

Cystatin C Is a More Reliable Biomarker for Determining eGFR to Support Drug Development Studies

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Abstract

Glomerular filtration rate (GFR) is routinely used as a surrogate endpoint for the development of investigational drugs in clinical trials. GFR and staging of chronic kidney disease are typically assessed by measuring the concentration of endogenous serum biomarkers such as albumin and creatinine. However, creatinine is subject to high biological variability, and levels of creatinine do not rise until nearly 50% of kidney function is damaged, leading to inaccurate chronic kidney disease staging and false negatives. A newer biomarker for GFR, cystatin C, has been shown to be subject to less biological interference and more sensitive to early declines in kidney function. Cystatin C has also been shown to outperform creatinine as an indicator of true GFR and to add information about the occurrence of acute kidney injury. Comparison studies of cystatin C and creatinine continue to demonstrate its increased accuracy and sensitivity for changes in true GFR. While challenges remain for use of cystatin C, international agencies and working groups continue to validate cystatin C as a biomarker and accompanying GFR estimating equations for diagnostic and drug development use. In this review, we summarize these comparison studies, regulatory and industry guidelines, and clinical trial case studies for use of cystatin C in drug development.

Keywords

creatinine, chronic kidney disease, glomerular filtration rate, renal impairment

Direct measures of glomerular filtration rate (GFR) can be determined with chromium 51–labeled ethylenediaminetetraacetate, technetium Tc 99m diethylenetriaminepentaacetic acid, inulin, or iohexol substrates, but these assays are expensive, cumbersome, and not readily available across medical and clinical sites.^{1–3} For more than 50 years, endogenous creatinine concentration, a biomarker of kidney function, has been used as an estimate of GFR (eGFR) in medical and clinical research settings because of its ease of measurement. Creatinine is generated at a constant rate by muscle and is completely cleared by renal excretion. Urine creatinine clearance is rarely used as a marker for GFR in clinical research because approximately 10%–20% of excreted creatinine is secreted by the proximal tubules, which can overestimate GFR.⁴ Elevated serum creatinine concentrations are typically observed in patients with kidney disease and can be a marker of renal impairment when an investigational drug affects renal excretion.

However, creatinine has limitations as a renal biomarker and is subject to high analytic variability. The primary analytical method of measurement combines creatinine with picric acid to produce a colorimetric compound. This reaction is affected by interference from endogenous and exogenous substances common in many patient populations, such as glucose, bilirubin, plasma proteins, and antibiotics.^{5,6} Furthermore, serum

creatinine is affected by the large biological variability associated with sex, age, ethnicity, and muscle mass. As shown in Table 1, several eGFR formulas to correct for these confounding factors in serum creatinine measurement, such as Cockcroft-Gault, Modification of Diet in Renal Disease (MDRD), and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), have been developed.^{7,8}

Yet limitations surrounding sensitivity and specificity still exist.⁴ For example, while the eGFR MDRD equation accounts for many of these intrinsic factors, the magnitude of change in muscle mass can vary among special populations and this equation does not account for other physiological changes such as illness, inflammation, sarcopenia, and deconditioning that affect muscle mass.⁹ Therefore, researchers and regulatory bodies continue to explore and validate novel renal biomarkers. Here, we review recent studies demonstrating the use of an alternative biomarker, cystatin C, as

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Table 1. Accuracy and Precision of GFR Estimating Equations Utilizing Creatinine and Cystatin C

Equation	Biomarker Used	Bias, mL/min/1.73 m ²	Precision at 30% eGFR	Accuracy at 30% eGFR
CKD-EPI	Creatinine	3.7	87.2	—
CKD-EPI	Cystatin C	3.4	85.9	—
CKD-EPI	Creatinine and cystatin C	3.5	91.8	—
MDRD	Creatinine	5.5	80.6	—
Cockcroft-Gault	Creatinine	11.4	69	—
Schwartz (pediatric populations)	Creatinine	6.1	16.9	88.1
Combined Schwartz (pediatric populations)	Creatinine and cystatin C	6	16	96.5

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease. Data obtained from Refs. 7,21,32

a measure of eGFR. While the benefits of cystatin C have been discussed previously in a medical setting, this review focuses on the application of cystatin C in drug development.¹⁰⁻¹²

Physiology of Cystatin C for eGFR Measurements

Cystatin C is a small protease inhibitor produced in all nucleated cells and is an alternative filtration biomarker less influenced than creatinine by muscle mass and biological factors.^{11,13} Investigation of the structure and promoter of cystatin C has demonstrated that the gene is a housekeeping gene in several nucleated tissue types, and large patient cohort studies have failed to correlate the serum level to any nonrenal pathophysiological state.¹² As it is stably secreted from all human nucleated cells, cystatin C is not subject to changes in diet, muscle mass, or age that drastically change serum creatinine levels.¹⁴⁻¹⁶ Similar to creatinine, the 13-kilodalton protein is freely filtered in the glomerulus. However, unlike creatinine, 99% of filtered cystatin C is reabsorbed and degraded by proximal tubular cells in a functioning kidney.

