Cystatin C as a biomarker for estimating glomerular filtration rate

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Purpose of review
Glomerular filtration rate (GFR) is rarely measured in clinical practice because of the complexity of the measurement. As such, kidney function is typically estimated using validated study equations, which use readily available data including age, sex, race, and serum creatinine as filtration marker. Contemporary research suggests that cystatin C may be an improved alternative to creatinine for inclusion in GFR estimating equations. The purpose of this article is to evaluate the benefits and limitations of using cystatin C as a biomarker of filtration.

Recent findings
Cystatin C has fewer non-GFR determinants, when compared with serum creatinine. Use of serum cystatin C avoids the limitations related to both diet and muscle mass that affect serum creatinine. Cystatin C may be more accurate than serum creatinine in estimating GFR, and is more strongly associated with all-cause mortality and cardiovascular events.

Summary
Cystatin C has some advantages over serum creatinine in estimating GFR. The use of cystatin C as a confirmatory biomarker in deciding medication dosages or as a confirmatory test in patients with an uncertain diagnosis of chronic kidney disease may be beneficial.

Keywords
chronic kidney disease, creatinine, cystatin C, glomerular filtration rate, risk prediction

INTRODUCTION

Estimating the glomerular filtration rate (GFR) is critical in the diagnosis, staging, and prognostication of chronic kidney disease (CKD) [1]. In addition, low GFR is also a potent predictor of cardiovascular disease, frailty, increased risk of hospitalizations, and early mortality [2,3]. The gold standard in measured GFR (mGFR) utilizes the urinary plasma clearance of exogenous filtration markers such as inulin or iothalamate; however, this is not routinely performed because of the complexity of measurement, and is usually only advised as a confirmatory test [4]. More often, GFR is estimated in a laboratory setting using readily available information, including age, sex, race, and measurement of serum creatinine as the biomarker of filtration [5,6]. However, serum creatinine is an imperfect biomarker, as it is known to be affected by diet, muscle mass, certain medications, rapidly changing kidney function, and active secretion by the kidney [1,7]. Cystatin C has been suggested as a potential alternative to serum creatinine, as it potentially has fewer non-GFR determinants. Recent findings suggest that GFR may be more effectively estimated using cystatin C as a supplement or replacement for serum creatinine [8▪▪].

DETERMINANTS OF CYSTATIN C

Cystatin C, a nonglycosylated protein, is a biomarker of glomerular filtration. Cystatin C is a small molecule, 13 kDa in size [9], that is filtered from the blood through the glomerulus and catabolized, but not secreted, by the proximal tubular cells [10,11]. Cystatin C is produced by all human nucleated cells and has been demonstrated to be a gene of the housekeeping type, with levels of serum cystatin C

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Curr Opin Nephrol Hypertens 2015, 24:295–300
DOI:10.1097/MNH.0000000000000115

KEY POINTS

- eGFR is essential in the classification, staging, and prognostication of CKD, and is typically calculated using measured serum creatinine.
- Newer research suggests that cystatin C, a protein filtered through the kidneys, may serve as an improved marker of renal function in comparison with serum creatinine.
- Equations for eGFR incorporating cystatin C have shown to offer comparable or improved diagnostic accuracy to existing serum creatinine-based models; moreover, they have reduced the biases introduced by differences in patient lean body mass, age, sex, and race.
- Serum cystatin C may be useful as a confirmatory test for patients requiring adjustment of medication doses, or patients with CKD who are on the threshold of suggested guidelines for more costly nephrology interventions.

having shown no correlation to any pathophysiological state other than GFR [12]. The plasma level of serum cystatin C can be expressed as its level of generation from cells and diet and its subsequent elimination through the gut, liver, and kidneys (Fig. 1) [4].

