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Cystatin C as a marker for glomerular filtration rate in pediatric patients

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Abstract Cystatin C is a non-glycated 13-kilodalton basic protein produced by all nucleated cells. The low molecular mass and the basic nature of cystatin C, in combination with its stable production rate, suggest that the glomerular filtration rate (GFR) is the major determinant of cystatin C concentration in the peripheral circulation. Recently published studies have shown that cystatin C correlates more strongly than creatinine with GFR measured using the ^{51}Cr-EDTA clearance. The aim of this study was to evaluate serum cystatin C as a marker for GFR in children. GFR was determined on medical indications using the ^{51}Cr-EDTA technique in pediatric patients (2–16 years) in our renal unit. Simultaneously their cystatin C and creatinine concentrations were also measured. Of our 52 patients, 19 had a reduced renal function (<GFR 89 ml/min per 1.73 m^2) based on the ^{51}Cr-EDTA clearance. The correlation of cystatin C with the isotopic measurement of GFR tended to be stronger (r=0.89, P=0.073) than that of creatinine (r=0.80). Receiver operating characteristic analysis showed that the diagnostic accuracy of cystatin C was better (P=0.037) than that of creatinine in discriminating between subjects with normal renal function and those with reduced GFR. This study demonstrates that serum cystatin C has an increased diagnostic accuracy for reduced GFR when compared with serum creatinine. Hence, cystatin C seems to be an attractive alternative for the estimation of GFR in children.

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Introduction

Glomerular filtration rate (GFR) is the most-important functional parameter in pediatric nephrology. Plasma or serum creatinine is the marker most widely used to predict GFR. Unfortunately, the circulating creatinine concentration also reflects creatinine production, which is proportional to muscle mass [1]. This phenomenon reduces its value as a marker of GFR, especially in children. Creatinine also often fails to detect patients with modestly reduced renal function [2]. There is thus a need for a simple marker of GFR that is clinically more reliable than creatinine.

Several low molecular weight proteins have been studied as candidate markers of GFR. The difficulty with the low molecular weight proteins is that the production rate may vary due to infection, dietary factors, or liver disease. Human cystatin C is a low molecular weight protein that has been found in several human body fluids [3]. It is produced by all nucleated cells and its production rate is unaltered in inflammatory conditions [4]. As a small basic (pI=9.3) protein it passes easily through the glomerular basement membrane and is then catabolized by the renal tubular cells [5]. It seems to be eliminated from the circulation almost exclusively by glomerular filtration [6], which makes it a promising indicator of GFR. It is also age independent in children, except for infants under 1 year of age [7, 8]. Several studies in adult patients have shown that cystatin C correlates more or at least as strongly as creatinine with GFR [9–11], and two recently published studies have reported similar results in children [12, 13].

Receiver operating characteristic (ROC) analyses have become popular in recent years for evaluating the discriminatory power of a test [14–17]. The ROC plot displays graphically the relationship between the true-positive rate (sensitivity) and the false-positive rate (1-

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 aim of the present study was to evaluate cystatin C as a marker of renal function in children in comparison with creatinine using the ^{51}Cr-EDTA clearance [18] as the gold standard.

Patients and methods

Patients

Serum cystatin C and creatinine concentrations were studied in 52 pediatric patients (24 girls and 29 boys, aged 2–16 years, median 9.9 years) with renal diseases (Table 1), whose GFR was determined simultaneously on medical indications. Approval of the study was obtained from the ethics committee of Tampere University Hospital.

Methods

Serum cystatin C concentrations were determined with a particle-enhanced turbidimetric immunoassay (Dako, Glostrup, Denmark) using a Hitachi 704 analyzer as follows. Standard or sample (15 µl) and reaction buffer (335 µl) were pipetted into a cuvette and the blank value was measured after 5 min at 340 nm. Then 45 µl of the latex reagent was added and after 5 min incubation the absorbance was again measured at 340 nm, and the result calculated from the differences in absorbance readings. The precision was assessed by measuring two controls 20 times in a series and between series over a time period of 20 months. At a level of 1.5 mg/l the intra-assay coefficient of variation (CV) was 2.6% and the interassay CV 6.6%. At the level of 5.8 mg/l the intra- and interassay CVs were 0.9 and 3.2%, respectively. Creatinine was measured with a kinetic picrate method [19] using the same instrument. At a level of 100 µmol/l the intra-assay CV was 2.05% and interassay CV 3.17%. At a level of 180 µmol/l the intra- and interassay CVs were 1.06 and 1.02%, respectively. At 650 µmol/l the intra- and interassay CVs were 0.64 and 2.42%, respectively. The GFR was determined by the plasma clearance of ^{51}Cr-EDTA assessed by the single-injection method [18].

