**Review: Cystatin C: a Promising Marker and Predictor of Impaired Renal Function**

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**Abstract.** Cystatin C is a relatively stable protein in serum and heparinized plasma that shows promise as a convenient measure of glomerular filtration rate (GFR). However, it is becoming clear that the relationship between cystatin C and GFR can depend on the clinical presentation. Factors influencing cystatin C levels are those that affect the rate of synthesis of the protein, such as thyroid status and the use of steroids. As with all laboratory tests, results should be interpreted in the light of the method’s known limitations and in conjunction with other clinical and laboratory information. Nevertheless, accumulating evidence suggests that cystatin C is a useful biomarker for renal function, and may even be the method of choice in a range of clinical situations, from GFR surveillance in diabetics to the assessment of acute kidney injury in critically ill patients.

**Keywords:** acute renal failure, creatinine, cystatin C, glomerular filtration rate

**Introduction**

The limitations of serum/plasma creatinine as a measure of glomerular filtration rate (GFR) have led to an extensive search for a more sensitive laboratory marker of impaired renal function. Cystatin C, a 13 kD protein produced by a housekeeping type gene by all nucleated cells [1], was first investigated as a marker for GFR in 1985 [2]. This cysteine protease inhibitor is freely filtered by the glomerulus without steric restrictions, and does not appear to be secreted by the renal tubules [3,4]. Some of the limitations of serum creatinine, namely, the effect of gender, body muscle mass (height and weight), and diet do not appear to be factors that significantly influence serum cystatin C levels. Cystatin C is relatively stable, and can be rapidly, accurately, and precisely analysed by automated analysers, thereby meeting the practical requirements of a suitable laboratory test [1,5]. This review summarises recent clinical studies that have examined the utility of cystatin C as a marker of GFR. In addition, arising out of its role as a measure of renal function, the place of cystatin C as a prognostic marker for morbidity and mortality in a number of conditions is briefly explored.

**Cystatin C as a Marker of GFR**

One of the key questions is this: does cystatin C identify patients at risk of acute renal failure at an earlier stage than serum creatinine? It is well known that serum creatinine is an insensitive measure of renal function, only loosely corresponding to GFR [6]. Moreover, serum creatinine is not an accurate reflection of GFR in the non-steady state, and is influenced by many factors, including muscle mass, gender, diet, liver function, and age to mention a few. A number of studies have explored the clinical utility of plasma cystatin C as a diagnostic marker of GFR [7-15]. These studies follow on from earlier work that suggested that cystatin C showed promise as a marker of renal function [5,16-21].
The assessment of GFR is problematic in unstable, critically ill patients [22]. Herget-Rosenthal et al [7] prospectively studied 85 high-risk patients in the intensive care unit, assessing their renal function by the so-called RIFLE criteria (a scheme that stages renal function from risk through to end-stage renal failure (E), based on serum creatinine/GFR or urine output)[23]. In this scheme, “R” denotes risk of renal dysfunction, “I” denotes injury to the kidney, and “F” denotes failure of kidney function. These authors found that serum cystatin C detected acute renal failure (ARF) 1.5 days earlier than serum creatinine did, according to the R-criteria (serum creatinine increased by ≥50%) (p <0.001). Serum cystatin C concentrations rose more quickly than creatinine in ARF patients, although this was not confirmed by the study of Ahlstrom et al 2004 [24], also in critically ill patients. The receiver-operating characteristic (ROC) plot showed that a 50% or greater increase in serum cystatin C predicted renal replacement requirement, suggesting that serum cystatin C may be a valid marker in the early as well as late stages of ARF [7]. Cystatin C had only weak predictive power for hospital mortality [24].

A possible limitation of these two studies is that serum cystatin C and not GFR was used to define ARF. Nevertheless, the finding that serum cystatin C detected ARF by the RIFLE criteria one to two days earlier than creatinine is potentially important, because earlier detection of impending ARF may facilitate the development and trial of intervention strategies to ameliorate renal injury.

