotted on a scale of 0.1 mg/dL, the sensitivity of serum creatinine becomes readily apparent as GFR declines, as also shown in Fig. 1.

A report on patients with polycystic kidney disease showed that the mean increase in serum creatinine (from 0.5 to 1.0 mg/dL) was of the same magnitude as the mean decrease in GFR by iothalamate clearance (from 150 to 100 mL/min/1.73 m^2), as shown in Fig. 2 [7].

Note also that the reference ranges for serum creatinine (approximately 0.73-1.37 mg/dL = ratio 1.88), cystatin C (0.55-1.15 mg/L = ratio 2.09) and GFR (67-135 mL/min/1.73 m^2: ratio 2.01) all have about the same relative ranges [8, 9]. While both serum creatinine, cystatin C, eGFRs, and mGFRs (by creatinine, inulin, iothalamate) all have relatively large and proportionately similar reference ranges [8, 9], the within-individual variations for serum creatinine, cystatin C, and eGFR are much lower than for mGFR by creatinine clearance [5, 10], or by iothalamate and inulin methods [7, 11].

As expected, eGFRs calculated from creatinine or cystatin C correlate poorly with clearance measurements of GFR, either by creatinine, iothalamate or inulin clearance, especially in the normal range [5, 9, 11].

IDMS standardization of creatinine

Creatinine methods are now commonly standardized to an isotope-dilution mass spectrometry (IDMS) reference method [12], which should lessen the systematic bias between methods and improve agreement among both creatinine and eGFR results from different institutions. However, it will have no effect on either the imprecision of a particular creatinine method or on the inherent random differences between calculated eGFR and measured GFR.

Serum cystatin C

Plasma concentrations of cystatin C are reliable markers for detecting and monitoring the progression of kidney disease. Both creatinine and cystatin C are each affected by factors other than GFR, with creatinine affected by factors related to muscle mass (age, gender, and race), and cystatin C affected by age, inflammation, obesity, and diabetes [13]. Like serum creatinine, cystatin C concentrations were much more stable in individuals without renal disease than was GFR measured by creatinine clearance [5].

A possible advantage of cystatin C with minor glomerular damage is that, being a large molecule, blood levels of cystatin C might rise sooner than creatinine. Several reports also indicate that cystatin C is better than creatinine for predicting risk in cardiovascular disease [14], although other studies did not conclude that cystatin C improved risk prediction [8, 15]. Cystatin C is also a more costly, slower, and less available test than creatinine. Cystatin C now has a certified reference material available that should improve method standardization for that analyte.

The term "clearance"

"Clearance" is a misunderstood term originally used by physiologists to calculate the net volume of blood cleared of a specific substance per unit of time by the combination of glomerular filtration, renal tubular reabsorption, and tubular secretion. While "clearance" would logically be the rate at which a substance is removed from the blood, with units such as mmol/min, the actual units are volume/time, such as mL/min. Thus, the "clearance" is more correctly regarded as a measurement of the GFR, if the measured substance is freely filtered and neither reabsorbed nor secreted by the kidney tubules.

The clearance equation is:

Clearance = timed urine volume (mL/min) × (urine concentration / plasma concentration)

Both an accurately timed urine sample and a blood sample must be collected. The timed urine volume represents the concentrated volume of the original glomerular filtrate, while the ratio of the urine concentration to the plasma concentration of the substance such as creatinine or inulin represents the factor (approximately 100 fold) that the glomerular filtrate has been concentrated as it becomes urine.

Substances such as inulin and iothalamate are freely filtered by the glomeruli and neither reabsorbed nor secreted by the renal tubules. Since some creatinine is secreted into the renal tubule, creatinine clearance overestimates the GFR. However, the advantages of creatinine are that it is naturally in blood and its concentration in blood typically remains constant during the period of urine collection.