Cystatin C for Staging Chronic Kidney Disease

Chronic kidney disease (CKD) is defined as ≥ 3 -month abnormal kidney function or structure impacting health and is diagnosed based on cause, GFR, and albuminuria. GFR evaluation is a graded system, with the following eGFR (mL/min/1.73 m²) ranges: ≥ 90 for normal function (stage 1), 60-89 for mild decrease (stage 2), 45-59 for mild to moderate decrease (stage 3a), 30-44 for moderate to severe decrease (stage 3b), 15-29 (stage 4) for severe decrease, and < 15 for kidney failure (stage 5).¹⁷ CKD staging can also be useful for pharmacological decisions. The US Food and Drug Administration (FDA) recommends that dose instructions be indicated by CKD stage, expressed in both eGFR (mL/min/1.73 m²) and estimates of creatinine clearance (mL/min), if the drug is intended for patients

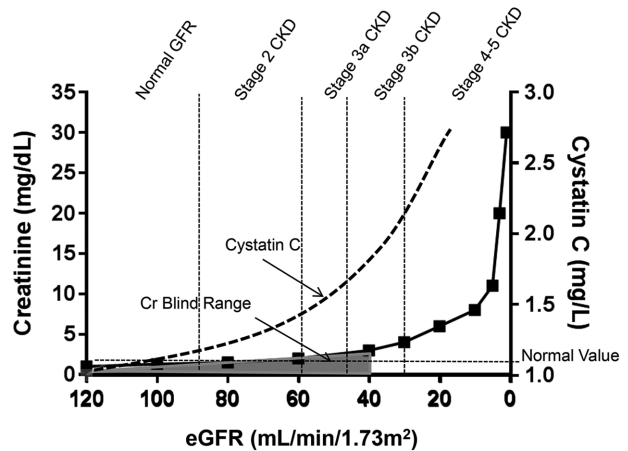


Figure 1. Increased sensitivity of cystatin C for eGFR changes avoids the creatinine blind range. Cr, creatinine; eGFR, estimated glomerular filtration rate.

with comorbid kidney disease and/or demonstrates altered pharmacokinetic properties in patients with renal impairment.^{4,18}

While true, or direct GFR, measurements rely on the clearance of an intravenous infusion of exogenous inulin or other probes, most eGFR formulas depend on endogenous creatinine levels.⁴ However, due to the limitations discussed above, creatinine is a poor biomarker of kidney function that is subject to high variability and low specificity.

In addition, serum creatinine has poor sensitivity to small changes in kidney function, especially in the early stages of kidney disease. Creatinine concentrations in serum do not significantly increase past established normal values until at least 50% of glomerular filtration has been impaired (stage 3b CKD), as shown in Figure 1.¹⁹ This observation has been termed the creatinine “blind range” (eGFR 40-59 mL/min/1.73 m²).⁸ Even adjusted eGFR equations that use creatinine, such as the MDRD, are inaccurate in subjects with low creatinine concentrations; values should only be reported by this method for patients with stage 3-5 renal disease (ie, < 60 mL/min/1.73 m²).²⁰ Cystatin C concentration has been shown in several large studies

to have superior sensitivity to changes in borderline kidney function in subjects with elevated eGFR levels of 70-90 mL/min/1.73 m².^{8,21} Moreover, current guidelines from the Kidney Disease: Improving Global Outcomes (KDIGO) research working group suggest measuring cystatin C in patients with creatinine eGFR 45-59 mL/min/1.73 m² to confirm the diagnosis.¹⁷ Cystatin C concentrations rose earlier than creatinine in longitudinal studies in patients with liver transplant, cardiac surgery, coronary angiography, or diabetic nephropathy.^{8,22,23} Detection of decreased kidney function and identification of CKD in its early stage is important in both diagnostic and clinical research settings, thus cystatin C should be utilized for this purpose.

Many population-specific studies and meta-analyses have been conducted to show cystatin C is a better indicator of GFR in several populations. Nephropathy is a common complication of diabetes, affecting nearly one-quarter of patients, and is the leading cause of end-stage renal disease.²⁴ In several studies in diabetes populations (type 1 and/or type 2), cystatin C better correlated with gold-standard direct GFR measures using clearance of chromium 51-labeled ethylenediaminetetraacetate or iohexol than did creatinine concentration or MDRD and was more sensitive to early changes in kidney function.^{12,25-27} Indeed, a recent meta-analysis of 9 studies found high sensitivity (88%) and specificity (85%) of serum cystatin C for predicting diabetic nephropathy and suggests that the biomarker may serve as an early signal of disease detection.²⁸ In another study, approximately 20% more samples using cystatin C-based estimating equations were within 10% deviation from isotopic GFR versus creatinine-based estimating equations.²⁶ Several creatinine-based eGFR equations overestimated GFR, potentially leading to false negatives or discrepant results. In a large National Health and Nutrition Examination Survey study, discordance between the classification of reduced kidney function by creatinine- or cystatin C-based CKD-EPI eGFR was approximately 12% in persons with diabetes and 5% in persons without diabetes.²⁹ Accurately monitoring early decreases in GFR is especially important in patients with diabetes because of their increased risk for kidney damage. Thus, several clinical trials use cystatin C as a secondary biomarker for renal function in diabetic populations.^{30,31}

