

Cystatin C: A New Standard to Measure GFR?

Introduction

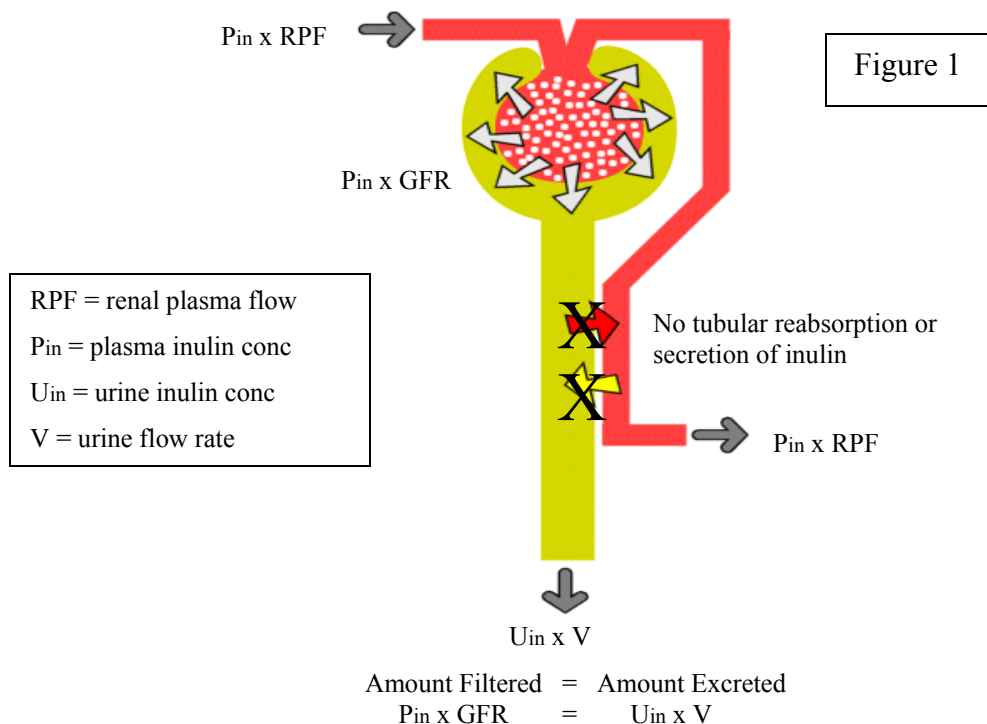
The clinical presentation of patients with kidney disease is highly variable. Some patients may present with symptoms directly related to the pathologic derangement of normal renal function (e.g. with hypertension and edema). Others may even present with signs such as hematuria or flank pain that may be indicative of direct injury to the kidney. However, it is most common that patients are noted to have elevated creatinine levels or abnormal urinalyses on routine examination. Once kidney disease is identified, an accurate measure of glomerular filtration rate (GFR) is essential to track progression of the underlying disease process.

Currently, measurements of serum creatinine (SCr) and estimation equations based on SCr are the most widely used methods to assess GFR. However, it has long been recognized that the use of SCr for this role has multiple limitations (as presented below). Subsequently, researchers have been in search of an alternative biomarker to assess GFR. In this review, I will focus on one of the most studied alternative markers of GFR, cystatin C. I will attempt to present whether cystatin C represents a feasible replacement for currently accepted SCr-based methods.

Measurement of glomerular filtration rate

Gold standard

It is not possible to measure GFR directly given that filtration occurs at a microscopic level and involves the function of millions of separate nephrons. However, GFR could be estimated from the urinary clearance of a marker that is filtered by the glomerulus but is neither secreted, reabsorbed, synthesized, nor metabolized by the kidney. One such marker that fits these criteria is inulin, a small polyfructose molecule. As illustrated below in Figure 1, the amount of inulin excreted in the urine per minute equals the amount of inulin filtered at the glomerulus [1]:



While inulin clearance is considered to be the gold standard for measurement of GFR, its use in the clinical setting is limited by multiple factors. As the substance is in short supply, it is relatively expensive and is difficult to assay. Moreover, typical protocols for measuring inulin clearance require continuous IV infusion, bladder catheterization, and collection of multiple blood samples [2]. With the need to obtain daily measurement of renal function in an inpatient setting to closely track progression of disease, such a complicated and cost-ineffective process has precluded inulin's use as an ideal analyte.