Methods for measuring GFR by plasma clearance

GFR may be calculated indirectly from the rate of removal of an exogenous substance in blood after the substance is injected intravenously, then blood specimens are collected at timed intervals after the injection. These methods have the advantage that no urine is collected.

Iohexol clearance. Iohexol solution is injected and allowed to distribute in the extracellular fluid, then blood samples are collected at timed intervals. The iohexol is measured and the GFR is calculated [16].

Iothalamate clearance. Iothalamate is injected subcutaneously and allowed to equilibrate for 45 minutes. Then a 7-mL blood sample is collected along with the patient emptying their bladder. After 45-60 min (accurately timed), another blood sample and a urine sample are collected. Then the urine and blood samples are analyzed for iothalamate [11].
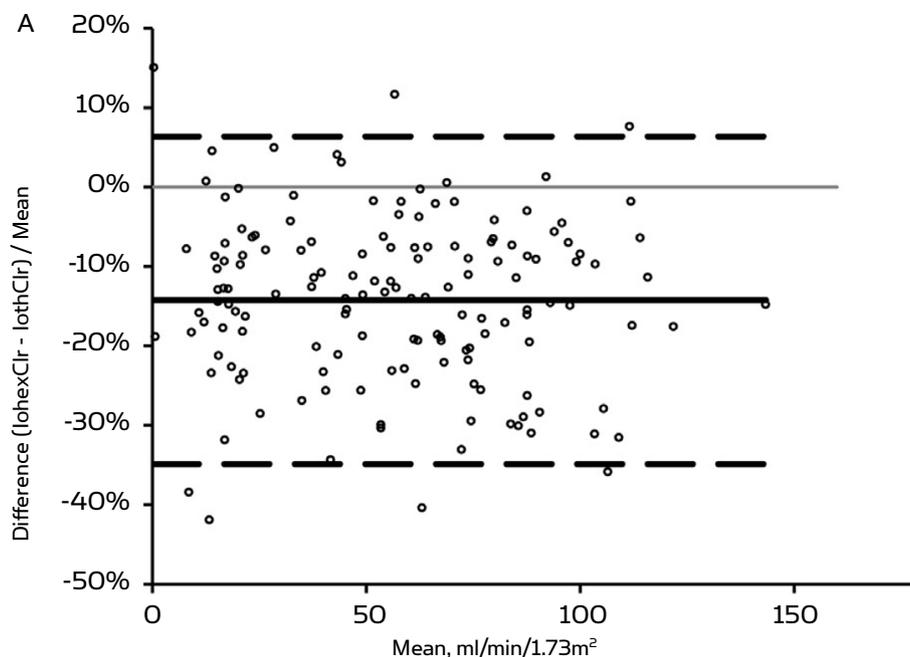

FIG. 3: Percent differences between measured GFRs: Iohexol vs iothalamate clearances. (from Seegmiller *et al*. Am J Kidney Dis 2016: 67: 49-55.

51Cr-EDTA method. Subjects are injected intravenously with ^{51}Cr-EDTA solution, then 6-mL blood samples are drawn from the opposite arm at 2, 3, and 4 h. The plasma concentrations of ^{51}Cr-EDTA are then measured in a gamma counter, with the log [^{51}Cr-EDTA] against time used to determine the GFR [17].

Although often regarded as "gold standard" tests, mGFRs are highly variable and often do not agree with each other [11, 18], as shown in Fig. 3, and do not agree with endogenous serum markers such as creatinine and cystatin C.

The variability of mGFRs appears to be related to factors such as tubular secretion of creatinine, inaccuracy in urine collection, matrix effects, analytical variation in serum and urine measurements, and higher daily variability of mGFR [5].

This variation between serum markers and measured GFRs appears to be inherent, as shown in plots of eGFR vs measured GFR, whether calculated from serum creatinine or cystatin C [5, 6, 7, 19]. The GFR is also affected by diet and hemodynamic alterations. Thus, mGFR has many shortcomings as a clinical marker to detect declining renal function in CKD.

