CYSTATIN C – A NOVEL MARKER OF GLOMERULAR FILTRATION RATE : A REVIEW

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ABSTRACT

Glomerular filtration rate is routinely assessed by measuring the serum markers such as urea nitrogen and serum creatinine. Although these markers are widely used to assess renal function but they do not perform optimally in certain clinical settings. There is thus a practical need for an easily automated alternative to plasma creatinine, which would be more specific, sensitive and reliable from the analytical and clinical view point. Compared with the above endogenous markers, and time consuming laborious tests, Cystatin C facilitates the recognition of abnormal renal function in children, as its reference range is constant beyond the 1st year of life. This review mainly focuses on the diagnostic performance of Cystatin C against other renal markers in the pediatric population and in specific subpopulations of patients.

KEY WORDS

Cys C – cystatin C; Scr – Serum creatinine, GFR – Glomerular Filtration Rate, ROC – Receiver Operating Curve, Crcl-Creatinine clearance..

INTRODUCTION

Glomerular Filtration Rate (GFR) is defined as the volume of plasma that can be completely cleared of a particular substance by the kidneys in a unit of time [1]. It is routinely assessed by measuring the concentrations of serum markers such as blood urea nitrogen and serum creatinine. Although widely used, these endogenous markers are not ideal and do not perform optimally in certain clinical settings. The other methods for determining GFR is to measure the clearance of exogenous substances such as inulin, iothexol, ^{51}Cr – EDTA, $^{99\text{mTc}}$ labeled diethylene triamine penta acetic acid (DTPA) or ^{125}I labeled iothalamate. These techniques are time consuming, expensive, labor – intensive and require administration of substances that make them incompatible with routine monitoring. Properties of an ideal endogenous blood substance to estimate GFR should include release into the blood stream at a constant rate, free filtration by glomerulus, no reabsorption or secretion by the renal tubules and elimination via kidneys.

Calculation of creatinine clearance by determining its concentration in timed urine collections and

simultaneously in blood correlates with standard exogenous methods better than serum creatinine [5]. However, collection of timed urine sample is cumbersome and prone to error in the out patient setting. Thus, despite their common use, blood urea nitrogen and serum creatinine have limitations as renal markers, and the search for an ideal endogenous marker of GFR continues [6]. There is thus a practical need for an easily automated alternative to plasma creatinine which would be more specific, sensitive and reliable from the analytical and clinical view point.

In the last 50 years, serum urea and serum creatinine estimation has become the most commonly used serum markers of renal function. However, urea concentration in the blood can vary with diet, hepatic function and numerous disease states [2]. Further more, rate of appearance of serum creatinine in the blood stream is related to muscle mass and its blood concentrations are affected by age and gender [3]. As plasma concentrations increase, tubular secretion of serum creatinine increases, leading to an over estimation of GFR in patients with moderate to severe decrease in GFR (<50 mL / min) [4]. Moreover, serum creatinine is also insensitive for detecting small decreases in GFR because of the nonlinear relationship between plasma concentration and GFR [5,7].

Low molecular weight proteins have been suggested to replace serum creatinine. Serum or plasma cystatin

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C (Cys C) may be a better marker for GFR than serum creatinine [8].

Cys C offers an advantage over creatinine because of its age and gender independence [10, 11]. Human cystatin C is a 13 Kda basic (PI = 9.3) non – glycosylated protein produced by all nucleated cells [9]. It contains 122 amino acids and it is a member of the family of cysteine proteinase inhibitors. It is the product of a "house keeping" gene expressed in all nucleated cells and is produced at constant rate [10]. Because of its small size and basic PI, Cys C is freely filtered by the glomerulus. It is not secreted, but is reabsorbed by tubular epithelial cells and subsequently catabolized so that it does not return to the blood flow [11]. This property negates calculations of Cys C clearance using urine concentrations of Cys C. The use of serum Cys C to estimate GFR is based on the same logic as the use of blood urea nitrogen and creatinine, but because it does not return to the blood stream and is not secreted by renal tubules, it has been suggested to be closer to the "ideal" endogenous marker.