In addition to inulin, multiple other markers have been identified to accurately measure clearance including iohexol, and radioactively tagged DTPA or EDTA. The methods established to measure these markers are not nearly as labor-intensive as for inulin [3]. Nevertheless, the need for exogenous administration of these markers makes their clinical application limited. Where they do play a role, however, is to serve as a gold standard for comparison of endogenous filtration markers.

Serum creatinine

Derived from the metabolism of creatine in skeletal muscle and from dietary meat intake, creatinine is released into the circulation at a relatively constant rate. It is freely filtered but neither metabolized nor reabsorbed within the tubules [4]. In the steady state, excretion of creatinine ($GFR \times SCr$) will be equal to creatinine production, which is assumed to remain constant. Therefore, serum creatinine should vary inversely with the GFR as demonstrated by the equation below:

$$GFR \times SCr = \text{constant (creatinine production)}$$

Because of its endogenous production, ease and cost-effectiveness of measurement, and inverse relationship to GFR, creatinine is the most widely used marker of filtration in current clinical practice. However, use of creatinine as a filtration marker is not without limitation.

Limitations of creatinine as a marker of GFR

Tubular secretion of creatinine

As mentioned above, creatinine is neither metabolized nor reabsorbed within the tubules. However, creatinine is secreted; in fact, it is thought that approximately 10 to 40 percent of urinary creatinine is derived as a product of proximal tubular secretion [5]. Therefore, serum creatinine levels may be either falsely elevated or reduced simply based on variations in tubular secretion. This has been shown to be the case in early renal failure where early decline in GFR is countered by increase in creatinine secretion, up to 50 percent more secretion than in the constant state [4]. Resultantly, SCr remains relatively constant until these secretory mechanisms are overwhelmed (typically at SCr of 1.5-2.0) and early decline in GFR ($>60 \text{ mL/min/1.73m}^2$) may go undetected, leading to what some investigators have referred to as a "creatinine-blind range" [6]. Conversely, in other states such as lupus nephritis, a rise in GFR as a result of effective treatment is not reflected in a decline in SCr because of a concomitant decline in tubular secretion [7]. In addition to disease states, tubular secretion is also modified by certain drugs (e.g.

trimethoprim and cimetidine). Therefore, increases in creatinine following initiation of these drugs may not represent a true reduction in GFR.

Compensatory hyperfiltration

With renal injury, the kidney compensates for loss in function by increasing filtration in the remaining normal nephrons [8]. Due to this mechanism, there is a relative preservation of GFR and, thus, a stable SCr value. While technically not a limitation of creatinine as a GFR marker (it correctly measures the adaptive hyperfiltration resulting in a normal GFR), this situation underscores how SCr cannot always be used as a reliable marker of renal injury or progression of disease.

Extrarenal creatinine loss

In normal subjects, extrarenal elimination of creatinine is not detectable. However, in patients with severe chronic kidney disease (GFR <15 mL/min/1.73m²), some studies have demonstrated up to a 68% removal of creatinine via extrarenal routes [9,10]. The mechanism for this removal is thought to be secondary to degradation of creatinine by bacterial flora due to an upregulation of bacterial creatininase activity [11]. Other potential mechanisms of extrarenal creatinine loss, such as via sweat and fecal losses, have been shown to be insignificant [12,13].

Variation in creatinine production

As shown above, the inverse relationship between SCr and GFR assumes a constant rate of creatinine production. However, the production of creatinine varies within people over time, typically secondary to changes in muscle mass or diet [6]. Amongst the population, variations of creatinine based on age, gender, and race make it difficult to establish a reference value that is truly generalizable.

