

Cystatin C is indispensable for evaluation of kidney disease

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Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this article.

ABSTRACT

The present minireview of the place of cystatin C in clinical medicine emphasizes, and discuss the evidence, that cystatin C-based GFR-estimating equations do not require the use of vague terms like race and sex, that cystatin C-based GFR-estimating equations are useful for both children and adults, including the elderly, that the best GFR-estimation requires simultaneous use of both cystatin C- and creatinine-based equations, that cystatin C-based GFR-estimating equations are superior to creatinine-based equations in predicting end-stage renal disease, cardiovascular manifestations, hospitalisation and death, and, finally that cystatin C is required to diagnose the new syndrome “Shrunken Pore Syndrome” with its high mortality and morbidity, even in the absence of reduced GFR. When automated laboratory equipment is available, the cost of cystatin C is comparable to that of enzymatically determined creatinine.

The conclusion is that cystatin C should be used at least as often as creatinine in clinical medicine.

INTRODUCTION

The introduction of creatinine as a marker of GFR started in 1926 with the publication of an article by Poul Brandt Rehberg: "Studies on kidney function. The rate of filtration and reabsorption in the human kidney" (1). Since then the use of creatinine has been a vital element of clinical medicine. Cystatin C was suggested to be a marker of GFR in 1979 (2) and a few articles published before 1994 supported its use as a GFR-marker (3-5). In 1994 an article with the title "Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate" was published (6), which initiated widespread studies of cystatin C as a marker of GFR. Today, October 2017, the search string in PubMed "Cystatin C AND (renal OR kidney)" produces more than 3500 titles. The information obtained in these 3500 investigations allows the conclusion that the low-cost analysis of cystatin C should be an integral part of the analysis spectrum for optimal evaluation of the kidney status of a patient.

This is because:

- A. Cystatin C-based GFR-estimating equations do not require the use of vague terms like race and sex
- B. Cystatin C-based GFR-estimating equations are useful for both children and adults, including the elderly
- C. The best GFR-estimation requires simultaneous use of both cystatin C- and creatinine-based equations
- D. Cystatin C-based GFR-estimating equations are superior to creatinine-based equations in predicting end-stage renal disease, cardiovascular manifestations, hospitalisation and death
- E. Cystatin C is required to diagnose the new syndrome "Shrunken Pore Syndrome" with

its high mortality and morbidity, even in the absence of reduced GFR.

CYSTATIN C-BASED GFR-ESTIMATING EQUATIONS DO NOT REQUIRE THE USE OF VAGUE TERMS LIKE RACE AND SEX

One of the main advantages of cystatin C compared to creatinine as a GFR-marker is that it is less dependent upon the body composition of a patient. For example, while muscle mass strongly influences creatinine, it does not, or only marginally, affect cystatin C (7-11).

Creatinine-based GFR-estimating equations therefore contain terms aiming at evaluating the muscle mass of a specific patient. These terms refer to the race and sex of the patient. Specific race-factors have been suggested for Afro-Americans (12), Japanese (13,14), Chinese (15), Koreans (16), and native Americans and Hispanics (17). But "race" is a very vague term, difficult to define and does not consider the problem that a major part of the world population represents persons with mixed ethnicity. In contrast, the cystatin C concentration varies only marginally with ethnicity and no vague race terms are therefore required in cystatin C-based GFR-estimating equations (18-20).

The mean muscle mass of females is lower than that of males and creatinine-based GFR-estimating equations therefore requires significant sex-related factors for females (21). However, the world is less and less sex-dichotomized and the existence of more than two sexes is now acknowledged in several countries (22). This ambiguity in applying creatinine-based GFR-estimating equations does not apply for some cystatin C-based GFR-estimating equations, since muscle mass only marginally, or not at all, influences the cystatin C-level and thus cystatin C-based GFR-estimating equations do not require factors for sex (20).