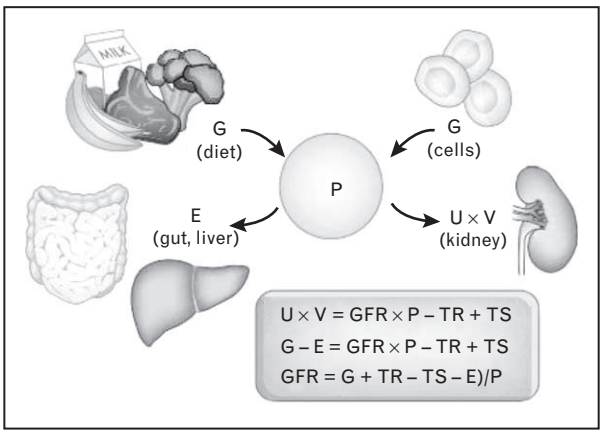

FIGURE 1. Determinations of estimated glomerular filtration rate (eGFR) [4]. Determinants of the serum level of endogenous filtration markers. The plasma level (p) of an endogenous filtration marker is determined by its generation (g) from cells and diet, extrarenal elimination (e) by gut and liver, and urinary excretion (UV) by the kidney. Urinary excretion is the sum of filtered load (GFR × P), tubular secretion (TS), and reabsorption (TR). In the steady state, urinary excretion equals generation and extrarenal elimination. By substitution and rearrangement, GFR can be expressed as the ratio of the non-GFR determinants (G, TS, TR, and E) to the plasma level.

Reductions in estimated GFR (eGFR) have been associated with reductions in concentration of serum cystatin C [11]. The concentration of serum cystatin C has also been shown to be unaltered in certain inflammatory conditions or other disorders of metabolism [13]. Furthermore, it has been suggested that because of the independence of cystatin C from many factors that affect serum creatinine, including age, sex, race, and muscle mass, an equation based on cystatin C may be more useful in detecting kidney disease in children, the elderly, and individuals with conditions affecting muscle composition [12]. In accordance with many of these findings, it is therefore hypothesized that cystatin C may be used as a novel biomarker alongside serum creatinine, or as a replacement for serum creatinine, to better identify kidney disease in the general population.

MEASUREMENT OF CYSTATIN C

Cystatin C can be ascertained using automated latex particle-enhanced turbidimetry assays, or through particle-enhanced immunonephelometry (PENIA) [14]. Research into the two methods has demonstrated that cystatin C ascertained with the PENIA method has a stronger correlation with GFR [15,16]. In addition, the PENIA method has been reported to be slightly more precise than particle-enhanced turbidimetry assay [17]. The importance of assuring immunoassay standardization as the measurement of serum cystatin C moves forward must also be considered [18].

ESTIMATION OF ESTIMATED GLOMERULAR FILTRATION RATE FROM CYSTATIN C – BENEFITS AND LIMITATIONS

eGFR is used in the diagnosis and clinical management of patients with CKD, as GFR is rarely measured [19]. eGFR is calculated using equations incorporating factors such as age, sex, race, and the measurement of serum creatinine, of which equations include the Modification of Diet in Renal Disease Study equation, or the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [5,6]. The addition of serum cystatin C in newer equations as a supplement to serum creatinine, or using cystatin C as a replacement for serum creatinine, may provide an augmented ability to predict the risk of adverse outcomes, including death, kidney failure, and cardiovascular events [8▪▪]. In fact, a previously published meta-analysis comparing the receiver operating characteristic curves of using both cystatin C and creatinine as a

reference test for GFR by measuring area under the curve, found that the area was 0.926 [95% confidence interval (CI) 0.826–0.960] for cystatin C and 0.837 (0.796–0.878) for serum creatinine ($P < 0.001$), demonstrating a marked improvement in the ability to measure kidney function [15].