Creatinine is a poor marker of renal function in neonates because the presence of maternal creatinine affects fetal creatinine measurement in the first 72 hours after birth, whereas cystatin C does not cross the placenta.³² Creatinine is also a poor marker in children and adolescents because creatinine production depends on muscle mass, which increases with growth and pubertal development, especially in boys.³³ Further-

more, the error produced by renal tubular secretion and non-renal elimination of creatinine is important for children because of their relatively low muscle mass and low serum creatinine. Serum concentration of cystatin C remains constant from around age 1 year to 50 years and is also suitable for use in utero and in neonates.^{11,32} Cystatin C-based eGFR equations outperform creatinine-based MDRD and Schwartz eGFR equations in children.³⁴ Because of this, a recent paricalcitol clinical trial study in pediatric populations used cystatin C as a confirmatory marker of kidney function.³⁵ These benefits of cystatin C were also shown in elderly patients.^{11,36} Furthermore, in patients who are obese (body mass index >30 kg/m²), creatinine-based eGFR calculation using the MDRD and CKD-EPI estimates underestimated GFR, whereas cystatin C-based measurement did not.¹⁰ Finally, while results have been mixed, some studies show improved estimation of GFR in severe liver disease because creatinine is biased by low creatine production and elevated bilirubin in these patients.³⁷

Specificity of Cystatin C

As noted, creatinine assays are marred by analytical and biological variability. A majority of studies comparing creatinine to cystatin C have shown that cystatin C has less biological variability and improved analytical specificity, with fewer interfering compounds.³⁸ From an analytical perspective, cystatin C assays have also significantly improved over the years, from radial immunodiffusion assays to rapid, automated, and more precise immunoturbidimetric assays approved by the FDA for diagnostic use. Cystatin C is also not subject to interference from dopamine and certain serum antibodies, which interfere even with improved enzymatic creatinine assays.

Cystatin C Better Predicts Adverse Outcomes of Decreased GFR

While increased accuracy, sensitivity, and specificity of cystatin C compared with creatinine to estimate GFR is valuable in clinical research, the main driver for increased use of cystatin C has been its role as a predictor of outcomes of decreased GFR, including risk of death and cardiovascular disease. In a large meta-analysis of 90,750 patients with CKD, Shlipak et al. demonstrated that reclassification of eGFR with cystatin C better correlated with risk for death, death from cardiovascular disease, and end-stage renal disease than with creatinine as the main variable.³⁹ In addition, a prospective study of 220 patients by Manzano-Fernández et al. found that cystatin C was a significant predictor of death and heart failure whereas serum creatinine was not.⁴⁰

Cystatin C was independently associated with mortality in patients with acute coronary syndrome along with other well-known predictors such as age and diabetes.⁴¹ Even cystatin C levels below the upper reference limits were associated with increased mortality, demonstrating that mild renal impairment is strongly correlated with adverse outcomes. Another indication affected by renal impairment is multiple myeloma. In a cohort of patients with multiple myeloma, cystatin C levels were elevated and reflected tumor burden.⁴² Furthermore, upon bortezomib treatment in relapsed patients, cystatin C was reduced. In all, the authors suggest that cystatin C has prognostic value in multiple myeloma. In clinical trials, particularly for drugs targeting kidney function and subsequently related diseases such as cardiovascular disease, cystatin C may provide prognostic value and added information on longitudinal health outcomes in addition to being a biomarker for GFR.

Equations for Estimating GFR Using Cystatin C

Due to the limitations of creatinine, eGFR equations incorporating anthropometric data are commonly used in clinical practice. Similar equations have been developed to estimate GFR from cystatin C. Several studies, summarized in Table 1, have compared cystatin C–based equations with the Schwartz, Cockcroft-Gault, and MDRD equations and found cystatin C–based equations to be superior estimates of GFR.^{7,8,21,43} These studies suggest that creatinine-based equations overestimated GFR, leading to false negatives, while cystatin C equations showed no bias. This was recently highlighted in a Japanese clinical trial involving patients treated with dolutegravir for antiretroviral HIV therapy.⁴⁴ Dolutegravir, an integrase inhibitor, affects creatinine transports and can potentially lead to increased serum creatinine. Results showed that cystatin C eGFR was more accurate than creatinine eGFR or serum creatinine alone in dolutegravir-treated patients with HIV when compared to direct inulin measures of kidney function. Therefore, an alternative biomarker such as cystatin C eGFR is suitable (and required) for kidney function monitoring of drugs that directly affect creatinine clearance pathway and, more broadly, offers improved accuracy for most situations requiring eGFR measurement. Interestingly, 3 equations have been developed that use both cystatin C and creatinine. These equations seem to outperform both types of single biomarker equations in small population studies and suggest that cystatin C may be used as a complementary biomarker rather than a replacement for creatinine.

Limitations of Cystatin C as a GFR Biomarker

While cystatin C offers significant benefits compared with creatinine, some challenges remain before it can be recommended as a complete replacement for creatinine-based estimation of GFR. Initial studies suggest that cystatin C does not vary from non-renal factors, however further investigation has shown some biological variability. In particular, thyroid diseases and the use of corticosteroids can alter cystatin C expression. Cystatin C levels are lower in the hypothyroid state even when creatinine levels are elevated and they are higher in the hyperthyroid state, suggesting that thyroid hormones influence regulation of cystatin C.⁸ High doses of corticosteroids may increase cystatin C, which is important to note because after renal transplant, patients often are prescribed glucocorticoids.^{45,46} In addition, body mass index and inflammation may affect cystatin C concentrations independent of kidney function and these factors must be considered when evaluating cystatin C levels in clinical practice.⁴⁷ Indeed, a positive association between proinflammatory C-reactive protein and cystatin C levels was observed, however, this study used creatine clearance to compare cystatin C and creatinine, which may have biased results against cystatin C.^{48,49} Nonetheless, the list of confounding diseases or conditions modifying cystatin C is relatively small compared with creatinine.⁸