Statistics

The data were evaluated with standard parametric tests using Microsoft Excel calculation programs (Version 5.0, Microsoft, Incline Village, Nev., USA). The ROC analyses and the maximum efficiency testing were performed using software purchased from Turku University (copyright owners Veli Kairisto and Allan Poola) [17]. Comparison of correlation coefficients was performed after z-transformation. *P* values below 0.05 were considered statistically significant.

Table 1 Etiology of the kidney disease in the children studied (*n*=52)

Diagnosis	*n*
Operative obstructive uropathy	17
Renal malformations	13
Chronic glomerulonephritis	7
Hereditary kidney diseases	7
Reflux nephropathy	4
Hemolytic uremic syndrome	3
Other	1

Results

Of our 52 patients, 19 had a reduced GFR (<89 ml/min per 1.73 m^2) [20]. Serum concentrations of creatinine and cystatin C were inversely related to GFR. This curvilinear relationship was linearized by using the reciprocals of the measured concentrations. There was a strong correlation between the reciprocal concentrations of cystatin C and GFR measured using the ^{51}Cr-EDTA clearance (r=0.89, P<0.001) (Fig. 1). The correlation between reciprocal concentrations of serum creatinine and GFR was somewhat weaker (r=0.80, P<0.001), although not significantly so (P=0.073). The correlation between GFR and the reciprocal cystatin C (r=0.90) tended to be stronger (P=0.08) than that between GFR and the reciprocal creatinine (r=0.75) in patients with a reduced GFR (n=19). The correlation between predicted creatinine clearance calculated from the formula of Schwartz et al. [21] and ^{51}Cr-EDTA was 0.81. The predicted creatinine clearance also correlated well with reciprocal cystatin C (r=0.85).

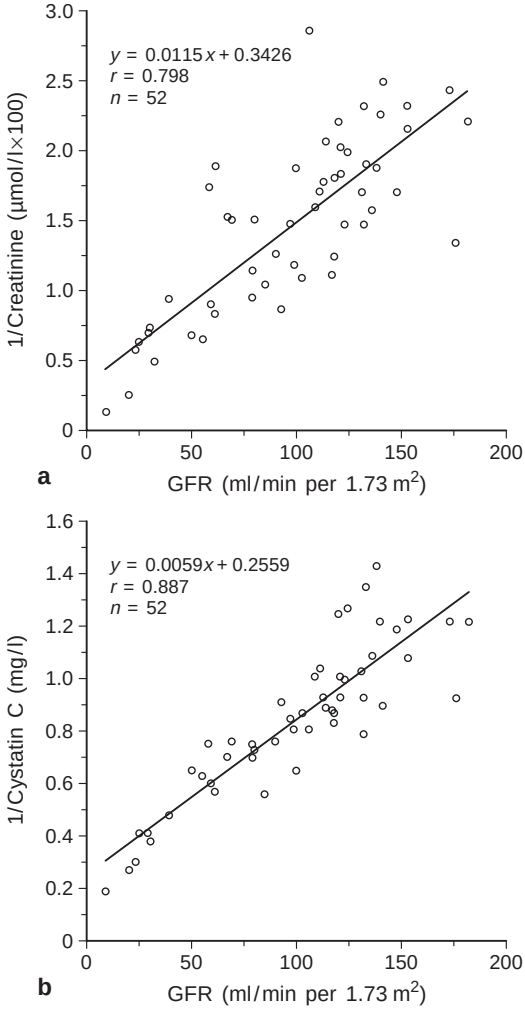

Fig. 1 Correlation between glomerular filtration rate (*GFR*) and reciprocal serum creatinine (**a**) and cystatin C (**b**) in 52 children with various renal conditions