In an analogy to another set of laboratory tests, the GFR is like blood glucose that fluctuates during the day, while the serum creatinine is like hemoglobin A1c. A physiologic analogy would be to compare GFR to cardiac output, which is the most important function of the heart, but is clearly a much less sensitive marker for detecting cardiac ischemia or necrosis than are blood levels of troponin.

Equations for calculating eGFR

Because of the challenges of measuring GFR by clearance or other tests, equations have been developed to calculate eGFR from serum creatinine and/or cystatin C with factors such as age, gender, race, and weight. For many years, the C-G formula [20] used a creatinine normalized for age, gender, and weight to estimate GFR based on creatinine clearance and is still the standard for calculating the dosage of drugs based on a patient's kidney function.

In 1999, the MDRD equation for eGFR was published. It was based on normalizing serum creatinine for age, gender, and race, with factors included to numerically resemble GFR [21]. The intent was to provide a kidney function parameter that would lessen the likelihood of chronic kidney disease (CKD) going unnoticed by non kidney
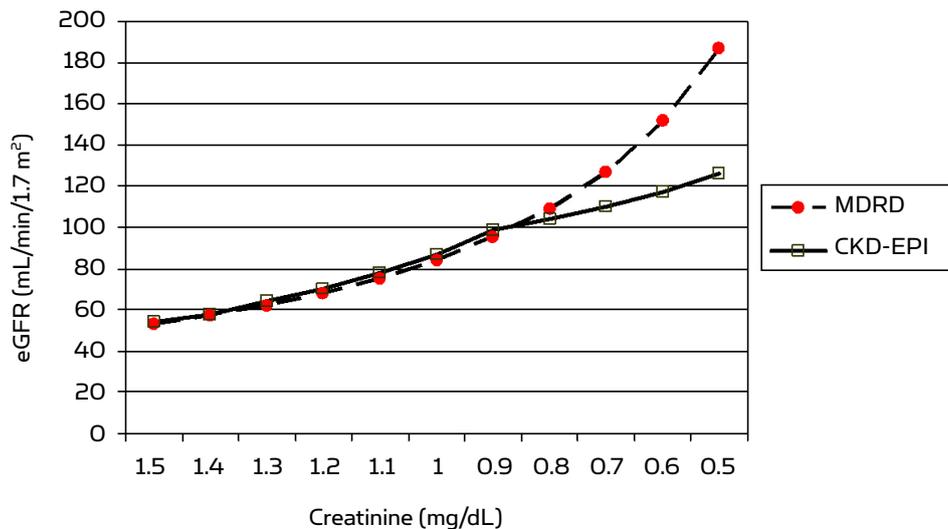

FIG. 4: eGFR calculated from serum creatinine by both the MDRD (dashed line with round dots) and CKD-EPI (solid line with squares) equations for a 50-year-old white male.

specialists. However, a significant clinical limitation of the MDRD eGFR was the recommendation to only report eGFRs below 60 mL/min/1.73 m^2.

This was done to avoid incorrectly classifying patients as having Stage 1 (GFR < 90 mL/min/1.73 m^2) or Stage 2 CKD (60-89 mL/min/1.73 m^2), but who had no apparent kidney disease, which resulted from the significant overlap between the normal range for mGFR (72-140 mL/min/1.73 m^2) and both Stage 1 and Stage 2 classifications of CKD. Furthermore, nephrologists commonly find the eGFR to be <60 mL/min/1.73 m^2 in elderly persons with no evidence of kidney disease and who have a serum creatinine that is stable at a slightly elevated level.

The CKD-EPI equation gives lower eGFR values than the MDRD equation in the normal ranges, as shown by a plot of eGFR values for a 50-year-old white male (Fig. 4).

