

CYSTATIN C, RENAL FUNCTION AND CARDIOVASCULAR RISK

Carola Wagner

Dade Behring Marburg GmbH – A Siemens Company, 35001 Marburg, Germany

Summary: Cystatin C is a novel serum marker of the glomerular filtration rate (GFR), a critical measure of normal kidney function. Unlike serum creatinine, cystatin C concentrations are independent of gender, age and muscle mass. As cystatin C shown no tubular secretion, it is a much earlier indicator of decreased GFR and allows the detection of mild reductions in GFR, which are not detected by creatinine. Cystatin C has been shown to be associated with future cardiovascular disease and deaths in a dose-dependent relationship that possibly reflects a very early stage of chronic renal dysfunction. In addition, »sub-clinically« elevated cystatin C concentrations in individuals without chronic kidney disease indicated by creatinine are an independent predictor of progression to chronic kidney disease, heart failure and all-cause mortality.

Keywords: cystatin C, GFR, chronic kidney disease, cardiovascular risk

Introduction

Cystatin C is a small 13 kD protein, which fulfills all the basic requirements for an endogenous filtration marker (1). Cystatin C is produced by all nucleated cells at a constant rate, regulated by a so-called »house-keeping« gene. The production rate of cystatin C is remarkably constant over the entire lifetime and elimination from the circulation is almost completely via glomerular filtration. In the absence of significant tubular damage, cystatin C is reabsorbed and metabolized by the proximal tubular epithelial cells and is not returned to the circulation. The cystatin C plasma concentration is independent from the muscle mass; thus, the strong association with sex and age as seen with creatinine is not observed for cystatin C. The increase of cystatin C with aging (> 50 years) reflects the natural decrease of renal function in advanced age. Only few circumstances have been identified which have an impact on cystatin C plasma concentrations. These are high-dose glucocorticoid therapy, and thyroid dysfunction (1).

Many studies have confirmed the high sensitivity and specificity for GFR estimation; in most studies cyst-

Kratak sadržaj: Cistatin C je novi serumski marker glomerularne filtracije (GFR), od kritičnog značaja za procenu normalne bubrežne funkcije. Za razliku od serumskog kreatinina, koncentracije serumskog cistatina C ne zavise od pola, starosti i mišićne mase. Cistatin C ne podleže tubulrnoj sekreciji, te je mnogo raniji indikator smanjenja GFR što omogućava otkrivanje i neznatnog smanjenja GFR, koje nije moguće otkriti pomoću kreatinina. Cistatin C je udružen sa budućim kardiovaskularnim oboljenjem što ukazuje i na rane stupnjeve hronične renalne disfunkcije. To znači da su »sub-klinička« povećanja koncentracija cistatina C u osoba bez hroničnog oboljenja bubrega indikovana kreatininom nezavisan prediktor progresije hroničnog bubrežnog oboljenja, srčanog oštećenja i smrtnosti.

Ključne reči: cistin C, GFR, hronično bubrežno oboljenje, kardiovaskularni rizik

tatin C was clearly superior to creatinine with regard to renal function assessment (1). As renal disease is closely associated with cardiovascular disease (and vice versa), cystatin C has shown a prognostic value not only for the further development of renal disease progression but also with respect to risk prediction for cardiovascular events and mortality (2–4).

Chronic kidney disease (CKD) – an increasing global public health burden

Epidemiological data from the US indicate that roughly 10% of the adult population show any form of CKD; studies from Europe, Australia and Asia confirm this high prevalence of CKD. The prevalence and incidence of patients on dialysis are increasing more and more rapidly, due to the fast increase of type 2 diabetes and hypertension, the two major causes of CKD. The major outcomes of CKD include not only progression to kidney failure but also complications of reduced kidney function and increased risk of cardiovascular disease (CVD). Patients with kidney disease are far more likely to die from CVD, than to develop kidney failure (5).