CYSTATIN C-BASED GFR-ESTIMATING EQUATIONS ARE USEFUL FOR BOTH CHILDREN AND ADULTS, INCLUDING THE ELDERLY

The strong correlation between muscle mass and creatinine poses a special problem concerning the use of creatinine-based GFR-estimating equations in childhood, since the muscle mass strongly increases with age. As a consequence, different equations generally have to be used for adults and children (23-25). In contrast, since the muscle mass does not influence cystatin C significantly many cystatin C-based equations work for both children and adults (20, 23-25). One of them is the CAPA-equation which has been shown to work from 1 - 50 years of age (20, 25 and unpublished observations by Grubb A, et al.). Another problem related to the use of creatinine-based equations is that the muscle mass in the elderly is often considerably reduced, so that it negatively affects the ability of these equations to demonstrate a reduced GFR in the elderly. In contrast, cystatin C-based equations are not significantly influenced by muscle mass and therefore useful in identifying reduced GFR also in the elderly with low muscle mass (26).

THE BEST GFR-ESTIMATION REQUIRES SIMULTANEOUS USE OF BOTH CYSTATIN C- AND CREATININE-BASED EQUATIONS

Although creatinine-based GFR-estimating equations are inferior in diagnostic performance compared to cystatin C-based equations for several populations, it has generally been shown that the best GFR-estimation requires use of both cystatin C and creatinine in the equation (27-31). The best estimates of GFR, produced by cystatin C-based equations, $eGFR_{\text{cystatin C}}$, produce values of which 80-85% are within $\pm 30\%$ of GFR measured by invasive gold-standard methods and similar figures are valid for the corresponding estimates, $eGFR_{\text{creatinine}}$, obtained by

creatinine-based equations (27-31). Equations using both cystatin C and creatinine might produce values of which 90-91% are within $\pm 30\%$ of GFR measured by invasive gold-standard methods (30,32). Still better results are obtained if the mean, $eGFR_{\text{mean}} = (eGFR_{\text{cystatin C}} + eGFR_{\text{creatinine}})/2$ of the estimates obtained by a cystatin C- and a creatinine-based equation are used, rather than complex equations containing both cystatin C and creatinine (32-34). This is due to that combined equations do not perform optimally in a number of clinical situations, for example, if the patient has an abnormally low muscle mass or is treated with a high dose of glucocorticoids. A strategy for GFR estimation based on the automatic use of a combined cystatin C and creatinine-based equation will, in these cases, have a worse diagnostic performance than a strategy that only uses the cystatin C- or creatinine-based GFR-estimating equation *not* influenced by the specific patient characteristics (33,34). Such a strategy thus requires that GFR is estimated by both a cystatin C- and a creatinine-based equation, producing $eGFR_{\text{cystatin C}}$ or $eGFR_{\text{creatinine}}$, and that the results are compared. If the two equations produce similar estimates, their average is a very reliable estimate of GFR. If the estimates do not agree and a specific factor known to disturb either the cystatin C- or creatinine-based estimate is present, only the estimate produced by the equation *not* disturbed by this factor, is used (33,34). As a matter of fact, since 1994, when cystatin C-based estimations of GFR were introduced in Lund in parallel with creatinine-based estimations, we have had 20-30 cases for which $eGFR_{\text{cystatin C}}$ and $eGFR_{\text{creatinine}}$ agreed, but disagreed with GFR measured by our invasive gold-standard procedure (plasma clearance of iothexol). In all cases, in which relevant information was available, the error was caused by technical problems in the execution of the gold-standard procedure. We therefore consider that, in practice, $eGFR_{\text{mean}}$ based upon agreeing

$eGFR_{\text{cystatin C}}$ and $eGFR_{\text{creatinine}}$ is at least as reliable as GFR measured by invasive gold-standard procedures (33,34). This strategy is described at the multilingual site www.egfr.se (35), which can also be implemented to calculate absolute GFR from relative GFR, which might be required in, *e.g.*, dosing of medicines cleared by the kidneys.