Another limitation of cystatin C is related to its application in eGFR equations. Initial eGFR-cystatin C formulas were developed from small and varying populations, often from a single center.⁸ In contrast, the MDRD equation with creatinine is derived from 1628 patients across multiple centers.⁸ Perhaps due to this limitation and variance among populations, some studies comparing eGFR equations find no improvement in accuracy when using cystatin C–based equations.⁴³ This has led to differing views as to which eGFR equations to employ. Some studies recommend that if cystatin C is measured as a secondary test based on the results of creatinine, then cystatin C–based eGFR equations should be calculated instead of combined equations so that creatinine level does not influence verification of a GFR.⁵⁰ Other studies suggest recalculating eGFR with combined equations to provide the most accurate results when cystatin C is measured as a secondary test.⁵¹ It is possible such uncertainty has limited the use of cystatin C in practice. Thus, further large population studies and meta-analyses are necessary across populations and disease groups to continue to validate the utility of cystatin C and calculate biological variability from non-renal conditions before widespread adoption.

Because cystatin C undergoes proximal tubular cell reabsorption, cystatin C levels in urine are theoretically a biomarker for acute kidney injury (AKI) due to deteriorated reabsorption functions.⁵² In addition, serum cystatin C reaches a steady state 3 times faster than does creatinine, making it a putative biomarker for diagnosis of AKI.^{53,54} However, validation of cystatin C as an AKI biomarker in practice has been inconsistent. A study of acute tubular necrosis found cystatin C and β 2-microglobulin to have the highest diagnostic accuracies.⁵⁵ In a study of 400 patients with CKD given contrast media, elevated serum cystatin C at 24 hours after contrast exposure was detected in 87 patients, with a better predictive value than creatinine (negative predictive value = 100%; positive predictive value = 39.1%).⁵³ By contrast, a multicenter intensive care unit patient cohort study found that both serum and urinary cystatin C had no diagnostic value for AKI.⁵⁶ Another study of approximately 1500 children and adults after cardiac surgery also found no association of cystatin C with AKI.⁵⁷ Further investigation of cystatin C in larger population studies is necessary to determine its value as a biomarker for AKI, and the recent development of validated, sensitive, and automated assays to measure urine cystatin C may help with this effort.⁵⁸ Currently, however, cystatin C is not validated as a diagnostic biomarker for AKI.

Compared with creatinine, there is no standardized measurement of cystatin C yet.⁷ To this end, the International Federation of Clinical Chemistry and Laboratory Medicine Working Group for the Standardization of Cystatin has been established and has developed an international reference standard in collaboration with the Institute for Reference Materials and Measurements.^{59,60} With the recent availability of this cystatin C standard reference material, the CKD-EPI investigators also developed new equations for standardized cystatin C alone or in combination with standardized creatinine, which are now recommended as a confirmatory test for GFR.⁶¹ These efforts to improve assay standardization and cystatin C estimating equations will require further modification of cystatin C eGFR equations for new standardized values.¹⁵

Nevertheless, the benefits of cystatin C discussed in this article, with its ability to outperform (analytical and clinical sensitivity and specificity) and add prognostic clinical outcome information to creatinine assays, has led industry agencies, including the Predictive Safety Testing Consortium Nephrotoxicity Working Group to recommend cystatin C for use as an early diagnostic biomarker in drug development.⁶²

In addition, it is generally believed that cystatin C is too expensive for routine use in clinical research. However, cystatin C assays are automated, similar to creatinine, minimizing labor and reagent costs, which are

reported to be approximately \$4 per test.⁵⁰ While this is roughly 3 times the cost of an enzymatic creatinine test (and costs may differ for clinical research assays not run in a clinical laboratory), it is significantly less than other common kidney biomarkers such as β 2-microglobulin and the benefits of accuracy and sensitivity justify its use, particularly for clinical research.

Recommendations for Clinical Diagnosis and Treatment of Kidney Disease

Over the past several years, many comparison studies and reviews detailing the benefits of cystatin C have been published by institutions and large agencies. However, creatinine will continue to be used due to its perceived cost-benefit ratio and familiarity. Nevertheless, as a transition to cystatin C is discussed and developed by the medical community, consideration to use cystatin C should be given for the use cases highlighted in this article. As recommended by KDIGO 2012 guidelines, a varied approach using the most appropriate marker and eGFR equation based on the patient's characteristics and clinical scenario may provide the best balance of cost and accuracy, as shown in Figure 2. Specifically, cystatin C should be used when creatinine assay-interfering substances or creatinine biological variability in the highlighted patient groups are suspected or, when monitoring of early or acute kidney injury, avoiding the creatinine blind range is necessary. Similar to creatinine, eGFR equations with cystatin C are recommended for diagnosis versus serum concentrations alone.¹⁷ While there are differing opinions on which eGFR equation to use and whether to use combined equations, clinicians should choose equations based on clinical characteristics, the population being studied, and the usage scenario.⁵⁰

Recommendations for Renal Impairment Studies in Drug Development

One final use case for cystatin C is pharmacokinetic, dosing, and toxicity studies for new drugs that are cleared by the kidney. The kidneys are the major organ for elimination of most drugs that are polar and not bound to plasma proteins. Preexisting renal dysfunction or impairment as a side effect of an investigational product can potentially prolong exposure to the drug and its toxic effects, thus the FDA and European Medicines Agency (EMA) have issued guidelines detailing when pharmacokinetic renal impairment studies are necessary. Examples include when a drug is renally excreted, secreted in bile (due to enterohepatic cycling ultimately leading to secretion in urine), administered to patients with renal disease, affected by dialysis, or is a cytokine, cytokine modulator, or antibody drug