These results were confirmed by the studies of Delanaye et al [25] and Villa et al [22] who also studied critically ill patients, but used 24 hr creatinine clearance as the reference method for GFR. Serum cystatin C levels (as the reciprocal) correlated strongly with the 24-hr creatinine clearance (r = 0.832, p <0.001) [22]. ROC plot analysis showed that cystatin C was a sensitive real time marker for renal impairment (defined as a GFR <80 ml/min/1.73 m²), was 88/97% for cystatin C versus 48/100 for creatinine.

The clinical significance of cystatin C has also been evaluated using non-creatinine-dependent measures of GFR [9,14,27]. Uzan et al [14] studied 52 subjects with GFR between 10 – 60 ml/min/1.73 m², and 52 healthy controls, with 99m Tc-DTPA clearance as the measure of GFR. Serum cystatin C levels were not influenced by gender, but increased with age, possibly reflecting the decline in GFR with age. Levels measured over a period of 3 mo were stable in the healthy subjects. A serum cystatin C value >1.36 mg/L was superior to both serum creatinine and β₂-microglobulin (another small protein that is freely filtered by the glomerulus) as a predictor of a GFR <60 ml/min/1.73 m², representing impaired kidney function. The sensitivity, specificity, and area under the curve values for cystatin C were 98%, 99%, and 0.99 respectively; by comparison, creatinine had values of 80%, 100%, and 0.97 when a cut-off of 103 µmol/L was used. Corresponding values for β₂-microglobulin (>2.51 mg/L) were 86%, 92%, and 0.94 respectively.

French workers [9] evaluated serum urea and cystatin C with clearance of iopromide as the reference method for GFR, in patients undergoing cardiac catheterization (n = 127). A cytostatin C value >1.3 mg/L exhibited 88% sensitivity and 96% specificity for detecting renal dysfunction, which was defined as iopromide clearance <80 ml/min/1.73 m². By comparison, creatinine had values of 63% and 80% for a cut-off value of >1.2 mg/dl (106 µmol/L) respectively. These workers concluded that cystatin C detected reduced GFR more reliably and at an earlier stage than serum urea in their patients, and that this was potentially useful in identifying patients at risk of contrast renal damage [9].

A related problem concerns the difficulties in detecting trends in renal function over time in patients with (for example) diabetes who may have normal or elevated GFR. Perkins et al [28] compared creatinine-based measurements with cystatin C, using cold iothalamate clearance as the reference method. During 4 yr follow-up, trends in iothalamate clearance correlated strongly (r = 0.77) with serum cystatin C (as the reciprocal), but poorly
with creatinine-based measures ($r = 0.35$). Moreover, all participants with negative trends in iothalamate clearance also had negative trends for $1/cystatin$ C, which suggests that serial cystatin C measurements may be useful in detecting early renal function decline in conditions such as diabetes [28].

National and international organizations are recommending that estimating equations be adopted as a way of overcoming some of the limitations of serum creatinine, because they incorporate known demographic and clinical variables as surrogates for the various physiologic factors that affect serum creatinine concentrations [29]. Grubb et al [10] compared several predictive equations (including the Modification of Diet in Renal Disease [MDRD] equation) [30] with iothalamate clearance for determining GFR in 536 patients aged 0.3 –93 yrs, and derived their own equation, based on plasma cystatin C. The equation $GFR \ (\text{ml/min}) = 84.69/cystatin\ C^{1.68}$ (ie, 84.69 divided by plasma cystatin C result in mg/L raised to the power of 1.68) included a factor for females (0.948) and prepubertal children <14 yr (1.384). This equation assessed GFR equally well or better than the MDRD equation for adults, or various equations commonly used for children. Based on this formula, a plasma cystatin C level of 1.23 mg/L corresponds with a GFR of 60 ml/min, the level recommended by the National Kidney Foundation’s Kidney Disease Quality Outcome Initiative (NFK-K/DQOI) for defining chronic kidney disease [31].