Chronic kidney disease does **not** cause pain – this is why CKD usually remains undetected for a longer period, until a screening test identifies the silent problem. When detected early, the further progression of CKD can be stopped or deferred if treated appropriately. For detection of CKD laboratory testing is the decisive step – both, decreased glomerular filtration rate (GFR) or increased albumin excretion in urine indicate presence of CKD.

At the time when CKD is diagnosed, most patients are still asymptomatic (with respect to kidney problems). Therefore, regular screening in high risk patients for CKD is the first step towards an improved prevention of end-stage renal disease (ESDR), and to decreased requirement of renal replacement therapy such as dialysis or transplantation.

Diagnosis and staging of CKD

In 2004 the KDIGO Controversies Conference issued the first international guideline on CKD, including definition and classification of CKD. While diagnosis of CKD requires either a decrease in GFR or an increase in albumin excretion in urine, the classification (= staging) of CKD only depends on GFR. Albuminuria represents a screening marker for kidney damage; other methods to identify (and specify) kidney damage are imaging abnormalities or pathological (bioptic) kidney abnormalities. Due to the low sensitivity of creatinine (Crea) based methods in the normal and slightly reduced GFR range, only GFR levels lower than 60 mL/min/1.73 m² are considered for the definition of CKD (6).

GFR determination provides the basis for detection and classification of CKD. The GFR is usually expressed in mL/min/1.73 m², and provides the volume of blood which is cleared per minute by the kidneys, standardized for the body surface, which is 1.73 m² for the average person.

The direct measurement of GFR by clearance of exogenously applied drugs such as certain radioactive substances (⁵¹Cr-EDTA, iothalamate) cannot be performed in daily routine due to cost- and labor-issues.

For estimation of GFR (eGFR) the KDIGO guideline recommends using a creatinine-based formula, preferably the MDRD formula, which corrects the creatinine level for age, sex and race. Creatinine levels

alone are considered not suitable as too insensitive (and unreliable due to the influence of age and sex). Creatinine clearance determination, which is not affected by age and sex interferences, is considered unreliable too due to the frequent urine sampling errors and the resulting high risk for incorrect results. However, all creatinine-based methods suffer from the influence of muscle mass (higher creatinine with higher muscle mass) and diet (high protein diet => higher creatinine). Furthermore, due to the low sensitivity of creatinine in the normal and slightly reduced GFR range only a reduction in GFR to 60 mL/min or lower is detected.

Cystatin C, a new GFR marker providing more sensitivity and overcoming many limitations of creatinine-based methods, is already listed as a promising candidate for new improved GFR formulas.

Cystatin C versus creatinine for renal function assessment

Creatinine measurement is the anywhere available method in clinical routine used for assessment of kidney function. However, creatinine has several limitations. For example, creatinine levels are directly correlated to muscle mass, and as a consequence plasma levels (and reference ranges) depend on sex and age. Different methods exist for its automated measurement, and International Standardization is available, but not yet widely introduced. Cystatin C provides a new alternative for GFR estimation. Fully automated methods are available, several studies have provided formulas for GFR calculation, and a standardization program is currently on the way.

From the methodological point of view a stable analyte is an important prerequisite. This is well given for both candidates, cystatin C and creatinine. As well, both analytes can be measured on fully automated analyzers, in random access and emergency cases. Furthermore, any method should be robust against possibly interfering substances. While no interfering factors have been identified for the cystatin C assay, many substances are known to influence creatinine assays, especially the most widely used Jaffe method. Examples of interfering factors are bilirubin, hemoglobin, ketones, high glucose or ascorbic acid levels, as well as several drugs.

Elimination exclusively via renal filtration is a further essential requirement for a GFR marker. While cystatin C fulfills this requirement well, creatinine can be alternatively secreted via the tubulus system. This alternative elimination pathway compensates for a decrease in GFR and keeps the serum creatinine level unchanged until GFR has declined to 60 mL/min/1.73 m². Creatinine levels only increase if the capacity of the alternate tubular secretion pathway is fully used; this is why there is a »creatinine-blind range« limiting the sensitivity and precision of creatinine in the normal and slightly reduced GFR range considerably.