Equations to estimate GFR

Recognition of some of the limitations of SCr as a filtration marker has led to the development of equations to more precisely estimate GFR. These equations incorporate demographic and clinical variables that are known to alter SCr levels, the thought being that adjustment for these variables will lead to more accurate calculations of GFR when compared to gold standard measurements. Cockcroft and Gault devised the first estimation equation in 1976, taking into account the decrease in creatinine production with age and the increase in production with lean body weight [14]:

$$\text{CCr (mL/min)} = \frac{(140 - \text{age}) \times \text{lean body weight (kg)}}{\text{SCr (mg/dl)} \times 72}$$

Subsequently, in 1999, Levey et al derived an equation from data on patients enrolled in the Modification of Diet in Renal Disease (MDRD) study who had baseline GFR measured using iothalamate clearance [15]. This equation takes into account a mix of six demographic and clinical variables and is adjusted for body surface area:

$$\text{GFR (mL/min/1.73m}^2\text{)} = 170 \times \text{SCr}^{-0.999} \times \text{Age}^{-0.176} \times \text{BUN}^{-0.170} \times \text{Alb}^{0.318} \times (0.762 \text{ if female}) \times (1.18 \text{ if black})$$

Derived in populations of predominantly hospitalized male patients and in patients with non-diabetic kidney disease with a mean GFR of approximately $40\text{mL}/\text{min}/1.73\text{m}^2$, respectively, the Cockcroft-Gault and MDRD equations have been rigorously evaluated for their performance in estimating GFR in all-comers in both CKD and healthy populations. Poggio, et al have shown that both equations are reasonably accurate when compared to iothalamate clearance in non-hospitalized patients with CKD with the MDRD equation slightly outperforming the Cockcroft-Gault [16]. However, as displayed in Figure 2, both equations are significantly less accurate in patients with normal GFR ($>60\text{mL}/\text{min}/1.73\text{m}^2$); moreover, the MDRD underestimates the measured GFR by 9 to 29 percent [16].

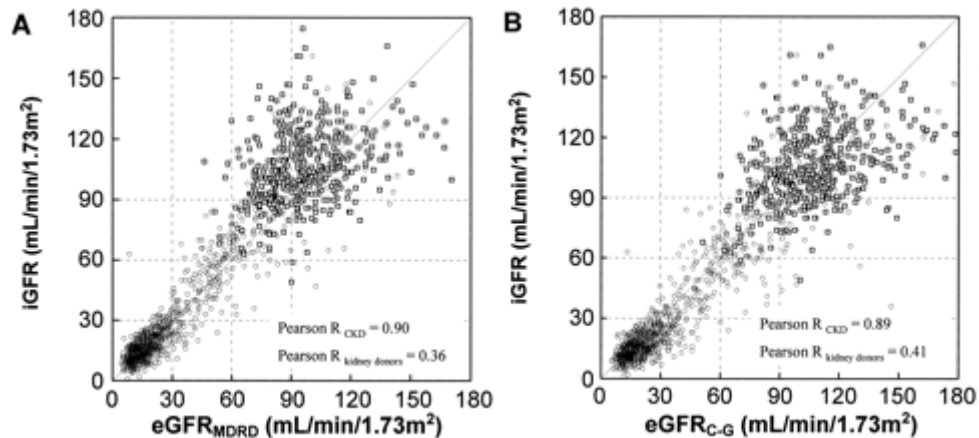


Figure 2 [16]. Association of estimated GFR (eGFR) with measured iothalamate GFR (iGFR) in outpatients with CKD (circles) and potential kidney donors (squares). (A) Association of iGFR with eGFR_{MDRD}. (B) Association of iGFR with eGFR_{C-G}. eGFR is plotted on the horizontal axis, and iGFR is plotted on the vertical axis.

Further criticism of estimation equations centers around the fact that their derivation was from a narrow and homogeneous patient population (mainly Caucasians with non-diabetic kidney disease). Further ongoing studies are currently evaluating whether these equations can be validated in a range of different populations, varying in ethnicity and etiology of kidney disease.