Grubb et al [11] also derived cystatin C-based formulae for comparison with the widely used Cockcroft and Gault (C&G) formula for creatinine clearance [32], which includes anthropometric variables to compensate for the inadequacies of creatinine as a marker of GFR. Iohexol clearance was used as the gold standard method. The formula $GFR = 99.19 / cystatin\ C^{1.713} \times (0.823 \text{ for women})$ had lower bias and higher accuracy in predicting GFR than the C&G formula, and was recommended for the initial estimation of GFR in a patient. In a recent paper, mild to moderate impairment of renal function was evaluated in 164 patients with chronic kidney disease, with $51\text{Cr-EDTA}$ clearance as the reference method [27]. Serum cystatin C had a significantly higher diagnostic accuracy for reduced GFR ($GFR <60 \text{ ml/min/1.73 m}^2$) than serum creatinine and calculated creatinine clearances (C&G formula), but (surprisingly) only in female patients. The MDRD formula [30] performed as well as cystatin C in identifying reduced GFR in this study. Cystatin C was found to be a more sensitive marker than serum creatinine for the early assessment of GFR changes induced by cisplatin-based chemotherapy [33].

A different approach was used by Sjostrom et al [13]. In deriving a cystatin C-based equation for GFR, these authors considered both the production rate and clearance (non-renal as well as renal) of cystatin C. Using iohexol clearance as the measure of GFR, they calculated the non-renal clearance of cystatin C to be 22.3 ml/min/1.73 m². Using a cohort of haemodialysis patients ($n = 70$) with zero renal clearance, they measured non-renal clearance to be 22.7 ml/min/1.73 m², thus confirming their calculated level. They also studied 381 patients with a GFR range of 12 – 151 ml/min/1.73 m². From their data, they proposed that the relationship between GFR and cystatin C can be described by the following simple formula:

$$GFR\ (\text{ml/min}/1.73\ m^2) = (124/cystatin\ C) – 22.3$$

where cystatin C is given in mg/L. Interestingly, in their haemodialysis group, the mean plasma cystatin C was 5.74 ± 6.6 mg/L, which, when inserted in the formula, gives a GFR of zero. This cystatin C value represents the highest value that should be encountered, based on the production rate of cystatin C (calculated to be 0.124 mg/min/1.73 m²) and non-renal clearance of 22.3 ml/min/1.73 m². By way of examples, a cystatin C value ≤1.2 corresponds to a GFR >80 ml/min/1.73 m², while a cystatin C value >1.5 mg/L corresponds to a GFR of <60 ml/min/1.73 m².

There are several limitations of this study. Firstly, iohexol is used as the measure of true GFR; the use of other substances would inevitably give slightly different values. Also, it is assumed that the production rate of cystatin C and the non-renal clearance rates are constant, and this may not be true under all circumstances. For example, the analysis of Luc et al [12] which examined plasma cystatin C in relation to the development of coronary heart disease, found that cystatin C was higher in cases of myocardial infarction-coronary death and angina versus controls, and that this...
difference was not explained by a decrease in GFR. They suggested that cystatin C participates in the inflammatory reaction, and indeed cystatin C was weakly correlated with a range of inflammatory markers including C-reactive protein (CRP). Other limitations have been identified (see discussion below). Nevertheless, the equation represents a useful advance in the determination of GFR, as it considers biologically relevant parameters in its derivation (production rate, non-renal clearance, and renal clearance).

How quickly does cystatin C reflect a sudden and sizable change in GFR? This question is important when considering the usefulness of cystatin C as a marker of acute kidney injury. Herget-Rosenthal et al [8] addressed this issue by studying patients undergoing uninephrectomy. This clinical situation provides the experimental opportunity to assess the response of cystatin C and creatinine to a sudden and dramatic change in GFR. Initially, the patients had a creatinine clearance of 105 ± 14 ml/min/1.73 m². Due to the nephrectomy, the patients lost 45 ± 3% of their renal function. Serum cystatin C increased one day after surgery, while serum creatinine increased after two days. Analysis showed that cystatin C concentrations increased 1.4 days earlier than creatinine. These authors concluded that cystatin C is useful as an early and accurate marker to detect rapid GFR decreases, as encountered in acute renal failure [8].