There have been demonstrable benefits to using cystatin C as a biomarker of filtration in eGFR estimating equations. eGFR using serum creatinine has been shown to have limitations in individuals with reduced levels of muscle mass [8▪▪]. In contrast, cystatin C has been shown to have no correlation with lean body mass ($R^2 = 0.01$, $P = 0.803$), whereas serum creatinine has shown a significant correlation with lean body mass ($R^2 = 0.54$, $P = 0.001$), suggesting a potential for improved assessment of renal function in individuals with varying levels of lean body mass using cystatin C [20]. In addition, serum cystatin C has been shown to be less affected by both age and race [21]. Similarly to lean body mass, serum creatinine is also influenced by another non-GFR determinant, meat (protein) intake, which in turn may be confounding outcomes related to cardiovascular events or all-cause mortality [22]. Cystatin C may also be beneficial for the determination of eGFR in individuals with only mild reductions in GFR (between 60 and 90 ml/min/1.73 m^2) whereas changes in serum creatinine are not observed and GFR estimating equations are more unreliable as a result [11,23].

Cystatin C has further benefits with respect to interindividual variability that improve its diagnostic accuracy in comparison with serum creatinine, with 25% of biological variability being explained by interindividual variability with cystatin C and 93% for creatinine (upper limit of the population reference interval for cystatin C being three to four standard deviations from the mean, whereas creatinine can reach up to 13 standard deviations from a healthy individual), demonstrating a reduced effect of diet, immune status, and genetic factors between individuals when using cystatin C [24,25].

Current research has considered adjusting models with serum cystatin C to modify the existing equations used to estimate GFR with serum creatinine, or recreating equations using serum cystatin C alone. Models incorporating cystatin C alone in these equations were shown to be not significantly altered by the addition of race as a variable, whereas the CKD-EPI equation using serum creatinine is adjusted for black race (multiplicative factor of 1.159), and the CKD-EPI equation using both serum creatinine and cystatin C is adjusted for black race (factor of 1.08) [26]. These equations are summarized in Table 1.

Table 1. Equations to estimate glomerular filtration rate

Equation	Expression	Reference
MDRD creatinine	$GFR = 175 \times Scr^{-1.154} \times age^{-0.203} \times [1.212 \text{ if black}] \times [0.742 \text{ if female}]$	Levey *et al.* [6]
CKD-EPI creatinine (female)	$GFR = 144 \times \min(Scr/0.7,1)^{-0.329} \times \max(Scr/0.7,1)^{-1.209} \times 0.993^{age} \times [1.159 \text{ if black}]$	Levey *et al.* [6]
CKD-EPI creatinine (male)	$GFR = 141 \times \min(Scr/0.9,1)^{-0.411} \times \max(Scr/0.9,1)^{-1.209} \times 0.993^{age} \times [1.159 \text{ if black}]$	Levey *et al.* [6]
CKD-EPI cystatin C ($Scys \leq 0.8$)	$GFR = 133 \times (Scys/0.8)^{-0.499} \times 0.996^{age} \times [0.932 \text{ if female}]$	Inker *et al.* [26]
CKD-EPI cystatin C ($Scys > 0.8$)	$GFR = 133 \times (Scys/0.8)^{-1.328} \times 0.996^{age} \times [0.932 \text{ if female}]$	Inker *et al.* [26]
CKD-EPI creatinine–cystatin C (female, $Scr \leq 0.7$ and $Scys \leq 0.8$)	$GFR = 130 \times (Scr/0.7)^{-0.248} \times (Scys/0.8)^{-0.375} \times 0.995^{age} \times [1.08 \text{ if black}]$	Inker *et al.* [26]
CKD-EPI creatinine–cystatin C (female, $Scr \leq 0.7$ and $Scys > 0.8$)	$GFR = 130 \times (Scr/0.7)^{-0.248} \times (Scys/0.8)^{-0.711} \times 0.995^{age} \times [1.08 \text{ if black}]$	Inker *et al.* [26]
CKD-EPI creatinine–cystatin C (female, $Scr > 0.7$ and $Scys \leq 0.8$)	$GFR = 130 \times (Scr/0.7)^{-0.601} \times (Scys/0.8)^{-0.375} \times 0.995^{age} \times [1.08 \text{ if black}]$	Inker *et al.* [26]
CKD-EPI creatinine–cystatin C (female, $Scr > 0.7$ and $Scys > 0.8$)	$GFR = 130 \times (Scr/0.7)^{-0.601} \times (Scys/0.8)^{-0.711} \times 0.995^{age} \times [1.08 \text{ if black}]$	Inker *et al.* [26]
CKD-EPI creatinine–cystatin C (male, $Scr \leq 0.9$ and $Scys \leq 0.8$)	$GFR = 135 \times (Scr/0.9)^{-0.207} \times (Scys/0.8)^{-0.375} \times 0.995^{age} \times [1.08 \text{ if black}]$	Inker *et al.* [26]
CKD-EPI creatinine–cystatin C (male, $Scr \leq 0.9$ and $Scys > 0.8$)	$GFR = 135 \times (Scr/0.9)^{-0.207} \times (Scys/0.8)^{-0.711} \times 0.995^{age} \times [1.08 \text{ if black}]$	Inker *et al.* [26]
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CKD-EPI, chronic kidney disease epidemiology collaboration; MDRD, Modification of Diet in Renal Disease; Scr, serum creatinine (mg/dl); Scys, serum cystatin C (mg/l).