Table 2 The diagnostic efficiency of serum cystatin C and creatinine for reduced glomerular filtration rate using different cut-off limits

	Cut-off limit	Sensitivity	Specificity	PPV	NPV	Diagnostic efficiency
		%				
Cystatin C	1.31	100	97	95	100	98
(mg/l)	1.55	63	100	100	83	87
Creatinine	91	74	97	93	86	88
(μmol/l)	114	53	100	100	79	83
	56	100	55	56	100	71

PPV, Positive predictive value; NPV, negative predictive value

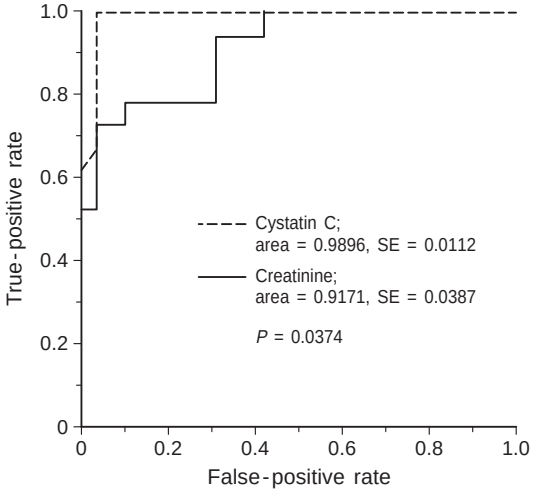

Fig. 2 Nonparametric receiver operating characteristic plots for the diagnostic accuracy of concentrations of cystatin C and creatinine in distinguishing between normal (≥89 ml/min per 1.73 m^2) and reduced GFR (<89 ml/min per 1.73 m^2) in 52 pediatric patients

ROC analysis showed that the diagnostic accuracy of cystatin C was significantly better (P=0.037) than that of creatinine (Fig. 2) in discriminating between subjects with normal renal function and those with reduced GFR.

In this patient group 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, while 100% specificity was achieved with an upper cut-off limit of 1.58 mg/l. The maximum efficiency for creatinine (88%) was reached using an upper cut-off limit of 91 μmol/l (Table 2).

Discussion

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. Typically, serum creatinine measurements, plasma clearance of inulin, or ^{51}Cr-EDTA and renal creatinine clearance have been used to assess GFR. Plasma clearance of inulin or ^{51}Cr-EDTA are expensive and time-consuming tests, which makes them quite impractical for clinical use. In our laboratory the reagents for measuring cystatin C cost about 3 US$, which is more than reagents for creatinine but of course much cheaper than ^{51}Cr-EDTA measurement. The plasma creatinine clearance is a relatively reliable method, but exact urine collection has turned out to be demanding in clinical practice, especially in children. Therefore the quantification of serum creatinine is the most widely used test for predicting GFR. From a theoretical point of view, cystatin C has several advantages over creatinine as a marker of GFR. The production rate of creatinine is variable, because it is determined mainly by muscle mass, and also the elimination of creatinine is complex. In addition, the most commonly used methods for the determination of creatinine have interference problems [22, 23]. The production of cystatin C, in contrast, is determined by a single gene. The structure of the cystatin C gene and its promoter has been defined, and the gene seems to be of the housekeeper type that is compatible with a stable production rate in all nucleated cells [24]. The low molecular weight and basic nature of cystatin C, in combination with its stable production rate, indicates that the concentration of this protein in peripheral blood is mainly determined by the GFR.

A series of studies in adult patients have suggested that cystatin C correlates with GFR as strongly as creatinine or even more strongly [4, 9, 10, 25]. Depending on the number of patients and the 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 [12]. In the present study the diagnostic accuracy of 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. [10]. In our study 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. This corresponds well to values found in children. Bökenkamp et al. [13] used a cut-off concentration of 1.39 mg/l for cystatin C and Helin et al. [12] determined reference values of cystatin C to be 0.63–1.33 mg/l for children over 1 year of age. 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).

In conclusion, this study demonstrates that serum cystatin C offers a more-efficient diagnostic tool than serum creatinine in children with renal disease. The turbidimetric method presented here is a practical and easy alternative for the routine determination of serum cystatin C.

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