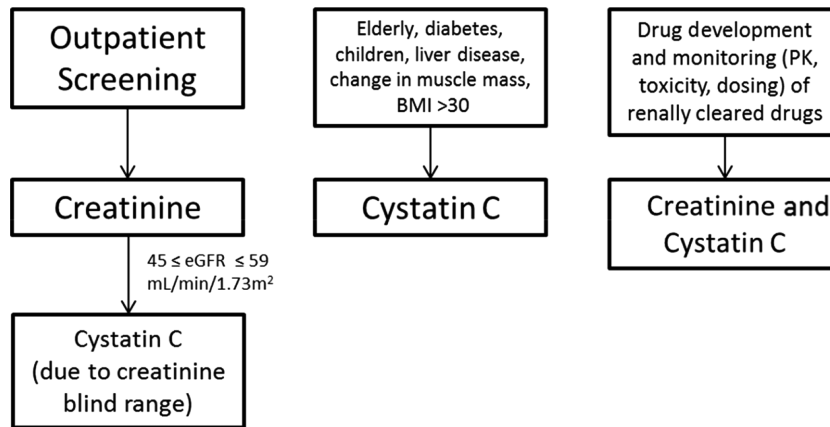


Figure 2. Recommendations for renal biomarker to use in different clinical settings. BMI, body mass index; eGFR, estimated glomerular filtration rate; PK, pharmacokinetics.

conjugate.⁴ To date, most clinical studies measuring cystatin C as a biomarker of renal impairment use it as a confirmatory marker.^{63,64} However, the evidence supports the use of cystatin C as a primary biomarker to determine kidney function in addition to typical measurement of serum creatinine with combined GFR estimating equations. Because of the creatinine blind range, early renal side effects of drugs cleared by the kidney may not be detected by measuring serum creatinine. Cystatin C can detect drug-induced glomerular alterations more readily because of its earlier rise in concentration compared with creatinine. In addition, the increased precision and accuracy of cystatin C in estimating GFR may allow more accurate dosing of these therapeutics.

As a safety biomarker for drug dosing, cystatin C was recently evaluated in a phase 3 randomized controlled trial for dyslipidemia in which pemafibrate, a novel selective peroxisome proliferator-activated receptor α modulator, was compared to the reference fenofibrate.⁶⁵ There are known safety concerns with fenofibrate treatment in elderly patients due to the risk of increased serum creatinine levels resulting in hospitalization.⁶⁶ The clinical study demonstrated minor fluctuations in both serum creatinine and cystatin C over 24 weeks with pemafibrate compared with the significant increase in these values with fenofibrate treatment. Because patients with CKD typically present with confounding diseases such as dyslipidemia, once approved, pemafibrate may be a suitable alternative agent for this population.⁶⁵ In another case report, dosing of an aminoglycoside antibiotic cleared by the kidney corrected for eGFR-creatinine resulted in overestimation of GFR, accumulation of the drug, and, ultimately, AKI in an elderly man. The patient also sustained liver injury, and studies have shown that in advanced liver disease, use of creatinine leads to an overestimation of GFR.³⁷ To remedy this, cystatin C is

recommended for more accurate GFR estimation and dosing guidance.⁶⁷ Therefore, KDIGO encourages that for the safety of patients with CKD, in posology decisions regarding medications with a narrow therapeutic window, where precision is required, GFR estimations should use cystatin C if direct measurement is not available.⁶⁸

Cystatin C is formally qualified by the FDA and EMA for clinical trials.⁶² These regulatory agencies also provide some input on the use of cystatin C as a renal impairment biomarker. While the adopted 2016 EMA guidelines for pharmacokinetic renal impairment studies do not specifically address the measurement of cystatin C,⁶⁹ an earlier draft did indicate preference for cystatin C as a secondary marker in addition to direct measures of GFR.⁷⁰ Also, it is anticipated that serum creatinine with cystatin C and corresponding eGFR calculations could be an acceptable measure of kidney function for future clinical trials in patients at risk for CKD.⁶⁹ Furthermore, the FDA does encourage the use of cystatin C to estimate GFR in pediatric patients with renal impairment in pharmacokinetic studies.¹⁸ While only limited guidance on the use of cystatin C is incorporated into agency recommendations, continued discussions with regulatory authorities and drug development partners is necessary for further advancement.

It is important to note that most assays for the measurement of cystatin C are performed in a Clinical Laboratory Improvement Amendments–certified clinical laboratory and based on *in vitro* diagnostic assays. The primary purpose of a diagnostic assay is to distinguish patients with disease from healthy patients. However, according to the most recent 2013 FDA Bioanalytical Method Validation Draft Guidance, when biomarkers (such as cystatin C or creatinine) are measured to support a regulatory claim for a drug (eg, improvement in renal function compared with an alternate therapeutic, pivotal determination of safety, support

of labeled dosing), full bioanalytical compliance and validation are necessary.⁷¹ Moreover, these FDA guidelines, for the first time, stated that use of diagnostic kits may not ensure reliability of the method for drug development purposes unless site-specific bioanalytical method validation is performed. While these guidelines are not final, they suggest that future new drug applications using biomarker data run in a clinical laboratory to support a regulatory claim may not be acceptable.

