CLN'S LAB 2004: FROM BASIC TO ADVANCED SERIES

Lab Estimation of GFR

Creatinine Clearance, Creatinine, or Cystatin C?

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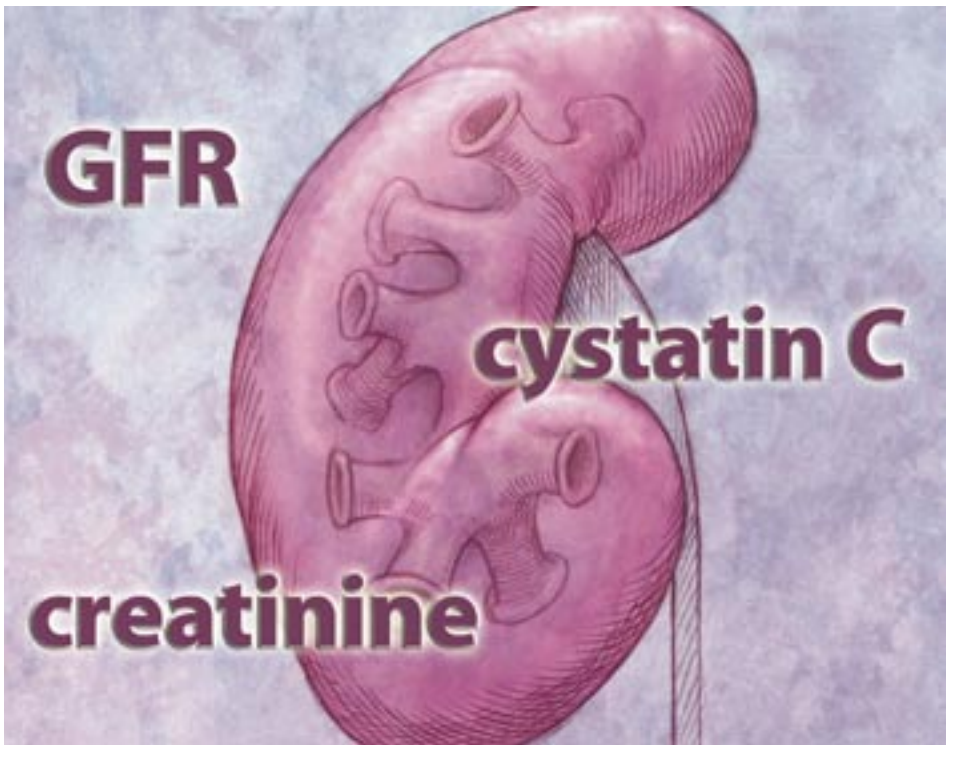
Glomerular filtration rate (GFR) is one of the most important parameters used in the assessment of renal function. Clinicians use GFR to detect suspected kidney disease in patients, as well as to monitor patients with known disease. In addition, clinicians use the results of GFR as an aid in planning renal replacement therapy for patients with advanced kidney disease and in adjusting drug dosage of pharmacologic agents that are excreted renally.

Underscoring the importance of the assessment of renal function is the fact that an increasing number of Americans—at least 8 million, currently—are estimated to have substantial kidney impairment. In fact, more people die annually from kidney failure than from either colon cancer, breast cancer, or prostate cancer. Additionally, it is well known that the presence of chronic kidney disease or kidney failure significantly increases a patient's risk of cardiovascular disease.

The recent Kidney Disease Outcomes Quality Initiative (K/DOQI) practice guidelines, which include a new classification of chronic kidney disease (Table 1) based on GFR stratification, emphasize the importance of the laboratory measurement. In order to reduce the associated morbidity and mortality, the guidelines promote GFR assessment in the patient-evaluation process as a means of detecting disease—especially incipient disease—and evaluating persons at various stages of kidney disease.

Given the increasing number of patients with kidney impairment, as well as the new emphasis on GFR called for in the K/DOQI guidelines, the demand on laboratories for GFR assessment is likely to grow. Consequently, it is essential for laboratorians to understand the differences between the various methods for assessment of GFR in order to aid clinicians in deciding which method should be used for a particular clinical situation.