As the limitations of SCr and SCr-based equations were identified, a great deal of interest was given to the search for an alternate endogenous marker of GFR. Multiple small molecules, all of which are freely filtered, have been suggested. Of these, cystatin C has been the most widely studied molecule, both for its intrinsic properties to serve as a marker of GFR and for its performance versus SCr in estimating measured GFR.

Cystatin C

Properties of cystatin C as a filtration marker

Cystatin C (cys-C) is a low-molecular weight protein that is part of the cystatin family, a group of inhibitors of cysteine protease, a lysosomal enzyme involved in the degradation of protein. Of the 12 members of this family, cys-C has been shown to be unique as it is produced by all nucleated human cells [17]. The gene that codes for Cys-C

is a housekeeping gene, meaning that the molecule's production remains relatively stable [18]. There have, however, been two factors shown to alter production of Cys-C. Large doses of glucocorticoids (studied primarily in the transplant population) have been shown to increase production of cys-C [19]. Thyroid function has also been shown to affect rates of cys-C production; cys-C levels are lower in the hypothyroid state and higher in the hyperthyroid state [20]. When compared to SCr, there are several possible advantages of cys-C as an endogenous marker of GFR (Table 1). As stated above, the production of cys-C is constant with the exception of hyper- or hypothyroid states and administration of high-dose glucocorticoids; this is not the case for SCr, which can vary according to muscle mass and diet. Cys-C is also reportedly unaffected by age, sex, or body composition [21]. Moreover, whereas SCr has been shown to be actively secreted in the proximal tubule as discussed above, cys-C is freely filtered, not secreted, and completely catabolized by proximal tubular cells without any reabsorption back into the bloodstream [22].

Table 1 [23]. Comparison of creatinine and cystatin C as filtration markers

	Creatinine	Cystatin C
Molecular properties		
Weight	113 Da	13 000 Da
Structure	Amino acid derivative	Nonglycosylated basic protein
Physiological determinants of serum level		
Handling by the kidney	Filtered, secreted and excreted in the urine	Filtered, reabsorbed and catabolized; not well studied
Generation	Varies, according to muscle mass and dietary protein; lower in elderly, women and white people	Thought to be constant by all nucleated cells; variation in cystatin levels, independent of GFR, may be due to generation
Extrarenal elimination	Increases at reduced GFR	Preliminary evidence that increases at reduced GFR
Use in estimating equations for GFR		
Demographic and clinical variables as surrogates for physiological determinants	Age, sex, race related to muscle mass	Unknown
Accuracy	Accurate for GFR <60 ml/min/1.73 m ²	Unknown
Assay		
Method	Colorimetric or enzymatic	PENIA
Assay precision	Very good Except at low range	Precise throughout range
Clinical laboratory practice	Multiple assays	Single dominant method, not on most autoanalyzers, not standardized
Reference standard	Widely used nonstandard calibration IDMS at NIST	None at present

GFR, glomerular filtration rate; PENIA, particle-enhanced nephelometric immunoassay; IDMS, isotope dilution-gas chromatography; NIST, National Institute for Standards and Technology. Reproduced with permission from [6].