CYSTATIN C – BASED GFR-ESTIMATING EQUATIONS ARE SUPERIOR TO CREATININE-BASED EQUATIONS IN PREDICTING END-STAGE RENAL DISEASE, CARDIOVASCULAR MANIFESTATIONS, HOSPITALISATION AND DEATH

One important reason to estimate GFR in a patient is to decide whether the patient suffers from chronic kidney disease or not, and to classify the degree of the chronic kidney disease, if present. Both $eGFR_{\text{cystatin C}}$ and $eGFR_{\text{creatinine}}$ work well for this purpose. However, another important aspect of the estimation is how well it predicts the consequences of kidney disease, *e.g.*, end-stage renal disease, cardiovascular manifestations, hospitalisation and death, since this knowledge influences decisions concerning the intensity of the treatment modalities. In this respect, $eGFR_{\text{cystatin C}}$ and $eGFR_{\text{creatinine}}$ differ, because the published scientific studies virtually unanimously show that $eGFR_{\text{cystatin C}}$ is significantly superior to $eGFR_{\text{creatinine}}$ (36-39).

The cause for the superiority of $eGFR_{\text{cystatin C}}$ as a risk marker is unknown, but observational studies have shown that inflammation, old age, male gender, greater weight, and cigarette smoking correlate with higher cystatin C levels (40). But statistical correlations in observational studies do not prove causal connections. A study of elective surgery of patients demonstrated a postoperative sharp rise in inflammation of the patients, with large increases in the levels of CRP of all patients, but with no increase in the cystatin C levels, thus rejecting the hypothesis

that inflammation causes a raise in the production of cystatin C (41). The correlations between inflammation, old age, male gender, greater weight, and cigarette smoking and cystatin C might be due to that all these factors promote the development of atherosclerosis, also in the renal arteries, thus producing a decrease in GFR and an increase in cystatin C (41). These correlations therefore speak in favour of cystatin C as a GFR-marker and not against it.

CYSTATIN C IS REQUIRED TO DIAGNOSE THE NEW SYNDROME “SHRUNKEN PORE SYNDROME” WITH ITS HIGH MORTALITY AND MORBIDITY, EVEN IN THE ABSENCE OF REDUCED GFR