Methods for GFR Assessment

Because there are no direct means to measure GFR, laboratories rely on measurement of endogenous or exogenous substances in plasma and/or urine to provide an estimate. These methods include measuring the urinary or renal clearance of a particular substance, measuring a plasma or serum marker of GFR, or using an estimation equation for GFR. For a substance to be used in the measurement of GFR by urinary clearance methods, it should ideally exhibit several properties: 1) it should be physiologically inert; 2) it should be freely filtered and not be bound to protein; and 3) it should not be reabsorbed, secreted, synthesized, nor metabolized by renal tubules.

Consequently, the amount of the analyte filtered by the kidneys should equal the amount excreted in the urine as described by the following equation, where Px and Ux are the plasma and urine concentrations, respectively, of the ideal substance, GFR is glomerular filtration rate, and V is the urine flow.

$$GFR \times Px = Ux \times V$$

Rearranging this equation gives:

$$GFR = \frac{Ux \times V}{Px}$$

The absolute amount of filtrate produced under normal conditions tends to vary in direct relation to body size; and therefore, GFR is often normalized by body surface area.

Non-isotopic and isotopic clearance method. Inulin has historically been regarded as the gold standard for measurement of GFR. It is a fructose polymer with a molecular weight of approximately 5 kDa. Assessment of GFR by inulin clearance requires a continuous intravenous infusion, during which plasma and timed urine samples are obtained. It is possible to measure inulin using a resorcinol-based spectrophotometric method. However, inulin clearance is cumbersome and expensive to perform, and is usually limited to clinical research use.

An alternative to inulin clearance is the measurement of radiolabeled agents such as ^{125}I-iothalamate, ^{51}Cr-EDTA, or ^{99}Tc^{m}-DTPA. In the U.S., the radiolabeled form of the radiocontrast agent iothalamate is typically used, and GFR measurements using this methodology compare closely to inulin clearances. The widespread availability of ^{125}I-iothalamate, the close correlation with inulin clearance, and the ease of measuring ^{125}I-iothalamate in plasma and urine, all make urinary clearance of iothalamate the preferred method for precise and accurate assessment of GFR.

But while radiolabeled iothalamate simplifies the measurement of GFR, the expense of the analyte, the resources needed to administer it and accurately collect samples, the concerns for the safety of the patient and the health worker, and the regulatory requirements for administering and handling of radioactive substances have limited its widespread use in clinical programs. However, when precise assessment of GFR is needed in patient evaluation or care, iothalamate clearance should be considered. In order to obviate the issues related to the handling of radioactive materials, researchers have developed new methods to measure non-radiolabeled iothalamate by capillary electrophoresis and HPLC. Both methods have been shown to correlate closely with measurements of the ^{125}I-labeled material.

Plasma clearance methods. While urinary clearance has been the preferred method for accurate measurement of GFR, obtaining timed urine collection can be difficult. For this reason, simplifying the procedure by estimating GFR based on the plasma disappearance of an intravenously administered marker, such as ^{125}I-iothalamate, ^{51}Cr-EDTA, or iohexol, is another option. It is beyond the scope of this article to review the details of plasma disappearance methods using these markers; however, it is important to note that one significant drawback of plasma disappearance relates to appropriate sample timing. For example, while obtaining blood samples one to four hours following the injection of a marker may be adequate for those with normal or near-normal GFR, patients with severely impaired GFR may require sampling up to 24 hours in order to accurately detect decreases in marker concentration. Also, plasma disappearance methods require an estimation of the volume of distribution of the marker, which is often difficult to determine accurately.

Laboratories may also estimate GFR with the same radionuclide markers that are used in renal imaging, such as ^{99}Tc^{m}-DTPA, ^{99m}Tc-mercaptoacetyltriglycine (MAG^{3}), or ^{123}I-iodohippurate. Quantitative assessment of the rate of disappearance of these markers through external radionuclide detection is much less precise than other more standard renal clearance methods, although it may be useful for comparing relative renal function between contralateral kidneys in a given individual.