Therefore, for bioanalytical drug development studies, most cystatin C assays acquired as research use only or as in vitro diagnostic use commercial kits must be adapted to meet FDA bioanalytical method validation guidelines and should follow recent guidelines for biomarker validation.^{71,72} Briefly, the assay must be devised to have at least 6 non-zero calibration points, including the expected lower and upper limits of quantitation instead of limit of detection. The quality controls (QCs) should also include the lower and upper limits of quantitation in addition to the QCs that span the rest of the calibration curve and include QCs of endogenous creatinine in study matrix. Following these guidelines with cystatin C ensures the highest accuracy, sensitivity, precision, and specificity in determining eGFR in drug development.

Conclusion

Cystatin C offers advantages over creatinine as a biomarker for renal function in clinical research. First, greater accuracy of GFR estimation has been consistently demonstrated compared with creatinine alone. This improved accuracy is especially prominent in several population groups, including patients with diabetes, neonates, children, patients with change in muscle mass, and elderly patients. Regulatory agencies recommend the use of cystatin C in addition to creatinine in some population-specific studies. Cystatin C also demonstrates improved specificity, as biological factors such as muscle mass, illness, and inflammation do not seem to affect its expression. Compared with creatinine, cystatin C is elevated in serum earlier on the CKD spectrum, allowing detection of a mild decrease in renal function from a therapeutic or disease state. This is crucial for dosing renally cleared drugs that have a narrow therapeutic window. Finally, in large population studies, cystatin C has been shown to be a better predictor of clinical outcomes that result from decreased GFR.

In clinical research, accurate, specific, and sensitive measurements of GFR may mean the difference between an efficacious candidate and a failed candidate or the presence or absence of renal toxicity. While limitations exist, the clinical trials highlighted in this review demonstrate validated use of cystatin C as

a renal biomarker, and continued discussion among industry partners and regulatory agencies, as well as agencies working to standardize cystatin C assays and cystatin C estimating equations is expected to increase its use in clinical research.

Declaration of Conflicting Interests

SK, SP, and RI are employees of Celerion, a clinical research organization.

References

1. Rahn KH, Heidenreich S, Brückner D. How to assess glomerular function and damage in humans. *J Hypertens*. 1999;17(3):309–317.
2. Stevens LA, Levey AS. Measured GFR as a confirmatory test for estimated GFR. *J Am Soc Nephrol*. 2009;20(11):2305–2313.
3. Thomas C, Thomas L. Renal failure — measuring the glomerular filtration rate. *Dtsch Arztebl Int*. 2009;106(51-52):849–855.
4. Paglialunga S, Offman E, Ichhpurani N, Marbury TC, Morimoto BH. Update and trends on pharmacokinetic studies in patients with impaired renal function: practical insight into application of the FDA and EMA guidelines. *Expert Rev Clin Pharmacol*. 2017;10(3):273–283.
5. Kroll MH, Hagenruber C, Elin RJ. Reaction of picrate with creatinine and cepha antibiotics. *Clin Chem*. 1984;30(10):1664–1666.
6. Panteghini M. Enzymatic assays for creatinine: time for action. *Clin Chem Lab Med*. 2008;46(4):567–572.
7. Levey AS, Inker LA, Coresh J. GFR estimation: from physiology to public health. *Am J Kidney Dis*. 2014;63(5):820–834.
8. Herget-Rosenthal S, Bokenkamp A, Hofmann W. How to estimate GFR-serum creatinine, serum cystatin C or equations? *Clin Biochem*. 2007;40(3-4):153–161.
9. Hojs R, Bevc S, Ekart R, Gorenjak M, Puklavec L. Serum cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of kidney function. *Nephrol Dial Transplant*. 2006;21(7):1855–1862.
10. Mysliwiec P, Jasiewicz P, Hady HR, et al. Creatinine or cystatin C - which is a better index of renal function in morbid obesity? *Adv Med Sci*. 2013;58(2):376–381.
11. Filler G, Bokenkamp A, Hofmann W, et al. Cystatin C as a marker of GFR - history, indications, and future research. *Clin Biochem*. 2005;38(1):1–8.
12. Pucci L, Triscornia S, Lucchesi D, et al. Cystatin C and estimates of renal function: searching for a better measure of kidney function in diabetic patients. *Clin Chem*. 2007;53(3):480–488.
13. Abrahamson M, Olafsson I, Palsdottir A, et al. Structure and expression of the human cystatin C gene. *Biochem J*. 1990;268:287–294.
14. Baxmann AC, Ahmed MS, Marques NC, et al. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clin J Am Soc Nephrol*. 2008;3(2):348–354.
15. Köttgen A, Selvin E, Stevens LA, et al. Serum cystatin C in the United States: the Third National Health and Nutrition Examination Survey (NHANES III). *Am J Kidney Dis*. 2017;51(3):385–394.
16. Tangri N, Stevens LA, Schmid CH, et al. Changes in dietary protein intake has no effect on serum cystatin C levels independent of the glomerular filtration rate. *Kidney Int*. 2011;79(4):471–477.