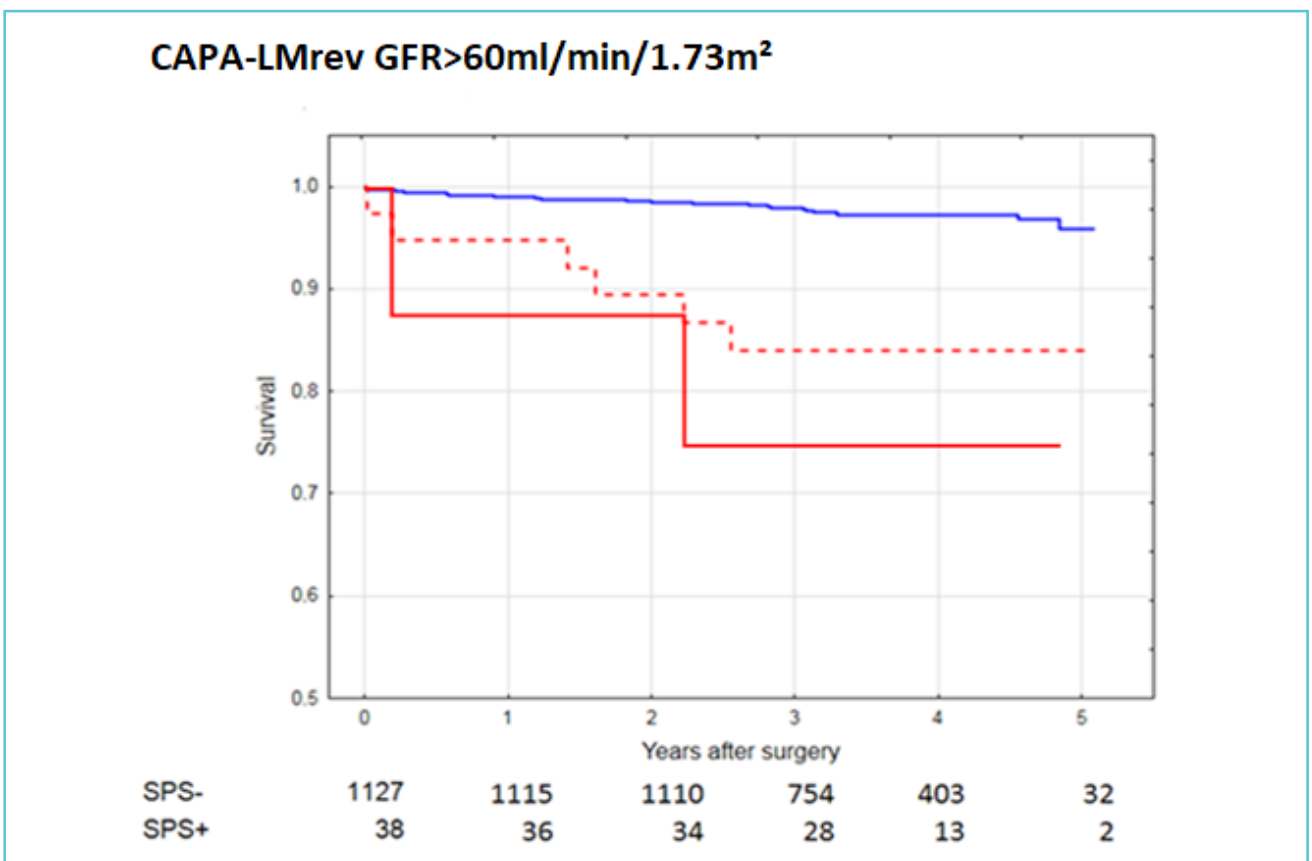
The use of $eGFR_{\text{mean}}$ and the simultaneous comparison of $eGFR_{\text{cystatin C}}$ and $eGFR_{\text{creatinine}}$ as the best way to estimate GFR in clinical practice (32-34,42) identifies a number of patients with significant differences between $eGFR_{\text{cystatin C}}$ and $eGFR_{\text{creatinine}}$. Part of these differences can be explained by factors, such as muscle wasting or treatment with large doses of glucocorticoids, known to invalidate the GFR estimations based on creatinine or cystatin C (33). But the majority of the patients with such differences between $eGFR_{\text{cystatin C}}$ and $eGFR_{\text{creatinine}}$ do not display any known such factor and their $eGFR_{\text{mean}}$ is, despite the differences between $eGFR_{\text{cystatin C}}$ and $eGFR_{\text{creatinine}}$, still the best way to estimate GFR (41).

Most of the patients displaying these differences has a pattern of $eGFR_{\text{cystatin C}}$ and $eGFR_{\text{creatinine}}$ in which $eGFR_{\text{cystatin C}}$ is lower than $eGFR_{\text{creatinine}}$ (42,43). When the levels of low-molecular mass proteins other than cystatin C, *e.g.*, β_2 -microglobulin, β -trace protein, and retinol-binding protein, were determined in patients with $eGFR_{\text{cystatin C}} \leq 60\%$ of $eGFR_{\text{creatinine}}$, it was observed that the concentration ratios of these proteins to creatinine were, like the cystatin C-creatinine ratio, higher, than in patients in whom $eGFR_{\text{cystatin C}} \approx eGFR_{\text{creatinine}}$ (43).

The genes for these proteins are located at different chromosomes and have different regulation elements and the synthesis of these proteins is not generally known to be influenced by factors affecting the production of cystatin C (43). This strongly indicates that the production of these proteins and cystatin C is not co-regulated and therefore cannot explain the concordant increases of their plasma levels. But the concurrent increase can be explained if the proteins have a common clearance mechanism by glomerular filtration and that this is

reduced by shrinking of the glomerular pores (43). Therefore, the observation that $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ in a patient indicates the presence of a new syndrome, tentatively called "Shrunken Pore Syndrome" (43). The explanation that creatinine and other small molecules do not simultaneously increase in concentration would then be, that their sieving coefficients are still close to unity (*i.e.*, one) despite the shrunken pores resulting in reduced sieving coefficients for proteins similar in size to cystatin C (43-45).