Although the improved agreement of the CKD-EPI eGFR to mGFR was modest, going from 80.6 % of the MDRD eGFRs to 84.1 % of the CKD-EPI eGFRs being within 30 % of the mGFR, the greater value of the CKD-EPI eGFR could ultimately be if all values of eGFR are reported [19].

Because blood concentrations of creatinine/cystatin C are relatively stable over time in persons with normal kidney function and mGFR is a renal filtration measurement that fluctuates continually, it appears inevitable that all comparisons of eGFRs to mGFRs will show large variation. As such, the eGFR should be interpreted as an independent parameter for detecting and monitoring kidney function. Furthermore, reporting all values of eGFR would allow it to become a useful guide for interpreting creatinine and/or cystatin C results.

Equations using cystatin C to estimate GFR

Because of higher expense and less availability, eGFR based on cystatin C (eGFR$_{cysC}$) will probably not replace eGFR by creatinine. However, studies suggest that having both eGFR$_{creat}$ and eGFR$_{cysC}$ can improve the risk prediction for end-stage kidney disease [21]. If both eGFR$_{creat}$ and eGFR$_{cysC}$ are available, a discrepancy between the two could indicate the need for further testing, such as with iohexol clearance [22].

For comparing the clinical utility of the eGFR equations to measurements of GFR, a most relevant point was reported by Grubb that over 15 years of study, the combined creatinine/cystatin C eGFR disagreed with their "gold standard" iohexol procedure only 10 times. In each case, the discrepancy was due to technical problems with the iohexol procedure [23].

Shortcomings of staging kidney disease based on GFR

A significant clinical problem of basing CKD on stages according to the GFR is the large overlap between the normal range for mGFR of 72-140 mL/min/1.73 m^2 [9] and both Stage 1 (GFR < 90 mL/min/1.73 m2) and Stage 2 CKD (60-89 mL/min/1.73 m^2) classifications of CKD. As noted earlier, nephrologists commonly find the eGFR to be slightly below 60 mL/min/1.73 m^2 in elderly persons with no evidence of kidney disease who have a serum creatinine that is stable at a slightly elevated level.

While the CKD-EPI eGFR improves average agreement with mGFR in the normal range and could allow all values of eGFR to be reported [19], it did not overcome the problems with the staging system of CKD based solely on the GFR.

To improve the clinical utility of the CKD stages, Stage 3 was subdivided into Stage 3a (45-59 mL/min/1.73 m^2) and Stage 3b (30-44 mL/min/1.73 m^2), and urine albumin or protein was added in the classifications. Thus, if a patient were in Stage 3a based on their GFR, but had normal excretion of protein in their urine and no other indications

of kidney disease, that would warrant continued monitoring of that patient for changes, but not necessarily aggressive therapy for CKD [24]. Peralta et al report that using the three more easily measured parameters (eGFR$_{creatinine}$, eGFR$_{cystatin C}$, and urine albumin:creatinine ratio) improved the prediction of mortality and end-stage kidney disease [25].

Summary

For acute kidney injury, serial measurements of either serum creatinine or eGFR on the same individual should improve detection of changes in kidney function in CKD. However, serial measurements may not be available in settings such as emergency medicine where a single creatinine result would be interpreted relative to a population reference range. While this could be a significant clinical opportunity for a single eGFR measurement (being a creatinine or cystatin C adjusted for age, gender, and race) to be interpreted with a narrower reference range, it will be necessary to report all values of eGFR.

Because serum creatinine and cystatin C have different homeostatic patterns over time, all eGFR equations for predicting mGFR from creatinine or cystatin C, in addition to any systematic bias, will show wide variation compared to mGFR. However, if the eGFR is reported at all values, it would become more clinically useful for detecting both chronic and acute changes in kidney function. Given the significant technical, cost, and clinical limitations of a measured GFR, the eGFR based on serum creatinine and/or cystatin C along with a urine protein or albumin could become the new "gold standard" for detecting and monitoring kidney disease.

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