Serum Creatinine and Creatinine Clearance

For decades, laboratories have measured creatinine (113 Da) and its renal clearance as a means to assess kidney function. A product of the hydrolysis of creatine and phosphocreatine in muscle, creatinine is proportional to muscle mass, which largely

Table 1. Stages of Chronic Kidney Disease

Stage	Description	GFR (ml/min/1.73m^2)
1	Kidney damage with normal or ↑ GFR	≥90
2	Kidney damage with mild ↓ GFR	60–89
3	Moderate ↓ GFR	30–59
4	Severe ↓ GFR	15–29
5	Kidney failure	<15 (or dialysis)

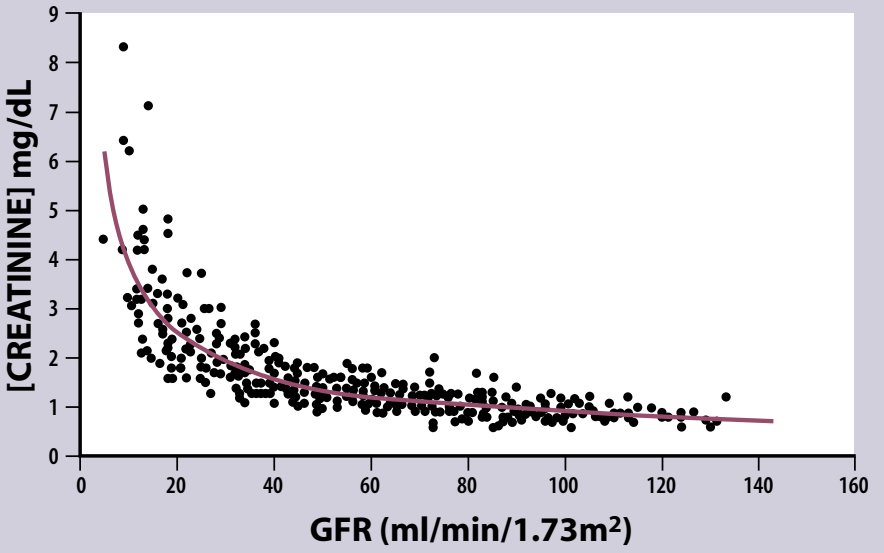


Figure 1. Relationship between Serum Creatinine and GFR.

accounts for age- and gender-related differences in serum creatinine, independent of differences in GFR. Another creatinine source is dietary ingestion of cooked meat.

Although serum creatinine is the most frequently utilized marker for kidney function, it unfortunately lacks sensitivity for early detection of decreases in renal function. This is underscored by the relationship between serum creatinine and GFR when the latter is measured by inulin or iothalamate clearance (Figure 1). GFR may decline by as much as 50% of normal before the serum creatinine increases to values above the normal reference range.

Measurement of creatinine. Colorimetric measurement of a complex formed between alkaline pricrate and creatinine, known as the Jaffe reaction, is the most common method for creatinine measurement. The accuracy of this method has been limited by a number of known interferences—acetoacetate, ascorbic acid, fructose, pyruvate, cephalosporins, and other endogenous noncreatinine chromagens—that can cause falsely elevated creatinine values, while high levels of bilirubin can decrease values. However, these interferences can be reduced by measuring the rate of color development, which is how many autoanalyzers perform the assay.

Laboratories have also used enzymatic assays with either creatinine iminohydrolase (creatinine deaminase) or creatininase (creatinine amidohydrolase) to measure creatinine levels. Both enzymatic assays have significantly fewer interferences in comparison to the Jaffe assay, although there have been reports of interference from a few drugs. For example, 5-fluorocytosine interferes with the iminohydrolase assay, and dopamine/dobutamine interferes with the creatininase assay.

Currently, standardization for the measurement of serum creatinine is lacking. As such, there is significant lab-to-lab variability in the creatinine measurements, as well as significant variation in the reported normal range for serum creatinine. To address these issues, the National Kidney Disease Education Program (NKDEP) has formed a Laboratory Working Group to develop standards for serum creatinine measurements.