Figure 1A Survival after coronary artery bypass surgery for patients with $GFR > 60\text{ mL/min per }1.73\text{ m}^2$ with and without Shrunken Pore Syndrome (SPS)



$eGFR_{cystatin\ C}$ was estimated using the CAPA equation and $eGFR_{creatinine}$ using the LMrev equation.

The cut-off level for SPS was $eGFR_{cystatin\ C} \leq 70\%$ of $eGFR_{creatinine}$ (red broken line) or $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ (red unbroken line).

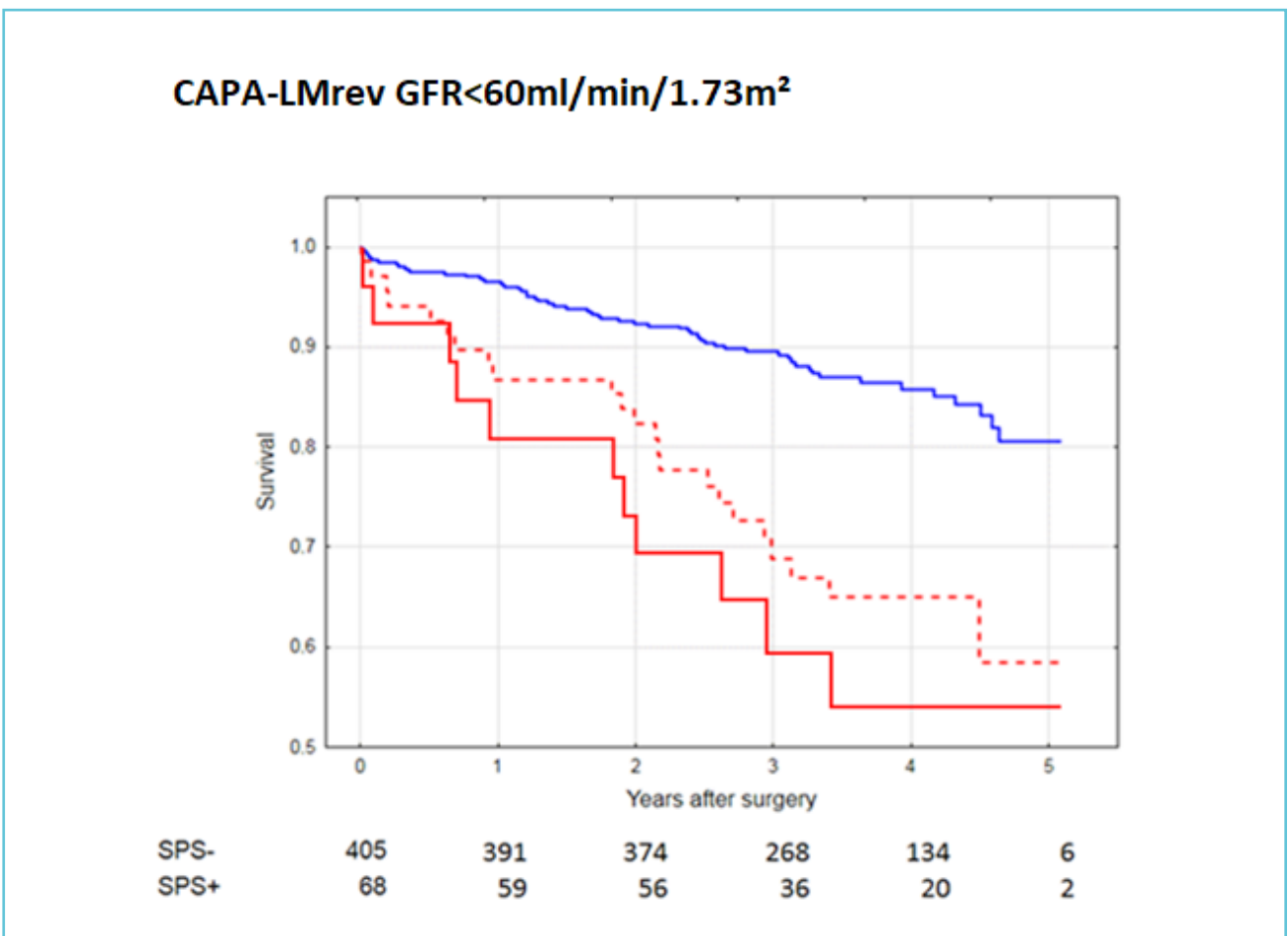
The unbroken blue line indicates the mortality of patients without SPS ($0.90 < eGFR_{cystatin\ C} / eGFR_{creatinine} < 1.10$).