Table I Classification of CKD

Stage	GFR (mL/min/1.73 m ²)	Description
1	≥ 90	normal or elevated GFR
2	60–89	mild GFR reduction
3	30–59	moderate GFR reduction
4	15–29	severe GFR reduction
5	< 15	renal failure

The ideal GFR marker should not be influenced by age, sex, body weight or other patient criteria allowing easy result interpretation. Cystatin C shows only a minor, not clinically relevant difference between men and women. Creatinine levels differ considerably between both sexes, requiring separate reference range. As creatinine levels are directly related to the lean body mass (= muscle mass), lower concentration are found in women compared to men, as well as elderly compared to younger individuals. The decrease of muscle mass with aging can mask the decrease of renal function with aging when considering creatinine levels for renal function testing. Therefore, all creatinine based formulas (but not cystatin C based formulas) require age and sex (and race) to compensate for these factors.

With cystatin C a constant relationship between analyte and GFR is observed; the decline of renal function with aging is reflected by increasing cystatin C levels in elderly in a sensitive manner. Up from 1 year of age cystatin C levels have reached adult levels, whereas creatinine levels increase as long as the muscle mass is growing in children and teenagers. Thus, a unique reference range can be applied for cystatin C for males and females up from 1 year of age (7), whereas the interpretation of creatinine requires age and sex specific reference ranges.

Clinical sensitivity and calculation of eGFR

Regarding the clinical requirements, a close correlation to reference methods such as ^{51}Cr -EDTA or iothalamate clearance and a high sensitivity are key. Because treatment is more efficient as earlier in the course of disease it starts, sensitivity especially to early stages of kidney disease as well as sensitivity to declining renal function are crucial. For calculation of eGFR, a validated formula should be available.

In the meta-analysis by Roos (27 studies with a total of 2007 patients) (8) cystatin C and creatinine were compared for their diagnostic accuracy against a reference method. The summary ROC curve indicates a considerably higher sensitivity for cystatin C compared to creatinine (for the same specificity). As well, the diagnostic odds ratio (DOR = measure of the overall test's accuracy) is higher for cystatin C; however, the difference did not reach statistical significance. The meta-analysis concluded that cystatin C is the more accurate marker for detection of renal impairment.

The sensitivity for early stages of renal disease for creatinine is far from perfect. As creatinine can be cleared to a certain extent via tubular secretion, plasma creatinine is insensitive to mild to moderate reductions in GFR («creatinine-blind» range). The comparison of creatinine against cystatin C and β -trace protein (β Tp), another small, freely filtered protein, clearly shows the higher sensitivity of CysC and β Tp in these clinically relevant early stages of disease, the difference versus creatinine being significant up from 70–80 mL/min (9).

With aging GFR decreases; this age-dependent loss of renal function is accelerated by comorbid conditions, such as atherosclerosis or hypertension. In the elderly, plasma creatinine is an unreliable indicator of GFR, as the daily production of creatinine is diminished due to a reduced muscle mass, resulting in overestimation of GFR with age. Compared with young subjects, mean GFR measured by inulin clearance was modestly but significantly less in elderly subjects. Mean plasma creatinine was identical in both groups, whereas mean cystatin C was significantly greater in the elderly ($p < 0.001$) (10). Reduced eGFR in the elderly should not be considered as normal, simply because it is common.

Any GFR assessment should sensitively reflect a progressive loss of renal function over time. Such a longitudinal assessment of GFR to detect systemic decreases in renal function was addressed by Perkins (11) who investigated 30 patients with type II diabetes with a follow-up over 4 years. Study participants had normal or elevated GFR at baseline, and were followed by cystatin C and creatinine testing, as well as iothalamate clearance determination every year. The trend in renal function over time was determined for each individual by use of linear regression. In contrary to creatinine and the MDRD formula, the individual trends (= slope over time) of cystatin C were strongly correlated with the trends seen for iothalamate clearance. Serial measures of cystatin C thus accurately detected trends in renal function in patients with normal or elevated GFR, that creatinine based methods could not identify. Cystatin C was considered to be a practical, inexpensive, and more accurate alternative for investigating trends in renal function.