Determination of Cystatin C:

The first immuno assay to quantify cys C was developed by Lofberg and Grubb [12]. The methods include to detect cys C are:-

1. RIA (detection limit of 30 mg/l) [12].
2. Fluorescent and enzymatic immuno assays The detection limit of these assays ranged from 0.13 to 1.9 mg/l [13, 14, 15].
3. Nephelometric assay (the reference range was 0.8 – 1.03 mg/l) [20].
4. Particle enhanced turbidometric immuno assay [18, 19].

The reference intervals were identical for males and females [16, 17, 18]. More recently automated homogenous immuno assays utilizing latex or polystyrene particle coated with cys C – specific antibodies were developed [19]. Estimation of serum Cys C by particle enhanced turbidometric immuno assay seems to be simple to perform compared to other methods, as they are time consuming and expensive for routine determination of serum Cystatin C. This assay is generally more precise than the earlier methods and reference intervals seem to be more consistent than those reported from earlier assays. Finally, the Particle enhanced turbidometric immuno assay is practical and easy alternative for routine determination of serum Cystatin C.

Only hyperbilirubinemia was associated with higher cystatin C concentration. However, it has been shown that bilirubin concentrations up to 700 $\mu\text{mol/l}$ do not interfere with the immunoturbidometric assay for

cystatin C [23]. Despite normalization of bilirubin levels after the first weeks of life, higher cystatin C concentration persisted for several months, further excluding assay interference by hyperbilirubinemia.

Receiver Operating Characteristic (ROC) analyses have become popular in recent years for evaluating the discriminatory power of a test [24, 25]. The ROC plot displays graphically the relationship between the true positive rate (sensitivity) and the false positive rate (specificity) over all possible decision values. The decision value is the variable test value that is used to discriminate between apparently healthy and affected subjects. The ROC analysis showed that the diagnostic accuracy of cystatin C was significantly better than that of creatinine in discriminating between subjects with normal renal function and those with reduced GFR [23].

Reference Range:

Kyhse – Andersen et al [18] calculated a reference range of 0.61–1.21 mg/l for cystatin C measured in 27 adults with a GFR above 80 ml/min per 1.73 m². Newman et al [23] determined a cut – off concentration of 0.25 mg/l for a GFR below 72 ml/min per 1.73 m² in 20 patients. In recent cross – sectional study, Norund et al [17] found a reference range of 0.7 – 1.21 mg/l in 120 adults 20 – 50 years of age. Pergande and Jung [19], however, reported much higher cystatin C concentrations (upper limit of normal 2.75 mg/l in men and 2.29 mg/l in women). This difference is probably due to different calibrator material. Arend Boken Kamp et al [26] gave the reference limit [26] (0.38 mg/l in a pediatric series of 216 urological patients (mean age 11 years).

Gender and external disease did not alter serum cystatin C concentrations. This is in accordance with two previous studies [23, 17]; however, others [19] found higher levels in males. It was found that cys C is higher before the age of 3 months and after the age of 70 years [21, 22]. The decrease in GFR and increased cys C value above the age of 70 years was found in some studies [22]. This property made it attractive to further examine cys C in certain groups of patients.

Cystatin C in pediatric patients:

The estimation of the GFR is an important part of the clinical evaluation of renal function and of the management of renal diseases in children. Plasma clearance of inulin or 51Cr EDTA are expensive and time consuming tests, which makes them unattractive for clinical use. A series of studies in adult patients have suggested that cystatin C correlates with GFR as strongly as creatinine or even more strongly [18, 23, 27].

Cys C has been postulated to have an advantage over

S Cr in pediatric populations because of the low muscle mass in children, which leads to very low Scr values, where increased assay imprecision is present. Therefore, it can be difficult to accurately detect small changes in GFR with S Cr in children < 4 years of age in whom normal S Cr values are only 2.0 – 4.0 mg/L. On the other hand, plasma concentration of Cys C appears to be rather constant in children > 1 year of age and similar to that of adults [38, 39, 40, 41]. For example in one study, the reference intervals for serum Cys C was 0.51 – 0.95 mg/L among 125 healthy children between the ages of 1 and 14 years [38] which is virtually identical to adult reference intervals. These authors also showed that immediately after birth, Cys C values were approximately twice those of older children and adults, but that they reached a mean value of 0.95 mg/L by 1 – 2 months of age. There are few studies [42, 43, 44] that showed increased Cys C values in the blood of premature infants.