Performance of cys-C versus SCr in patients with native kidney disease

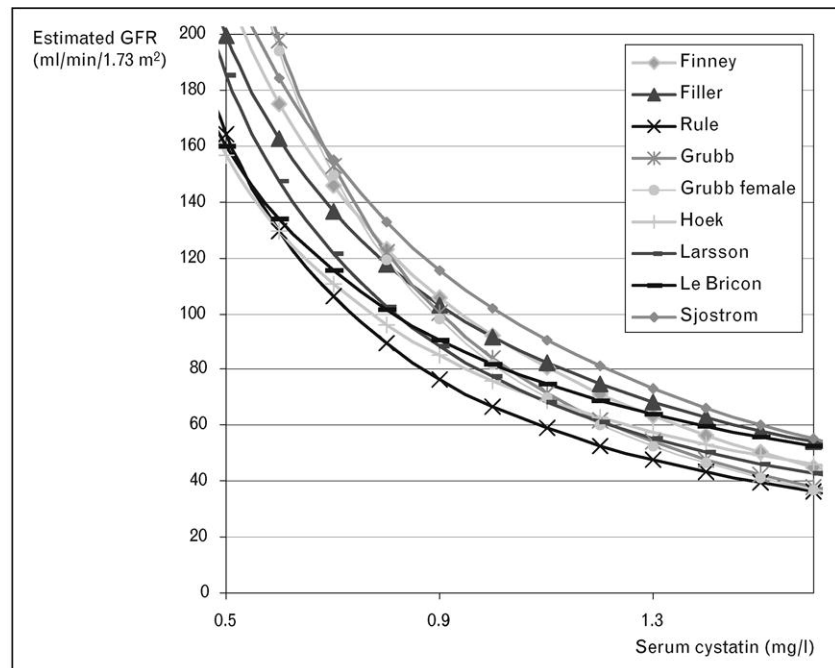
Since 1985, 29 studies have been performed to compare cys-C and SCr to reference GFR (measured predominantly by inulin clearance or radioactive isotope scan) in patients with native kidney disease. Of these studies, 17 showed superiority of cys-C over SCr while the remaining 12 studies showed no statistical significance; it should be noted that no study demonstrated superiority of SCr [23]. However, many question the power of these results given methodological flaws in these studies. The sample size in all studies was relatively small, less than 100 in over half of the studies and exceeding 300 in only one study. Moreover, across all studies, authors used different cutoffs to define decreased GFR (<60 versus <90 mL/min/1.73m²); use of a higher GFR cutoff could skew results toward superiority of cys-C due to the aforementioned "creatinine-blind range." Lastly, individual studies used different assay methods for both cys-C and SCr. This is a significant flaw given that it has been demonstrated that the correlation between GFR and

the reciprocal of cys-C is significantly stronger when measured with one particular assay [24]; the importance of standardized assays has also been shown for use with SCr [25].

As with SCr, several groups have recently developed equations to calculate estimated GFR (eGFR) from serum cys-C levels; unlike SCr-based equations that are adjusting for age and anthropometric data, these equations were calculated solely on linear regression models between the reciprocal of cys-C and a gold standard measurement of GFR [26]. However, the eGFR calculated from these cys-C based equations varies widely from one equation to another as shown in Figure 3 below.

Figure 3 [27]. Estimated glomerular filtration rate (ml/min/1.73 m²) calculated from the published cys-C-based GFR estimating equations for levels of serum cys-C in the range from 0.5 to 1.6 mg/L

At levels of cystatin C of 0.5, 1 and 1.5 mg/l, maximal differences in glomerular filtration rate estimates from the various equations are 111, 35 and 20 ml/min/1.73 m², respectively. GFR, glomerular filtration rate.



Multiple studies have compared the performance of these equations against the most commonly used SCr-based equations, namely the MDRD and Cockcroft-Gault equations. In all but one study, the authors concluded superiority of cys-C based equations. Nonetheless, there was disagreement among studies about which equation performed best; an equal number of authors concluded superiority of each of the following equations: the Grubb, Filler, and Hoek. Overall, performance seemed to be skewed in favor of the cys-C equations because study populations were more similar to those in which these equations were derived as opposed to the population used to derive the MDRD and Cockcroft-Gault formulas [23]. The lack in uniform superiority amongst cys-C based equations, possibly due to the population in which each equation was derived and validated, makes it impossible to advocate their use as a replacement for current SCr-based equations.