17. Journal O, The OF, Society I, Nephrology OF. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Off J Int Soc Nephrol*. 2012;2(1).
18. FDA. Guidance for Industry. Pharmacokinetics in Patients With Impaired Renal Function — Study Design, Data Analysis, and Impact on Dosing and Labeling. May 1998. <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072127.pdf>. Accessed April 3, 2018.
19. Villa P, Jiménez M, Soriano M-C, Manzanera J, Casasnovas P. Serum cystatin C concentration as a marker of acute renal dysfunction in critically ill patients. *Crit Care*. 2005;9(2):R139–R143.
20. Helou R. Should we continue to use the Cockcroft-Gault formula? *Nephron Clin Pract*. 2010;116(3):c172–c186.
21. Ma Y-C, Zuo L, Chen J-H, et al. Improved GFR estimation by combined creatinine and cystatin C measurements. *Kidney Int*. 2007;72(12):1535–1542.
22. Mazur MJ, Heilman RL. Early detection of acute renal failure by serum cystatin C: a new opportunity for a hepatologist. *Liver Transplant*. 2005;11(6):705–707.
23. Ishibashi Y, Yamauchi M, Musha H, et al. Impact of contrast-induced nephropathy and cardiovascular events by serum cystatin C in renal insufficiency patients undergoing cardiac catheterization. *Angiology*. 2010;61(8):724–730.
24. de Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J. Temporal trends in the prevalence of diabetic kidney disease in the United States. *JAMA*. 2011;305(24):2532–2539.
25. Willems D, Wolff F, Mekhali F, Gillet C. Cystatin C for early detection of renal impairment in diabetes. *Clin Biochem*. 2009;42(1-2):108–110.
26. Chudleigh RA, Ollerton RL, Dunseath G, et al. Use of cystatin C-based estimations of glomerular filtration rate in patients with type 2 diabetes. *Diabetologia*. 2009;52(7):1274–1278.
27. Beauvieux MC, Le Moigne F, Lasseur C, et al. New predictive equations improve monitoring of kidney function in patients with diabetes. *Diabetes Care*. 2007;30(8):1988–1994.
28. Zhou B, Zou H, Xu G. Clinical utility of serum cystatin C in predicting diabetic nephropathy among patients with diabetes mellitus: a meta-analysis. *Kidney Blood Press Res*. 2016;41(6):919–928.
29. Tsai C-W, Grams ME, Inker LA, Coresh J, Selvin E. Cystatin C- and creatinine-based estimated glomerular filtration rate, vascular disease, and mortality in persons with diabetes in the US. *Diabetes Care*. 2014;37(4):1002–1008.
30. Jin S-M, Han KA, Yu JM, et al. Probucol in albuminuric type 2 diabetes mellitus patients on renin-angiotensin system blockade: a 16-week, randomized, double-blind, placebo-controlled trial. *Arterioscler Thromb Vasc Biol*. 2016;36(10):2108–2014.
31. Jabbour S, Seufert J, Scheen A, et al. Dapagliflozin in patients with type 2 diabetes mellitus: a pooled analysis of safety data from phase IIb/III clinical trials. *Diabetes Obes Metab*. 2018;20(3):620–628.
32. Filler G, Guerrero-Kanan R, Alvarez-Elias AC. Assessment of glomerular filtration rate in the neonate: Is creatinine the best tool? *Curr Opin Pediatr*. 2016;28(2):173–179.
33. Filler G, Lepage N. Should the Schwartz formula for estimation of GFR be replaced by cystatin C formula? *Pediatr Nephrol*. 2003;18(10):981–985.
34. Grubb A, Nyman U, Björk J, et al. Simple cystatin C-based prediction equations for glomerular filtration rate compared with the modification of diet in renal disease prediction equation for adults and the Schwartz and the Counahan-Barratt prediction equations for children. *Clin Chem*. 2005;51(8):1420–1431.
35. Webb NJA, Lerner G, Warady BA, et al. Efficacy and safety of paricalcitol in children with stages 3 to 5 chronic kidney disease. *Pediatr Nephrol*. 2017;32(7):1221–1232.
36. Pottel H, Hoste L, Dubourg L, et al. An estimated glomerular filtration rate equation for the full age spectrum. *Nephrol Dial Transplant*. 2016;31(5):798–806.
37. Beben T, Rifkin DE. GFR estimating equations and liver disease. *Adv Chronic Kidney Dis*. 2015;22(5):337–342.
38. Ferguson TW, Komenda P, Tangri N. Cystatin C as a biomarker for estimating glomerular filtration rate. *Curr Opin Nephrol Hypertens*. 2015;24(3):295–300.
39. Shlipak MG, Matsushita K, Ärnlöv J, et al. Cystatin C versus creatinine in determining risk based on kidney function. *N Engl J Med*. 2013;369(10):932–943.
40. Manzano-Fernandez S, Januzzi JL, Boronat-Garcia M, et al. β -trace protein and cystatin C as predictors of long-term outcomes in patients with acute heart failure. *J Am Coll Cardiol*. 2011;57(7):849–858.
41. Jernberg T, Lindahl B, James S, et al. Cystatin C: a novel predictor of outcome in suspected or confirmed non-ST-elevation acute coronary syndrome. *Circulation*. 2004;110(16):2342–2348.
42. Terpos E, Katodritou E, Tsiftakis E, et al. Cystatin-C is an independent prognostic factor for survival in multiple myeloma and is reduced by bortezomib administration. *Haematologica*. 2009;94(3):372–379.
43. Levey AS, Tighiouart H, Simon AL, Inker LA. Comparing newer GFR estimating equations using creatinine and cystatin C to the CKD-EPI equations in adults. *Am J Kidney Dis*. 2017;70(4):587–588.
44. Yukawa S, Watanabe D, Uehira T, Shirasaka T. Clinical benefits of using inulin clearance and cystatin C for determining glomerular filtration rate in HIV-1-infected individuals treated with dolutegravir. *J Infect Chemother*. 2018;24(3):199–205.
45. Taglieri N, Koenig W, Kaski JC. Cystatin C and cardiovascular risk. *Clin Chem*. 2009;55(11):1932–1943.
46. Knight EL, Verhave JC, Spiegelman D, et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int*. 2004;65(4):1416–1421.
47. Stevens LA, Schmid CH, Greene T, et al. Factors other than glomerular filtration rate affect serum cystatin C levels. *Kidney Int*. 2009;75(6):652–660.
48. Risch L, Herklotz R, Blumberg A, Huber AR. Effects of glucocorticoid immunosuppression on serum cystatin C concentrations in renal transplant patients. *Clin Chem*. 2001;47(11):2055–2059.
49. Westhuyzen J. Cystatin C: a promising marker and predictor of impaired renal function. *Ann Clin Lab Sci*. 2006;36(4):387–394.
50. Shlipak MG, Mattes MD, Peralta CA. Update on cystatin C: incorporation into clinical practice. *Am J Kidney Dis*. 2013;62(3):595–603.
51. Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. 2012;367(1):20–29.
52. Vanmassenhove J, Vanholder R, Nagler E, Van Biesen W. Urinary and serum biomarkers for the diagnosis of acute kidney injury: an in-depth review of the literature. *Nephrol Dial Transplant*. 2013;28(2):254–273.
53. Briguori C, Visconti G, Rivera N V, et al. Cystatin C and contrast-induced acute kidney injury. *Circulation*. 2010;121(19):2117–2122.
54. Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am J Kidney Dis*. 2002;40(2):221–226.