Creatinine clearance. Although creatinine is not protein-bound and is freely filtered by the glomerulus, it has several other properties that make creatinine clearance measurements less than ideal for GFR assessment. For example, a component of urinary creatinine is derived from renal tubular secretion. This contributes to the well-known observation that creatinine clearances overestimate true GFR (Figure 2). The tubular excretion of creatinine tends to increase disproportionately as GFR decreases, causing an increasing discrepancy between true GFR and measured creatinine clearance. In addition, although the extra-renal clearance of creatinine may be negligible in normal subjects, it increases in patients with advanced renal failure. Other factors that can affect creatinine secretion independently of changes in GFR, and therefore the calculation of creatinine clearance, include the inhibition of creatinine secretion by the medications cimetidine and trimethoprim.

Typically, laboratories measure creatinine clearance from a 24-hour urine collection and a serum sample obtained during the urine collection. Several factors contribute to imprecision and errors in this method: tubular secretion that tends to cause overestimation of GFR; conversion of urinary creatinine to creatine when urine is stored for prolonged periods, especially at high temperatures and low pH; and inconsistencies in obtaining a complete and accurate 24-hour urine collection. These factors result in significant variability and inaccuracies, and consequently, the new K/DOQI guidelines do not recommend routine GFR assessment using a 24-hour creatinine clearance.

Because the drug cimetidine inhibits tubular creatinine secretion, it has been suggested that cimetidine administration be used during creatinine clearance measurement in order to obtain a more accurate measure of GFR. Cimetidine, a H_2 receptor antagonist, is relatively safe and inexpensive. Although this approach may improve the accuracy of GFR estimation by this method, the inaccuracies inherent with obtaining a complete 24-hour urine collection remain.

Prediction Equations to Estimate GFR

The NKDEP and National Kidney Foundation recommend that laboratories use prediction equations to estimate GFR from serum creatinine in patients with chronic kidney disease and in those at risk for chronic kidney disease (Table 2). While the Cockcroft-Gault equation provides a reasonable assessment of kidney function, it tends to overestimate GFR because the formula was derived from creatinine clearance measurements. It also does not adjust for differences in creatinine production between individuals of the same age and gender. Despite the drawbacks of this equation, which was empirically derived nearly 30 years ago, it has gained widespread use for the estimation of creatinine clearance.

More recently, some groups have advocated the Cockcroft-Gault equation as an alternative prediction equation. Derived from the Modification of Diet in Renal Disease (MDRD) study, researchers assessed GFR using ^{125}I-iothalamate in more than 500 adult individuals with varying degrees of kidney disease. They derived several empiric equations for the study and validated them in an additional large group of individuals. The equation providing the greatest correlation with GFR requires several variables including serum creatinine, urea, and albumin in addition to age, gender, and race. However, researchers have found that an abbreviated MDRD equation using only serum creatinine, age, gender, and race has similar accuracy and precision (Table 2). While this equation is not as easily calculated as the Cockcroft-Gault equation, it can be programmed into most calculators, computers, and LIS systems, and several Web sites provide ready access to the equation. A particular advantage of the abbreviated MDRD equation is that it does not require the measurement of patient weight for the calculation.

Although the MDRD equation was derived from a relatively wide range of GFRs, the source data did not include normal subjects. Recent studies have confirmed that this equation correlates poorly with normal GFRs, likely as a result of the insensitivity of serum creatinine to early changes in GFR. Additionally, inter-laboratory differences in calibration of creatinine assays have their greatest impact in the near-normal range and therefore lead to greater inaccuracies in estimation equations in this GFR range. Consequently, the NKDEP recommends that the MDRD prediction equation be used to report values above 60 ml/min/1.73m^2 as "above 60 ml/min/1.73m^2" and not as an exact number.

For infants and children, laboratories commonly use the Schwartz formula and the Counahan-Barratt formula (Table 2). Even though both equations lack precision, they are convenient to use, requiring only serum creatinine and body length measurement. For many clinical purposes, these equations provide an estimate of GFR that is sufficient.