Depending on the number of patients and methods used for measuring cystatin C, creatinine and GFR, the correlations have varied in different studies. The correlation between serum cystatin C concentration and GFR was significantly stronger ($P < 0.05$) than that between creatinine concentration and GFR in a recently published study performed in pediatric patients [28].

Elisa et al [30] showed in their study that serum cystatin C was superior to that of creatinine in discriminating between children with normal and reduced GFR. Sensitivity and specificity of serum cystatin C were higher than the corresponding characteristics of serum creatinine, and similar results have also been reported in adult patients by Kyhse – Andersen et al [18]. Boken Kamp et al [29] used a cut off concentration of 1.39 mg/l for cystatin C and Helin et al [28] determined reference values of cystatin C to be 0.63 – 1.33 mg/l for children over 1 year of age.

Where as the best diagnostic efficiency (98%) for a reduced GFR was reached when an upper cut off limit of 1.31 mg/l was used for cystatin C in a pediatric population study performed by Elisa et al [30]. Earlier studies have reported cystatin C values to be constant after the 1st year of life. A Harmoinen et al [37] showed that children between 1 and 3 years in age have slightly, but statistically significant higher cystatin C values than older children. The pre-term infants had higher cystatin C concentrations (mean 1.88 mg/l SD 0.36 mg/l) than the full term (mean 1.70 mg/l SD 0.2 mg/l, $P=0.01$). The reference interval for pre term infants calculated non parametrically was 1.34 – 2.57 mg/l and for full term infants 1.36 – 2.23 mg/l. The cystatin C concentrations decreased rapidly after birth, and above 3 years of age did not depend on age [37]. During the 1st year of life the decrease in the plasma cystatin C correlated significantly with age. This decrease still continued, but more slowly during the next 2 years

reflecting the maturation of the kidneys [29]. Accordingly, serum cystatin C appears to represent a useful and simple tool both for the identification of children with reduced GFR (high sensitivity) and for the exclusion of children with normal GFR (high specificity).

Plasma creatinine is a poor marker of GFR, when renal function is normal or only slightly reduced [31, 32]. Its age dependency further reduces its value as a marker for GFR in children. Adjustment for height [33] may improve the usefulness of creatinine [17], but not necessarily [34]. Although a clear difference has been found between female and male creatinine concentrations in adults, [35,36] the values were identical in children of both sexes. Plasma creatinine might be useful for detecting temporal changes in renal function in individuals with established renal disease.

Ylinen et al [45] concluded that Cys C performed better than serum creatinine to estimate GFR based on the areas under ROC curves (0.9896 Vs 0.9171; $p = 0.04$). Using upper reference limits of 1.31 mg/L for Cys C and 10.3 mg/L for Scr, they estimate that the sensitivity and specificity were 100% and 97% for Cys C and 74% and 97% for Scr for detecting a GFR < 90 mL/min.

The available studies in children to date indicate that Cys C is atleast as useful as Scr to assess GFR, with several indicating that Cys C may perform better. However, the number of children under 4 years of age, for whom it is hypothesized that Cys C may be most effective. Larger prospective studies still need to be done to validate this hypothesis.

Cystatin C in other patient groups

There are two reasons to monitor renal function in cancer patients under going chemotherapy: a) direct damage to the renal tubules by chemotherapeutics and their metabolites in the presence of decreased GFR. For example, the dose of cisplatin must be reduced by one – half when GFR is < 60 mL/min [46].

Therefore, it should be beneficial to detect changes in renal function as early as possible.