For calculation of estimated GFR (eGFR), several formulas have been derived from different studies based on cystatin C:

- The formula published by Hoek in 2003 (12) provides an estimated GFR; as reference method ^{125}I -iothalamate clearance was used. The formula gives a body-surface adjusted eGFR as used for classification of CKD by KDIGO and KDOQI.

- The Larsson formula published in 2004 (13) in contrast provides an eGFR that is not body-surface adjusted, which is favored in certain applications. As reference method iothalamate clearance was used.

- The Arnl formula in fact was the first cystatin C based formula developed in 1999, based on a data set of more than 200 patients with inulin clearance. However, this formula was not published in a peer-reviewed journal until 2007 (14).

All these formulas provide results which closely agree in the normal and slight to moderately reduced GFR range, but can differ in the very high and low range, as the studies included only few samples in these ranges, with a resulting wider confidence range.

Recently data on cystatin C were published on three large US American cohorts of CKD patients. Se-

veral formulas were derived from these cohorts, which in turn were validated in a clinical population sample from Paris, France. The major finding was that cystatin C levels alone provide GFR estimates that are more accurate than serum creatinine level alone, and equivalent to those obtained by the MDRD formula. The GFR estimates by cystatin C were further improved by the addition of age, sex and race to formula; however, the contribution of these parameters to improvement of GFR estimation was much smaller than for creatinine.

Both markers, cystatin C and creatinine, provide independent information for GFR estimation. The most accurate estimates were provided by a formula combining cystatin C, creatinine, age, sex and race (15).

The recent review article by Herget-Rosenthal, Bökenkamp and Hofmann (7) on GFR estimation provides recommendations on how to use cystatin C and creatinine most efficiently, which in their view depends on the expected range of GFR, and patient characteristics as well as the clinical situation. The major advantage of cystatin C is its sensitivity for mild kidney disease (CKD stage 2 = 60–90 mL/min), a range where all creatinine based methods are unreliable (= creatinine-blind range). If CKD is already manifest (GFR < 60 mL/min) creatinine represents a suitable marker for further monitoring. However, over- or underestimation of GFR is observed in patients with grossly reduced (paralysis, amputation, cachexia) or elevated (body builders) muscle mass by creatinine-based eGFR. For patients with renal failure (< 15–20 mL/min) the recommended mean of creatinine clearance and urea clearance compensates for the over- and underestimation of GFR by the one and the other method. In patients at risk for or with acute renal failure high sensitivity for changes in GFR are most important. Here, cystatin C shows a clear advantage compared to creatinine, allowing the right diagnosis to be made already earlier.

Acute renal failure

In the intensive care setting, the development of acute renal failure (ARF) is a frequent and dangerous complication. Despite the advances in medicine, a high mortality rate of about 40% remained unchanged as treatment following the creatinine-based diagnosis comes too late to prevent non-reversible kidney damage. To improve clinical outcome, a more sensitive diagnostic marker is required which allows the sensitive and accurate detection of small decreases in GFR in the beginning disease.

The performance of cystatin C regarding the detection of ARF was demonstrated in a prospective study on 85 patients at risk to develop ARF, who had daily measurement of cystatin C and creatinine. 44 patients developed ARF according to the RIFLE classification based on creatinine increase. When applying the same rules to cystatin C as to creatinine, ARF was diagnosed 1 to 2 days earlier by cystatin C. The incre-

ase of cystatin C significantly preceded the increase of creatinine. ROC analysis, in which the 41 patients who did not develop ARF served as negative controls, indicated a high diagnostic value for detection of ARF already 2 days before diagnosis by creatinine (AUC 0.97 on day -1; AUC 0.82 on day -2) (16).