The biases provided by age, sex, and race that are adjusted in these equations have been shown to be small determinants of GFR in other research with respect to using cystatin C as a predictive biomarker, with equations using serum cystatin C alone in some instances providing estimates of GFR that are nearly as accurate as those provided by equations using serum creatinine that have been adjusted for these three covariates [27].

The use of cystatin C as a biomarker in eGFR calculations also has several limitations. Test-retest reliability of eGFR calculated using measured serum creatinine has been found to be better than the test-retest reliability of eGFR as calculated using cystatin C ($r = 0.84$ and 0.76, respectively, and $r = 0.82$ for both serum creatinine and cystatin C used in combination) [28]. There are also cost implications of using serum cystatin C assays in supplement or as a replacement to serum creatinine assays [29]. As it stands, the cost of a serum cystatin C assay is roughly 12 times as expensive as a serum creatinine assay (US $3.00 versus $0.25, respectively) [12]. This is potentially a manifestation of the use of cystatin C not being routinely applied in clinical practice [21]. Even if the costs of these assays came down, as expected with increased scale, these increased costs would have tremendous impact on healthcare expenditures as tests of kidney function are so widely utilized by care practitioners.

Ideally, because of increased costs, the use of serum cystatin C in eGFR estimating equations could be applied as a confirmatory test. In particular, this would be useful clinically for patients that require additional certainty in measuring kidney status. Examples of uses include the measurement of eGFR at the time of adjustment of medication dosages [9], or for deciding the course of treatment of patients on the threshold of suggested guidelines for more costly interventional nephrology care. The majority of pharmacokinetic and pharmacodynamic drug studies, however, have been done using serum creatinine and its estimating equations, so a transition to cystatin C-based equations would have significant switching costs and require change management initiatives to make clinicians comfortable in their routine application for these purposes.

CYSTATIN C AND CREATININE IN DETERMINING THE RISK OF ADVERSE OUTCOMES

Cystatin C alone, or in combination with creatinine, has been shown to strengthen the association between the risks of death and kidney failure. The extent of improvement in risk prediction for individuals who are reclassified using cystatin C in comparison with creatinine can be expressed using net reclassification improvement (NRI) [30], a measure that has been extensively used to evaluate the predictive ability of newer biomarkers [31]. One study found the NRIs for cystatin C compared with creatinine to be 0.23 (95% CI 0.18–0.28) with respect to death, and 0.10 (0.00–0.21) for end-stage renal disease, and 0.16 (0.12–0.21) for deaths from cardiovascular causes, therefore demonstrating an improvement in prediction for all outcomes [8▪▪].