Limitations of Cystatin C as a Marker of GFR

There are dissenting views regarding the application of cystatin C as a marker of kidney function in some clinical settings. Wulkan et al [34] questioned the use of this parameter in the intensive care unit because of changes in thyroid hormone metabolism in critically ill patients, the so-called non-thyroidal illness. Their hypothesis is that the production of cystatin C is under the influence of thyroid hormone, and therefore changes in thyroid function may influence serum cystatin C levels independently of renal function. In a paper published in 2003, these authors reported that cystatin C levels were low in hypothyroidism while creatinine levels were elevated, and in hyperthyroidism the reverse applied. Similar observations were made by Manetti et al [35], however, they noted that this was not a universal finding: 62% of their hyperthyroid patients and 76% of the hypothyroid patients had normal serum cystatin C values. However, the contribution of thyroid function to the measured cystatin C level was unequivocally demonstrated in a prospective study in which restoration of euthyroidism by therapy was associated with normalisation of serum cystatin C level [35].

The contribution of non-renal factors to serum cystatin C levels has recently been investigated [36]. The authors utilized data from the PREVEND study (a population-based study, n = 8058) and performed multivariate analysis to identify factors such as age, gender, smoking, and CRP independently associated with serum cystatin C levels. Creatinine clearance was determined from the average of two separate 24-hr urine collections. After adjusting for creatinine clearance, older age, male gender, greater weight, greater height, and cigarette smoking, higher CRP levels were independently associated with higher serum cystatin C levels. The authors concluded that multivariate serum cystatin C-based estimates of renal function were not superior to multivariate serum creatinine-based estimates. This is not surprising as a creatinine-based measure (creatinine clearance) was used to compare creatinine and cystatin-C-based measures, and is therefore intrinsically biased in favour of creatinine and against cystatin C. The use of an independent measure of GFR might well have yielded a different result.

Higher cystatin C values for factors such as age may actually reflect declining GFR with age (and therefore be a true reflection of renal function). Moreover, the observation that non-renal factors (such as age and weight) are associated with higher cystatin C levels does not necessarily count against the utility of cystatin C as a measure of GFR, as values were corrected for creatinine clearance in the multivariate analysis and not true GFR. Higher weight, greater height, and male gender are associated with raised plasma creatinine concentrations, which in turn would tend to result in a lower calculated creatinine clearance (\( \frac{P_{\text{creat}}}{\text{creatinine}} \) is in the denominator when creatinine clearance is calculated). Since plasma cystatin C is adjusted for creatinine clearance, cystatin C values would
appear to be raised in these situations. Nevertheless, the question remains: do non-renal factors contribute to the net measured cystatin C concentration? And the answer is almost certainly “yes.”

An important non-renal factor that has been identified is glucocorticoid therapy. While Herget-Rosenthal et al [7] found no difference in cystatin C concentrations in their intensive care unit (ICU) patients according to serum cortisol levels, other studies have shown associations between glucocorticoid therapy and increased concentrations of cystatin C [37]. This issue is important in renal transplant patients who are treated with high-dose glucocorticoid medication. Studies suggest that high doses of steroids increase serum cystatin C levels in the first days following renal transplantation [38,39]. As a result, GFR estimates based on cystatin C tend to underestimate the true GFR [40].

Whether this effect is transient or a feature of the transplant state is uncertain. In a study of children, Bokenkamp et al [38] showed that serum cystatin C levels were increased in renal transplant recipients compared with children without renal transplants, and having the same GFR (determined by inulin clearance). Conversely, the same cystatin C level in adult transplant patients compared to native kidney disease patients was associated with a 19% higher GFR in the former group of patients [40]. This study shows that GFR estimates may not be generalizable across all patient groups, and suggests that equations derived from populations with characteristics similar to the patient be utilized (if possible) to reduce sampling bias.