Cystatin C

Recently, researchers have advocated cystatin C as a superior marker to serum creatinine for GFR assessment. Cystatin C is

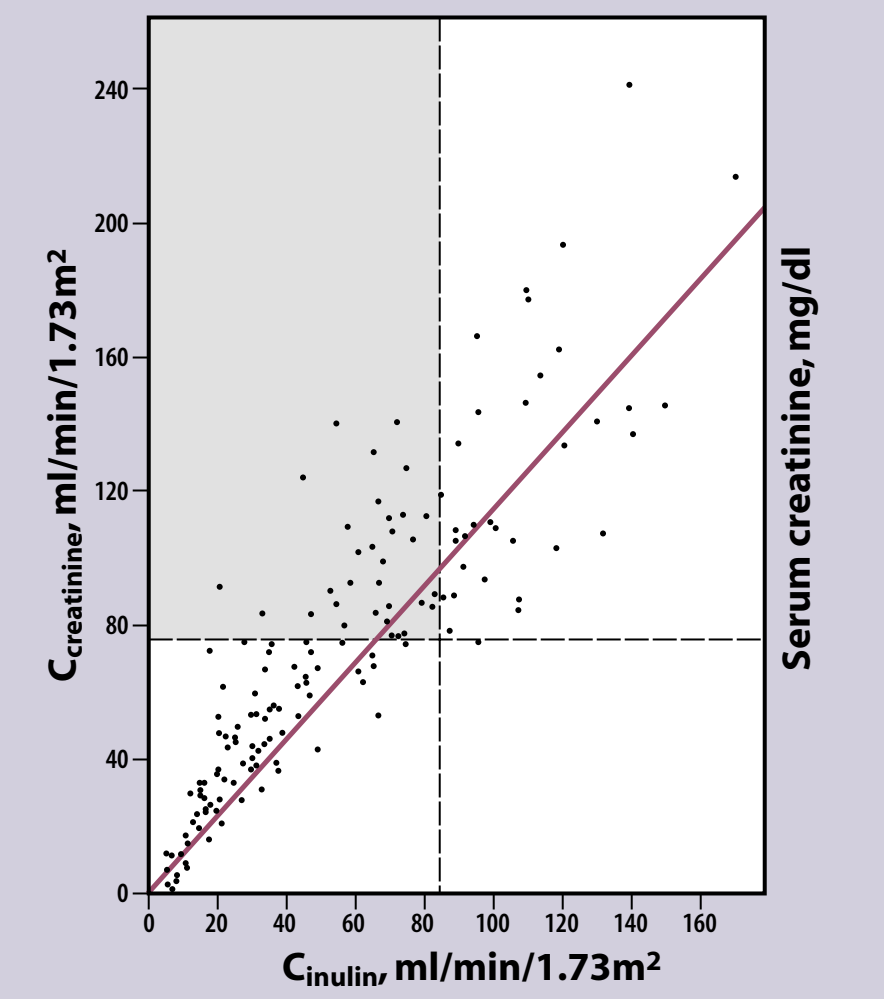


Figure 2. Relationship between Creatinine Clearance and GFR in Patients with Glomerular Disease. The horizontal and vertical dashed lines represents the lower limit for creatinine clearance (77 ml/min/1.73m^2) and inulin clearance (82 ml/min/1.73m^2), respectively, in the author's laboratory. The shaded rectangle represents patients with abnormal (low) GFR measured by inulin clearance, but normal creatinine clearance. The solid diagonal line is the line of identity.

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a low molecular weight, non-glycosylated basic protein (13 kDa) that is part of the cystatin superfamily of cysteine proteinase inhibitors. It appears to be synthesized by all nucleated cells, and its production rate is constant, being regulated by a housekeeper type gene. Additional properties that make it an ideal endogenous serum GFR marker are: it is not protein bound; it is freely filtered by the glomerulus; and it is not affected by muscle mass, diet, gender, or inflammation. Similar to many other low molecular weight proteins, cystatin C is reabsorbed and metabolized by the proximal tubules of the kidney after it is filtered, which means that very little analyte is present in excreted urine under normal conditions. Consequently, it is not possible to estimate GFR by timed urinary cystatin C clearance, similar to that used for creatinine clearance. On the other hand, increases in urinary excretion of cystatin C may be a marker for proximal renal tubule injury or dysfunction.