In addition to this, other research has shown that independent of GFR, serum cystatin C is a stronger predictor of both all-cause mortality and cardiovascular disease than serum creatinine; however, it was also found to be a somewhat weaker predictor for the outcome of kidney failure [22]. This improvement is further illustrated by the association between both eGFR measured by creatinine and eGFR measured with cystatin C, with the latter being significantly associated with cardiovascular mortality in patients with late-stage CKD ($eGFR < 30\,ml/min/1.73\,m^2$) when adjusting for other comorbid conditions [32].

When considering the use of cystatin C in addition to creatinine for the determination of the severity of CKD there has been a demonstrated improvement in the classification of patients with both mild and moderate CKD. Patients with an eGFR measured based on creatinine in the range of $45–74\,ml/min/1.73\,m^2$ were reclassified as having an mGFR as either greater or less than $60\,ml/min/1.73\,m^2$, with an NRI of 19.4% ($P < 0.001$), and, similarly, those with an eGFR from 45 to $59\,ml/min/1.73\,m^2$ as measured by equations with only serum creatinine had 16.9% of individuals reclassified as having an mGFR of $60\,ml/min/1.73\,m^2$ or higher [26]. An example of this improvement in detecting patients with more severe CKD and higher risk of all-cause mortality was demonstrated in a study that found patients whose CKD was defined by albumin-to-creatinine ratio (ACR) to have a 2.3-fold higher rate of death compared with patients with no CKD, whereas patients who had CKD defined by cystatin C had a 4.2-fold higher rate of death than the no CKD population, and using ACR and cystatin C in combination identified patients with a 6.6-fold higher rate of death, in contrast to CKD identified by serum creatinine in which mortality risk was similar to those with no CKD by either ACR, cystatin C, or creatinine [21]. The magnitude of this comparison is illustrated in Fig. 2. The same study had similar findings for patients developing kidney failure, with a four-fold risk in patients classified as having CKD with eGFR as measured with cystatin C in addition to creatinine and albuminuria [21].