Cystatin C as a Predictor of Outcome

The presence of impaired renal function has been associated with an increased risk of death in various patient groups, including outpatients [41]. Shlipak et al [42] compared cystatin C, creatinine, and estimated GFR by the MDRD equation [30] with the risk of cardiovascular events in a population-based cohort of elderly adults followed for up to 8 yr (n = 4637). Cystatin C was a stronger predictor of the risk of all-cause mortality and cardiovascular events than creatinine. They also identified three groups based on cystatin C levels: a low-risk group (cystatin C <1.0 mg/L), intermediate-risk group (1.0 – 1.28 mg/L), and a high-risk group (>1.28 mg/L). Compared to the low-risk group, the hazard ratio (adjusted) in the high-risk group for death from all causes was 2.05 (95%CI 1.73-2.40). The unadjusted ratio was 3.98 (95%CI 3.44-4.60). If these cystatin C concentrations are related to GFR using the equation of Sjostrom et al [13], then a change in GFR from >101.7 ml/min/1.73 m² to <73 ml/min/1.73 m² could be associated with a doubling of risk of cardiovascular events in this population cohort.

Serum cystatin C and creatinine levels have been investigated as predictors of outcome in patient with non-ST elevation acute coronary syndrome (n = 726) [43]. In Cox regression models including well-known predictors of outcome (age, diabetes mellitus, cardiac troponin T, CRP), cystatin C level was independently associated with mortality but not with risk of subsequent infarction. Compared to the lowest quartile, the risk of subsequent death was 1.8, 3.2, and 11.7 for the second, third, and fourth quartiles respectively. The corresponding ranges for cystatin C were <0.83, 0.83-0.99, 1.00-1.24, and >1.24 mg/L, respectively. The upper reference level for cystatin C, defined as the 97.5th percentile value in an apparently healthy population, was 1.12 mg/L for persons up to 65 yr, and 1.21 mg/L for subjects >65 yr [44]. Thus, cystatin C levels below the upper reference limits were associated with increased mortality in this patient cohort. These authors used an equation based on cystatin C to estimate GFRs (GFR =77.24 x cysC<sup>-1.2623</sup>), and obtained GFRs of >98, 78-98, 59-77, and <59 ml/min, respectively. This demonstrates that even mild renal impairment is strongly associated with increased mortality in patients with acute coronary syndrome, and prompted the investigators to suggest that plasma cystatin C measurement should substantially improve the early assessment of these patients over creatinine-based measures [43]. A similar finding was made in a cohort of 77-yr old men (n = 792) [45]. During 1 to 4 yr follow-up, cystatin C values were significantly correlated with overall mortality: compared to the lowest quintile, mortality was 3-fold higher in the top quintile for cystatin C [45].
Cystatin C as a Marker of Tubular Dysfunction

Because cystatin C is freely filtered in the glomerulus and is completely taken up by the proximal convoluted tubule [4], the primary interest in cystatin C has been as a marker for GFR. However, the involvement of the proximal convoluted tubule in the re-uptake process suggests that the measurement of urinary cystatin C might provide information on tubular function. Indeed, the measurement of specific proteins (especially enzymes) in urine has long been an important part of the differentiation of glomerular versus tubular proteinuria [46,47]. Interest in cystatin C is a relatively recent development. Like plasma cystatin C, urinary cystatin C is unaffected by age or muscle mass [48]. The urinary concentration of cystatin C in normal subjects is usually low (<120 µg/L) [48]. Concentrations are significantly higher in patients with tubular disease compared with those with glomerular disease or normal controls, suggesting that the measurement of cystatin C could allow for the detection of tubular dysfunction in the presence of pure and mixed nephropathies [49]. Urinary cystatin C may also have strong prognostic value for poor renal outcomes. A recent study of the prognostic value of urinary proteins in non-oliguric acute tubular necrosis (ATN) found cystatin C and β2-microglobulin to have the highest diagnostic accuracies in identifying patients requiring renal replacement therapy [47].

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References