Performance of cys-C versus SCr in transplant patients

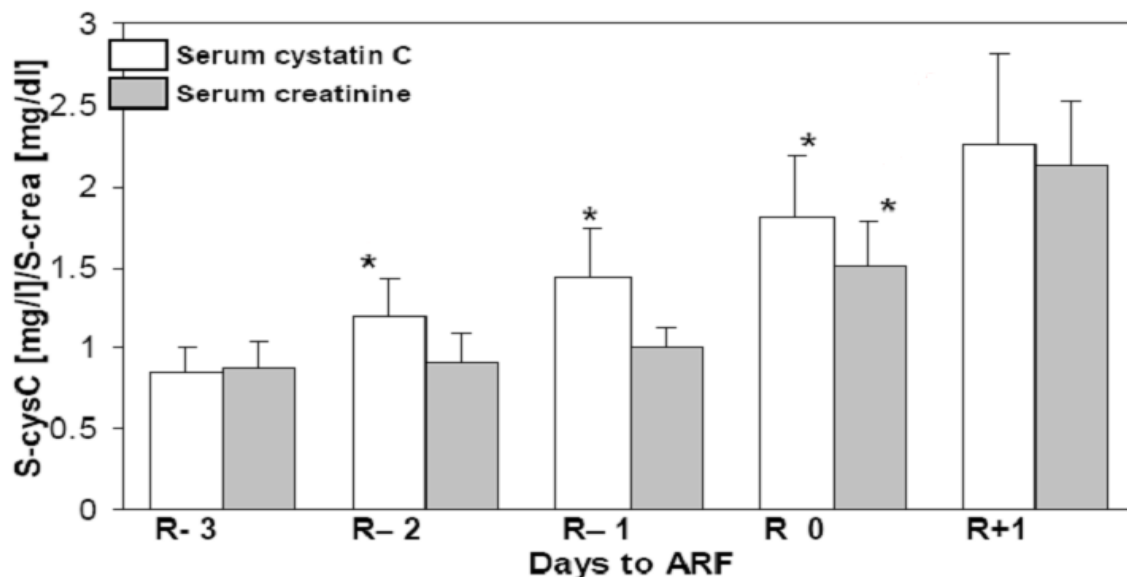
Given the need for an ideal filtration marker to accurately estimate GFR in transplanted kidneys in addition to native kidneys, several studies have compared the performance of cys-C and SCr in transplant patients. Between 1998 and 2004, a total of 14 studies were performed using the following different methods as gold standard measurements of GFR: 5 studies used creatinine clearance with 24-hr urine collection, 4 studies used radioactive uptake scan, 2 studies used inulin, 1 study used iohexol and 2 further studies employed the Cockcroft-Gault equation as the reference GFR. Of these studies, 10 demonstrated superiority of cys-C over SCr. Three studies showed no difference while one study actually reported superiority of SCr. Superiority did not seem to be related to method used for reference GFR; of the four studies indicating equality or superiority of SCr, each used a different measure to determine reference GFR [23].

Use of cys-C as a marker of early renal dysfunction

In addition to the study of cys-C in large patient populations, namely those with native kidney disease and transplant recipients, there has been a considerable body of research dedicated to studying cys-C as a marker in specific clinical scenarios. With the recognition that one of the greatest limitations of SCr is its accuracy at normal or high GFR, investigators have set out to study whether cys-C may perform better at estimating GFR in this range. Two particular clinical scenarios have been studied to answer this question, one in the inpatient and one in the outpatient setting: detection of early decline in GFR in acute renal failure (inpatient) and in diabetics (outpatient).

In a study performed by Herget-Rosenthal, et al, 85 ICU patients determined to be at high risk for acute renal failure (based on age >70, development of hemorrhagic, septic, or cardiogenic shock, diabetes, etc.) had serum creatinine and cystatin C levels measured daily. Of the 85 patients identified, 44 developed acute renal failure (defined as increase of baseline serum marker of $\geq 50\%$) and the other 41 patients served as controls. As shown in Figure 4, the increase in cys-C significantly preceded that of SCr [28].

Figure 4 [28]. Early detection of acute renal failure by measurement of cystatin C versus serum creatinine