The numbers below indicate patients with and without SPS, when the cut-off level was 70%.

It is noteworthy, that a similar mechanism previously has been suggested for the increase in plasma levels of low-molecular mass proteins in the third trimester of pregnancy (46-48) and for the development of still higher concentrations of low-molecular mass proteins in preeclampsia (49,50). This suggests that the (patho-)physiologic changes in late pregnancy and preeclampsia are similar to those occurring in patients with “Shrunken Pore Syndrome.”

As “Shrunken Pore Syndrome” was identified recently (43), only a few studies of its clinical consequences have been performed. The first investigation showed, that the long-term mortality in patients undergoing elective coronary artery bypass grafting was much higher in patients suffering from “Shrunken Pore Syndrome” than in patients without the syndrome (51). This was true both when the preoperative GFR was normal or reduced (Figure 1A and B). In this study, the

Figure 1B Survival after coronary artery bypass surgery for patients with GFR < 60 mL/min per 1.73 m² with and without Shrunken Pore Syndrome (SPS)



$eGFR_{\text{cystatin C}}$ was estimated using the CAPA equation and $eGFR_{\text{creatinine}}$ using the LMrev equation.

The cut-off level for SPS was $eGFR_{\text{cystatin C}} \leq 70\%$ of $eGFR_{\text{creatinine}}$ (red broken line) or $eGFR_{\text{cystatin C}} \leq 60\%$ of $eGFR_{\text{creatinine}}$ (red unbroken line).

The unbroken blue line indicates the mortality of patients without SPS ($0.90 < eGFR_{\text{cystatin C}} / eGFR_{\text{creatinine}} < 1.10$).

The numbers below indicate patients with and without SPS, when the cut-off level was 70%.

cystatin C-based CAPA-equation was used to produce $eGFR_{\text{cystatin C}}$ and the creatinine-based LM-rev-equation to produce $eGFR_{\text{creatinine}}$, as both these equations work not only for adults, but also for children (20,52,53). Interestingly, an increase in mortality was not only observed when $eGFR_{\text{cystatin C}} \leq 60\%$ of $eGFR_{\text{creatinine}}$, but also when $eGFR_{\text{cystatin C}} \leq 70\%$ of $eGFR_{\text{creatinine}}$ (Figure 1 A and B). Ongoing studies demonstrate that the long-term mortality in “Shrunken Pore Syndrome” increases inversely with the $eGFR_{\text{cystatin C}}/eGFR_{\text{creatinine}}$ -ratio, starting at 0.90. Recently published and ongoing studies in several different types of populations corroborate, that “Shrunken Pore Syndrome” is associated with significantly increased mortality and morbidity (54,55) and indicate that the syndrome also predicts higher risks for development of end-stage renal disease, cardiovascular manifestations and for hospitalisation.

CONCLUSION

The use of cystatin C (or $eGFR_{\text{cystatin C}}$) in addition to creatinine improves the estimation of GFR, makes it independent of vague terms like race and sex, and facilitates its use for children and the elderly. It also allows the identification of a new syndrome (Shrunken Pore Syndrome) associated with a high morbidity and mortality. When automated laboratory equipment is available, the cost of cystatin C is comparable to that of enzymatically determined creatinine. Cystatin C should therefore be used at least as often as creatinine in the clinical routine.

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