Kidney function and cardiovascular risk

The presence of chronic kidney disease is a strong cardiovascular risk factor. In fact, most patients with CKD die from cardiac events before progression to end-stage renal failure. In patients with acute coronary syndromes, an elevation of creatinine or reduction of eGFR is related to a poor prognosis. Thus, the question arose if cystatin C can predict cardiovascular events and poor outcome as well, probably with more sensitivity, and earlier in the course of disease.

The first major publication which looked for a link between cystatin C and cardiovascular disease was published 2004 by Jernberg (2). In a Swedish cohort of 726 patients with acute coronary syndrome, cystatin C levels measured at baseline were related to the mortality observed over the next 40 months. The risk of death during follow-up increased with increasing cystatin C. Patients within the fourth quartile for cystatin C had a 15-times higher mortality in univariate analysis compared to those in the first quartile, the prognosis value being significantly higher than for creatinine or creatinine clearance. In multivariate analysis including demographic data, previous CVD events, and the established cardiovascular risk markers troponin T (RR 2.2), NT-proBNP (RR 3.2) and CRP (RR 2.1), cystatin C was the best marker to discriminate between non-survivors and survivors (RR 4.3).

In a German cohort of patients with newly diagnosed coronary heart disease increased cystatin C was strongly and independently associated with future secondary events. Only cystatin C, but not creatinine or creatinine clearance, was related to adverse events during a 3-year follow-up. Patients in the top quintile of cystatin C had a more than 2-fold risk for secondary events compared to those in the bottom quintile, even after adjustment for a large number of potential confounders, such as inflammation or creatinine and creatinine clearance (3).

The Cardiovascular Health Study (CHS) is a community-based, longitudinal study of elderly adults in the US, sponsored by the NIH, which is designed to evaluate risk factors for the development and progression of cardiovascular disease. To be eligible, persons had to be at least 65 years of age, not institutionalized, expected to remain in the current community, and not under active treatment for cancer. From the total cohort of roughly 6,000 participants, about 4,500 samples were available for analysis of cystatin C. In the first papers published in 2005 on cystatin C in CHS the mean fol-

low up time was about 7 years, which has increased to now 9 years in more recent evaluations.

Within the extensive data set of the CHS, cystatin C was investigated with regard to risk prediction for several endpoints, in particular an association with all cause mortality, cardiovascular mortality and morbidity, incidence of heart failure, cognitive impairment, and progression to chronic kidney disease was investigated.

When comparing cystatin C and creatinine in the CHS for prediction of mortality in elderly persons, only cystatin C was found to be a strong and independent predictor of overall mortality (HR 2.05, multivariate analysis) (3). While the association of creatinine with mortality appeared to be J-shaped, for cystatin C a linear dose-response relationship was seen. As well, in this cohort of ambulatory elderly individuals cystatin C was an independent predictor of mortality from cardiovascular causes (HR 2.27). Furthermore, high cystatin C was associated with newly diagnosed myocardial infarction (HR 1.48) and stroke (HR 1.47). In contrast, only the highest 7% with respect to creatinine levels had a significantly increased risk for all-cause death, and no independent association with any of the other endpoints. Based on cystatin C levels, a low (<1.00 mg/L), intermediate (1.00–1.28 mg/L) and high (>1.28 mg/L) risk group were defined (3).

When CHS participants are subdivided into those with CKD (= MDRD-eGFR > 60 mL/min), those with normal kidney function (eGFR > 60 mL/min and cystatin C < 1.00 mg/L) and a group of patients with »pre-clinical kidney disease« (pre-CKD: eGFR > 60 mL/min, but cystatin C > 1.00 mg/L), a strong association between the kidney function and cardiovascular outcomes is seen, with those with pre-CKD (39% of participants) being at clearly increased risk compared to participants with truly normal kidney function. In addition participants with pre-CKD were at substantially increased risk for progression to chronic kidney disease during follow-up. These findings suggest that elevated cystatin C identifies a state of preclinical kidney disease that is highly prevalent in elderly individuals and points to increased risk for cardiovascular and renal disease (17).