The medical community has nevertheless shown a great deal of interest in evaluating cystatin C as a potentially more reliable serum marker for GFR. Most studies have shown cystatin C to correlate more closely to GFR than serum creatinine when an accurate measure of GFR such as iothalamate, inulin, or EDTA clearance is used as the reference GFR measure. Unlike serum creatinine, cystatin C is not influenced by muscle mass, and therefore, its level does not change during childhood. During late adulthood, cystatin C levels tend to increase with age, likely as a result of normal age-related decreases in GFR. Whether other factors such as glucocorticoid steroid use, tobacco use, thyroid function, or other unidentified factors affect cystatin C levels independent of GFR has yet to be definitively established. Until then cystatin C appears to be an attractive alternative to serum creatinine for assessment of renal function.

Currently, laboratories are using either particle-enhanced turbidimetry or particle-enhanced nephelometry to measure cystatin C. Both immunoassay methods allow rapid and precise measurements of cystatin C. However, in the U.S., the FDA has so far cleared only the nephelometric assay.

Table 2. Common Estimation Equations for Creatinine Clearance or GFR

Cockcroft-Gault Equation	$C_{CR}\ (ml/min) = \frac{(140 - Age) \times Weight}{72 \times S_{CR}} \times (0.85 \text{ if female})$
Abbreviated MDRD Equation	$GFR\ (ml/min/1.73m^2) = 186 \times S_{Cr}^{-1.154} \times Age^{-0.203} \times (0.742 \text{ if female}) \times (1.21 \text{ if African-American})$
Schwartz Formula	$C_{CR}\ (ml/min) = \frac{0.55 \times Length}{S_{CR}}$
Counahan-Barratt Equation	$GFR\ (ml/min/1.73m^2) = \frac{0.43 \times Length}{S_{CR}}$

Abbreviations and units: C_{Cr}, creatinine clearance; Age in years; Weight in kg; S_{Cr}, serum creatinine in mg/dL; Length, body length in cm.

See www.nkdep.nih.gov/GFR-cal.htm for equations.

Assessment of Renal Function: Matching the Right Test to the Patient

Clinicians need to understand the differences between the various methods for the assessment of GFR in order to decide which should be used for a particular clinical situation. Serum creatinine has been used for decades by clinicians for assessment of renal function, but it has multiple, significant limitations. Perhaps the greatest limitation of all serum markers is that they have an inverse relationship to GFR. Given this relationship, serum markers will be inherently less sensitive for detecting changes at the higher range of GFR in comparison to the lower end (see Figure 1), even when used in predictive equations.

It is also important to keep this relationship in mind when evaluating a patient for early changes in renal function. In situations where it is clinically important to detect early changes in GFR, a serum marker may not provide the sensitivity needed, and a clearance method may be more appropriate. The recent emphasis on using serum creatinine to estimate GFR by the NKDEP underscores some of the limitations of using serum creatinine itself as an index of GFR. The limitations of these prediction equations, namely that they are primarily useful for assessment of GFR < 60 ml/min/1.73m^2 and their imprecision across the entire range of GFR, must be kept in mind.

In some cases, a more precise determination of GFR may be indicated. Two examples are for the assessment of an individual seeking consideration for kidney donation or in those instances where creatinine production is significantly affected, independent of GFR, such as malnourished states and other severe muscle wasting conditions. In many clinical situations, it also may be important to detect kidney disease much earlier than 60 ml/min/1.73 m^2, the cut-point recommended for use of the MDRD equation by the NKDEP. In these instances, a more formal GFR assessment, such as isotopic or non-isotopic iothalamate, may be more appropriate.

Lastly, cystatin C holds great promise as a more reliable marker of renal function than the currently accepted creatinine clearance, primarily since it is not affected by muscle mass or diet. As such, it may be quite useful as an adjunct to renal function assessment, especially in clinical practices or situations where precise GFR measurements are not readily available.

SUGGESTED READINGS

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