55. Herget-Rosenthal S, Poppen D, Hüsing J, et al. Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis. *Clin Chem*. 2004;50(3):552–558.
56. Royakkers AANM, Korevaar JC, van Suijlen JDE, et al. Serum and urine cystatin C are poor biomarkers for acute kidney injury and renal replacement therapy. *Intensive Care Med*. 2011;37(3):493–501.
57. Koyner JL, Garg AX, Shlipak MG, et al. Urinary cystatin C and acute kidney injury after cardiac surgery. *Am J Kidney Dis*. 2018;61(5):730–738.
58. Szirmay B, Kustán P, Horváth-Szalai Z, et al. Novel automated immune turbidimetric assay for routine urinary cystatin-C determinations. *Bioanalysis*. 2018;10(6):377–384.
59. Ebert N, Delanaye P, Shlipak M, et al. Cystatin C standardization decreases assay variation and improves assessment of glomerular filtration rate. *Clin Chim Acta*. 2016;456:115–121.
60. First certified reference material for cystatin C in human serum ERM-DA471/IFCC. *Clin Chem Lab Med*. 2010;48:1619.
61. Delanaye P, Cavalier E, Cristol JP, Delanghe JR. Calibration and precision of serum creatinine and plasma cystatin C measurement: impact on the estimation of glomerular filtration rate. *J Nephrol*. 2014;27(5):467–475.
62. Dieterle F, Sistare F, Goodsaid F, et al. Renal biomarker qualification submission: a dialog between the FDA-EMEA and Predictive Safety Testing Consortium. *Nat Biotechnol*. 2010;28(5):455–462.
63. Jabbour SA, Hardy E, Sugg J, Parikh Shamik. Dapagliflozin is effective as add-on therapy to sitagliptin with or without metformin: a 24-week, multicenter, randomized, double-blind, placebo-controlled study. *Diabetes Care*. 2014;37(3):740–750.
64. Jodele S, Fukuda T, Vinks A, et al. Eculizumab therapy in children with severe hematopoietic stem cell transplantation-associated thrombotic microangiopathy. *Biol Blood Marrow Transplant*. 2014;20(4):518–525.
65. Ishibashi S, Arai H, Yokote K, Araki E, Suganami H, Yamashita S. Efficacy and safety of pemafibrate (K-877), a selective peroxisome proliferator-activated receptor alpha modulator, in patients with dyslipidemia: results from a 24-week, randomized, double blind, active-controlled, phase 3 trial. *J Clin Lipidol*. 2018;12(1):173–184.
66. YY Z, MA W, Manno M, et al. New fibrate use and acute renal outcomes in elderly adults: a population-based study. *Ann Intern Med*. 2012;156(8):560–569.
67. Rebollo N, Cepeda-Piorno FJ. Cystatin C for therapeutic drug monitoring. *Clin Chem*. 2015;61(6):807–808.
68. Levin A, Stevens PE. Summary of KDIGO 2012 CKD Guideline: behind the scenes, need for guidance, and a framework for moving forward. *Kidney Int*. 2014;85(1):49–61.
69. EMA. Guideline on the evaluation of the pharmacokinetics of medicinal products in patients with decreased renal function. *Ema*. 2015;44:1–15.
70. CHMP. Guideline on the clinical investigation of medicinal products to prevent development / slow progression of chronic renal insufficiency. *Ema*. 2014;44:1–11.
71. US Department of Health and Human Services, Food and Drug Administration. Guidance for industry: bioanalytical method validation. <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM368107.pdf>. Published September 2013. Accessed April 3, 2018.
72. Group W, Fda US, Burkey J, et al. Points to Consider Document: Scientific and Regulatory Considerations for the Analytical Validation of Assays Used in the Qualification of Biomarkers in Biological Matrices.