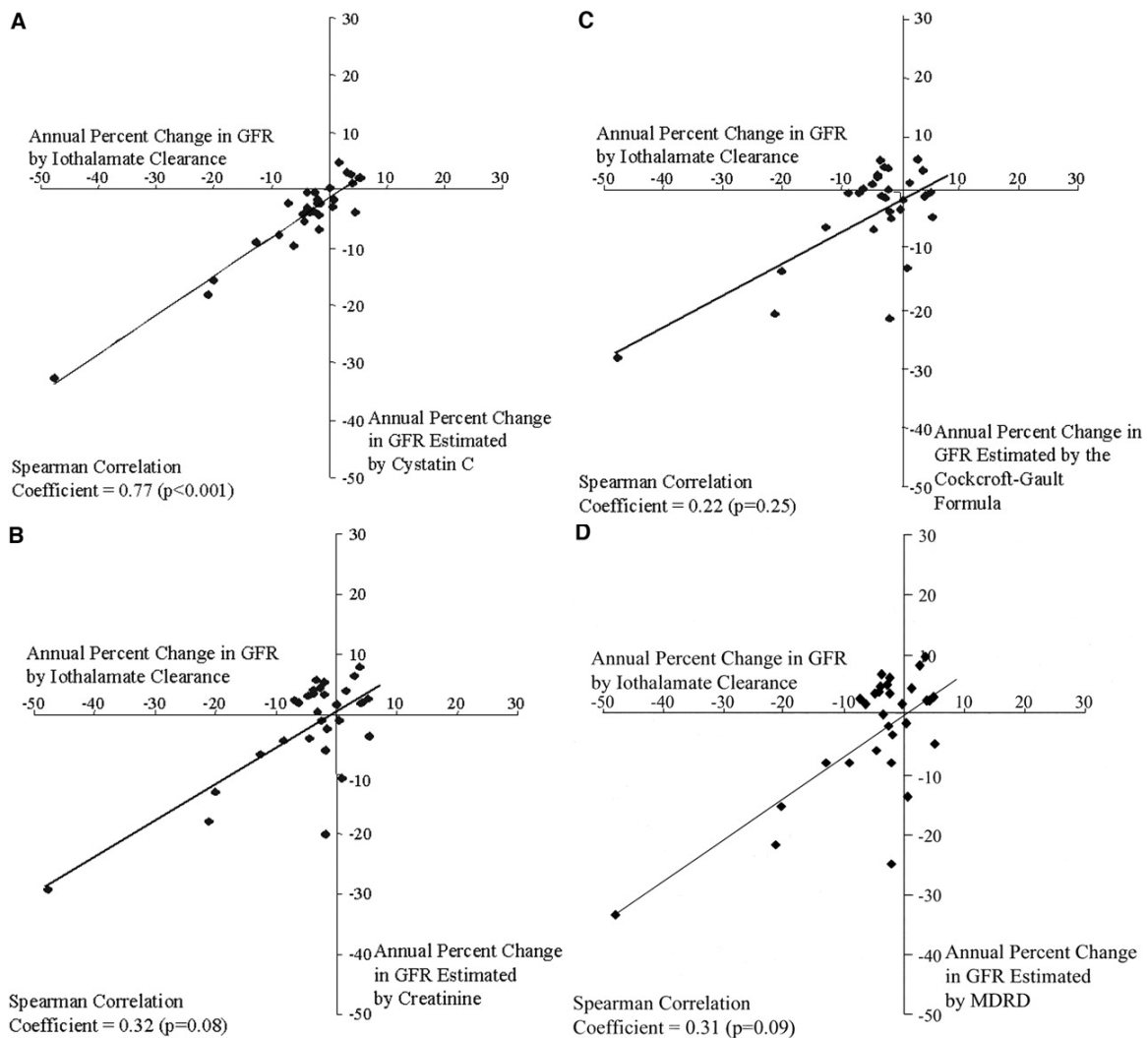


*Clinically significant ARF as determined by increase of baseline serum marker of $\geq 50\%$

Specifically, serum cys-C increased by $\geq 50\%$ of its baseline value 1.5 \pm 0.6 days before the same increase was observed in SCr. Thus, measurement of cys-C in patients at risk for developing acute renal failure has the potential to play a clinically important role in inpatient medicine. With the ability to better detect minor, early changes in GFR, preventative steps may be put into place sooner to limit progression of acute renal failure.