When looking to those participants in CHS without previous heart failure at baseline (18), cystatin C independently predicts the risk for development of heart failure during follow up. After adjustment for demographic factors, traditional and novel risk factors, cardiovascular status, and medication use, sequential quintiles of cystatin C were associated with a stepwise increase in risk for heart failure; quintiles of creatinine were not related to development of heart failure.

In a sub-population of CHS without prevalent CKD, defined by MDRD-eGFR < 60 mL/min/1.73 m², cystatin C was a strong predictor of death, cardiovascular disease, and progression to chronic kidney disease, whereas creatinine levels had almost no association with outcomes in this subpopulation. Individuals with pre-clin-

ical disease characterized by elevated cystatin C (>1.00 mg/L) showed a roughly 4-fold risk to progress to chronic kidney disease (17, 19).

Kidney function is known to decline with age; however, this age-related decline is highly variable and some older individuals have little change in kidney function while others have a rapid decline. Therefore, in the CHS also the relationship was investigated between cystatin C and aging success, defined as remaining free of cardiovascular disease, cancer, chronic obstructive pulmonary disease and having intact physical and cognitive functioning (20). In those CHS participants free of the mentioned conditions at baseline 41% reached a first event during the 6-years follow-up. After adjustment for several predictive factors, in the highest versus the lowest quartile of cystatin C a 27% reduction in successful life years was seen. The risk in the highest quartile was as strongly associated with unsuccessful aging as a decrease in ankle-arm index, left ventricular hypertrophy, or presence of diabetes mellitus. In addition to cardiovascular disease, cystatin C was strongly related to incident cognitive impairment and physical disability (HR 1.39).

All data presented above were from populations with increased risk for cardiovascular disease – patients with already prevalent disease or elderly individuals. Regarding the risk prediction of cystatin C in the general population up to now only one study has been published in abstract form (20). In the PREVEND study, a community-based, longitudinal study in the Netherlands, about 8500 individuals aged 28–75 years were followed over 3 years for death and cardiovascular events. While cystatin C was closely related to mortality, no such association was seen for creatinine. Any adjustment for other confounding factors such as age, sex, weight, smoking, CRP or creatinine did not change the relationship between cystatin C and mortality.

Conclusion

Generally, elevated cystatin C levels are a strong predictor of poor prognosis; elevated cystatin C not only being related to an increased risk for death and cardiovascular events, but also to development of heart failure, hypertension, diabetes, physical disability and cognitive impairment. The association of serum cystatin C with cardiovascular morbidity and mortality most likely reflects early renal dysfunction.

Elevated cystatin C levels > 1.0 mg/L seem to indicate a stage of »subclinical« kidney disease with a substantially increased risk for progression to chronic kidney disease. This higher sensitivity especially in the early stages of CKD, as well as to subtle changes of GFR as seen with beginning ARF or with progression of early diabetic nephropathy make cystatin C the better marker for renal function assessment.

The practical application of cystatin C testing is facilitated by a single reference range and robustness

against any interfering factors, which guarantee reliable results in a wide spectrum of patients.

Cystatin C can improve GFR estimation in general; however there are certain patient groups who benefit most. These are first of all patients without yet established CKD, but at increased risk to develop CKD, such as diabetic or hypertensive patients. In addition patients in question to develop a soon, rapid decline of renal function (as with development of acute renal failure) are candidates for cystatin C testing. As cystatin C is not influenced by muscle mass, GFR estimation in

children and elderly is improved and does not require special age-dependent reference ranges. Furthermore, in patients with advanced liver disease, cystatin c provides a more reliable and sensitive estimate of GFR. Finally, whenever the further prognosis of the patient needs to be evaluated, cystatin C provides independent information in addition to established risk markers.

»... serum cystatin C is a more appropriate and effective biomarker for the overall estimation of GFR than serum creatinine values« (21).

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