Stabuc et al [47] explored Cys C as an early indicator of renal damage in 72 patients receiving cisplatin chemotherapy for malignant melanoma, gastric cancer or ovarian cancer. All but 12 had a GFR < 78 mL/min by $^{51}\text{Cr-EDTA}$ clearance. The correlation to GFR was significantly better for Cys C than Scr and ROC analysis indicated that Cys C was a better indicator than Scr for predicting a GFR < 78 mL/min. The sensitivity and specificity were 100% and 87% respectively, for serum Cys C compared with 61% and 98% respectively, for Scr. They found that these results were independent of the presence of metastases and concluded that Cys C may be more useful than Scr for monitoring renal function during cisplatin therapy [47].

After renal transplantation, patients are at risk of acute damage may lead to more effective intervention. In a preliminary study, LeBricon et al [48] first suggested that Cys C was more sensitive than Scr for detecting decreases in GFR and delayed graft function in renal transplant patients. As in most studies, plasma Cys C measurements correlated well with Scr and Crcl. However, in the three cases of acute renal resection that were confirmed by biopsy, the increase in plasma Cys C values was more pronounced than that observed for Scr. For example, one patient had a 100% increase in Cys C Vs a 40% increase in Scr 5 days before biopsy – confirmed acute rejection. In this patient, as well as another with confirmed acute rejection and another with FK 506 toxicity, Cys C increased earlier and more rapidly than did Scr. The authors of the above study also concluded that Cys C was more sensitive than Scr and Crcl in post – renal transplant patients ($P < 0.01$) because no false – negative results for detecting impaired renal function (defined as $< 80 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$) in these transplant recipients were found, whereas plasma creatinine and Crcl produced ~ 25% false negatives.

The epidemiologic studies have show an dramatic increase in incidence and prevalence of end stage renal disease (ESRD) in patients with type 2 diabetes [49]. Accurate evaluation of glomerular filtration rate is thus of crucial importance in diabetic patients to direct early renal impairment. Early makers of diabetic nephropathy thus need to be identified. Although micro albuminuria is considered to be a risk factor for diabetic nephropathy and progressive renal insufficiency [50, 51], recent investigations have raised questions about its predictive value [52] owing to its variability and low predictivity for the underlying renal pathology.

Caroline perlemoine et al evaluated in their study [53] that cystatin C as a marker of renal impairment in a large population of diabetic patients. Cystatin C was well correlated with GFR, and its diagnostic accuracy was comparable to that of creatinine. The high sensitivity of cystatin C and its diagnostic accuracy at low creatinine levels makes it a good prediction of later renal impairment in diabetic patients, especially as the value of micro albuminuria in there patients has been questioned by recent work [53].

Buysschaert M, Joudi et al showed in their study that serum cystatin C better discriminates among a population of type 1 and 2 diabetic patients with regard to their estimated glomerular filtration rate when compared with conventional serum creatinine measurement [54].

Whereas Shimizu A, Horikoshi S, et al concluded that serum cystatin C was better than Scr in terms of sensitivity and specificity. It appears that the levels of serum cystatin C may predict early prognositc stages

of patients with type 2 diabetic nephropathy [55].

It has been observed that thyroid dysfunction may alter creatinine, which has been found to be increased in hypothyroidism and decreased in hyper thyroidism. Fuicker M. et al performed a study to evaluate whether changes in Cys C and creatinine are parallel during the treatment of hypo and hyperthyroidism [56]. The authors of this study concluded that thyroid dysfunction has a major impact on Cys C levels. Therefore, thyroid function has to be considered when Cys C is used as a marker of kidney function. In contrast to creatinine concentrations, cystatin C levels are lower in the hypothyroid and higher in the hyper thyroid state as compared with the euthyroid state [56].

Another interesting finding was observed in one study [57] that serum cystatin C may be used as a marker, not only for impaired renal function, but also for the degree of glomerular endotheliosis and increase in glomerular volume in pregnancy. It may be of value in the monitoring of pregnancies complicated by pre-eclampsia.

Thus, this review demonstrates that serum cystatin C offers a more efficient diagnostic tool than serum creatinine in adults and in children with renal disease.

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