Whereas the study by Herget-Rosenthal demonstrated the superiority of cys-C to detect early changes in renal function in an inpatient setting, Perkins et al set out to show similar results in an outpatient setting. They took a population of diabetics with a baseline GFR of over 120 ml/min/1.73m² as defined by iothalamate clearance and followed them for 4 years, drawing levels of SCr and cys-C and repeating measurements of iothalamate clearance at yearly intervals. Each study participant's trend in renal function, defined as annual percent change in iothalamate clearance, was determined and then compared to trends in cys-C and three SCr-based estimates of GFR (inverse of SCr, MDRD, and Cockcroft-Gault) [29]. The results of this comparison are best displayed below in Figure 5.

Figure 5 [29]. Correlation between estimates of the annual percentage change in renal function as determined from serial measurements of standardized iothalamate clearance and four indirect measures



As shown above, all subjects with declining GFR measured by iothalamate clearance also had a negative trend in eGFR as determined by levels of cys-C; these results were determined to be clinically significant ($p < 0.001$). Conversely, the three SCr-based methods of estimating GFR correlated poorly to trends in iothalamate clearance, more often overestimating true filtration rate [29]. Given that diabetic nephropathy is the single most frequent cause of end-stage renal disease in the world and that decline in renal function is associated with an increased incidence of cardiovascular morbidity and mortality, the ability to use a more sensitive marker for early renal impairment in diabetics would be extremely valuable.

Use of cys-C in chronic dialysis patients

Unfortunately, there is no data showing the usefulness of cys-C as a marker of the adequacy of dialysis therapy on an intersession basis. However, cys-C does have a role in the dialysis population. In chronic dialysis patients, both on hemo- and peritoneal dialysis, it has long been shown that residual glomerular filtration rate (rGFR) has a significant effect on morbidity, mortality, and quality of life [30]. The MDRD equation, which has been shown to be relatively accurate at low GFR (figure 2), is most widely used to estimate rGFR in dialysis patients given that 24-hour urine collection is a cumbersome task. Hoek, et al set out to study how a cys-C based formula would perform against the MDRD equation in measuring rGFR. Their findings were that the cys-C formula showed both better accuracy and precision in calculating rGFR than the MDRD formula [31]. Although a promising result for yet another use of cys-C, Hoek's study has one inherent flaw: the cys-C equation used for comparison against the MDRD was derived in the patient population he was studying. It is unclear whether this equation could be generalized to the rest of the dialysis community with similar results.

Conclusions

In order to adequately diagnose and treat kidney diseases, it is imperative to obtain accurate measurements of GFR. Current SCr-based measures of eGFR have multiple limitations that have prompted the search for alternate filtration markers. The observation that cys-C serum concentration is determined mainly by renal function and is reportedly independent of height, age, gender, and body composition has made it a widely studied surrogate marker of GFR. As shown above, cys-C based measures of estimating GFR have trended toward superiority over SCr based methods in patients with both native kidney disease and status-post transplant. Moreover, cys-C has been shown to be especially useful in patient populations with normal or mildly reduced GFR (≥ 60 ml/min/1.73m²) to detect early decline in function.

Nevertheless, doubts remain surrounding the ability of cys-C to replace SCr as the most widely used marker of GFR. The majority of studies showing the superiority of cys-C to SCr recruited only a small number of patients and many authors question whether the results of these studies can be generalized to the rest of the population. Of more concern, there are several reports that suggest factors other than GFR may affect cys-C concentration. One study showed that age, male gender, greater weight, height, cigarette smoking, and higher C-reactive protein levels increase the production and/or catabolism of cys-C [32]; another study has also demonstrated that lean mass affects cys-

C concentrations [33]. In addition to demographic variables that alter cys-C levels, some disease states or drugs have been shown to do the same. As mentioned above, states of thyroid dysfunction as well as administration of high dose glucocorticoids affect cys-C production; one isolated report has also suggested that HIV may raise cys-C levels [34] although this finding has not been validated. Finally, some question whether there is an extrarenal elimination of cys-C at reduced GFR – at least one study has drawn this conclusion [35]. Although cys-C shows promise as a potential replacement for SCr to estimate GFR, significantly more work will need to be done to address some of its potential limitations before its widespread use.

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