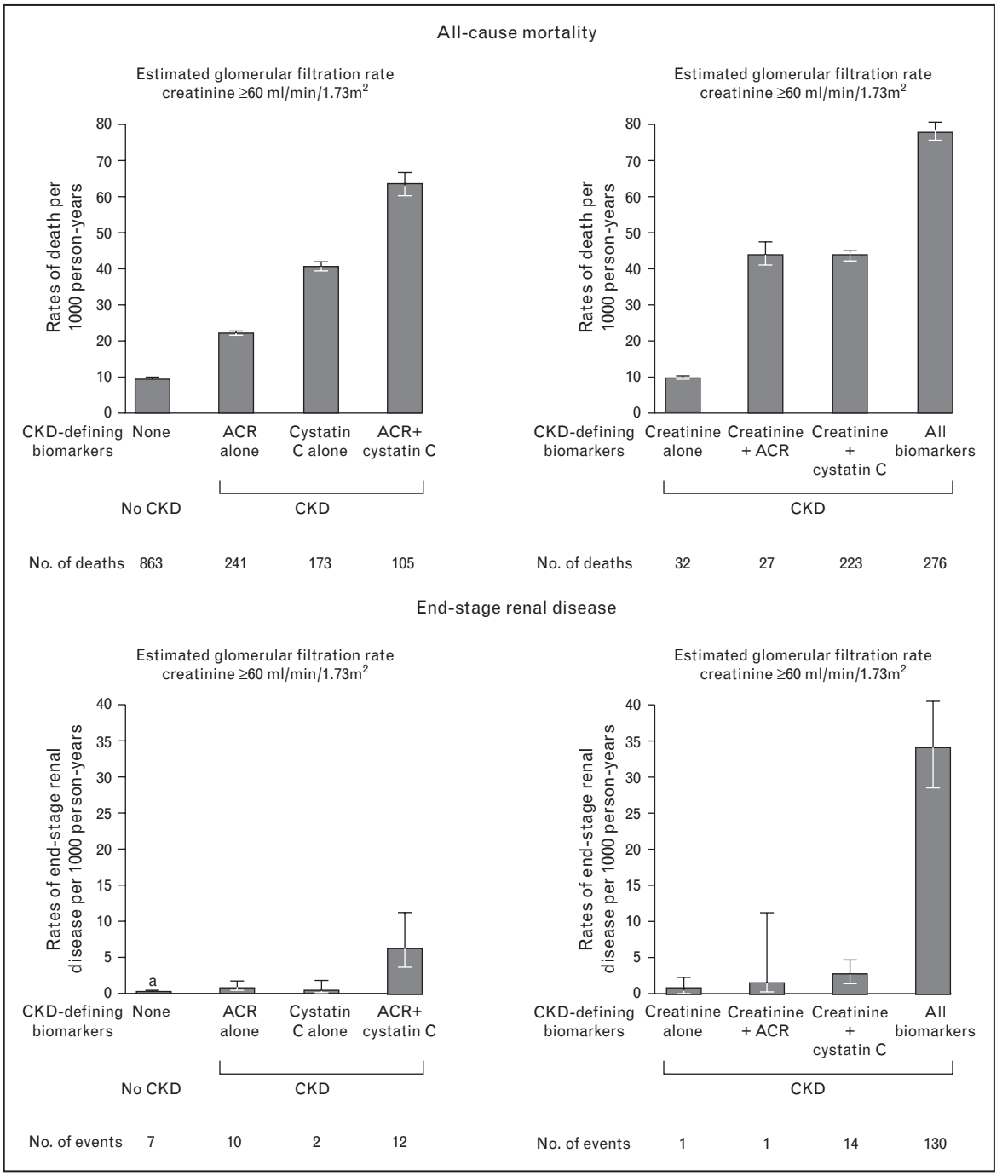

FIGURE 2. Association of chronic kidney disease (CKD) definitions with all-cause mortality and end-stage renal disease [21]. Error bars indicate 95% confidence intervals (CI). ACR, albumin-to-creatinine ratio. [a]No CKD from all biomarker measures: 0.08 (95% CI 0.04–0.17) per 1000 person-years.

CONCLUSION

Serum creatinine has been the longstanding biomarker of choice in estimating kidney function for the diagnosis, staging, prognosis, and treatment of patients with possible or progressing CKD. Cystatin C is a well investigated biomarker with clear advantages over serum creatinine in patients with extremes in muscle mass, weight, age, and other areas where estimating equations using creatinine have well documented limitations [1,7]. From a public health and clinical epidemiology perspective, cystatin C is an attractive supplement to serum creatinine in estimating risk of adverse outcomes, and the diagnosis of mild-to-moderate impairment in GFR.

The downside of cystatin C at present is the substantially higher costs of the assay and the massive change management initiative that it would take to educate clinicians, patients, and other stakeholders in the healthcare system of the proper use of this biomarker. It may be that in the general clinical setting for the majority of adult patients that the costs and effort required to make a wholesale switch to cystatin C currently does not represent good value for money. Focus should remain on the use of this biomarker in populations in which serum creatinine is clearly not accurate in estimating GFR or the risk of adverse outcomes.

Acknowledgements

None.

Financial support and sponsorship

T.W.F. is supported by a studentship from the Manitoba Health Research Council (MHRC), now Research Manitoba. N.T. is supported by the KRESCENT New Investigator Award and the MHRC Establishment Award. The KRESCENT New investigator award is a joint initiative of the Kidney Foundation of Canada, Canadian Institute of Health Research and the Canadian Society of Nephrology.

Conflicts of interest

There are no conflicts of interest.

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