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Diagnostic sensitivity of serum cystatin for impaired glomerular filtration rate

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Abstract Recently, the reciprocal of cystatin C (Cys-C), a non-glycosylated 13-kilodalton protein that is produced by all investigated nucleated cells, was found to correlate closely with glomerular filtration rate (GFR). In order to determine the diagnostic validity in children for the detection of impaired GFR, venous blood samples from 381 children (aged 1.7–18 years) with various renal pathology referred for ^{51}Cr-EDTA clearance investigations were obtained for measurement of Cys-C as well as β_2-microglobulin (β2-MG) and serum creatinine. Two hundred and sixteen children with clearance values >90 ml/min per 1.73 m^2 constituted a control group, with a normal GFR. In the control group, Cys-C values were normally distributed with a mean of 0.94±0.27 mg/l and an upper reference limit (97.5th percentile) of 1.47 mg/l. In all children, there was a positive correlation between ^{51}Cr-EDTA clearance and the reciprocal of Cys-C (r=0.64, P<0.0001), β_2-MG (r=0.59, P<0.0001), creatinine (r=0.55, P<0.0001), and the height/creatinine ratio (r=0.73, P<0.0001). Receiver-operating characteristics analysis showed that there were no significant differences between these three parameters for discriminating between patients with normal and reduced GFR, although there was a tendency towards the best diagnostic sensitivity of the GFR estimate according to the Schwartz formula. We conclude that for the detection of mildly impaired GFR, a full clearance study cannot be replaced by measurement of serum Cys-C or β2-MG concentrations.

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Key words ^{51}Cr-EDTA clearance · Cystatin C · β_2-Microglobulin · Schwartz formula · Diagnostic sensitivity

Introduction

Serum creatinine is the most widely used marker to predict the glomerular filtration rate (GFR). In childhood, there is an age and muscle mass dependency of serum creatinine, and it remains difficult to assess a normal GFR accurately, and even the use of the body length/creatinine ratio is not reliable [1–3]. Recent studies have suggested that serum or plasma cystatin C (Cys-C) may be better markers for GFR than serum creatinine [4–6]. Likewise, β_2-microglobulin (β2-MG) has also been previously advocated as a better predictor of GFR [7]. However, to date there has been no proof in pediatric patients that diagnostic sensitivity of either parameter is indeed better than serum creatinine, particularly in patients with only mildly impaired renal function. Furthermore, the scarce data available compare mainly Cys-C and β2-MG with serum creatinine rather than using a height/creatinine ratio (e.g., Schwartz formula) that reflects the renal function in children more precisely [2]. Therefore, in this study, we analyzed the diagnostic sensitivity and specificity of both parameters in comparison with the GFR estimate, using the Schwartz formula and the Cr-51 EDTA clearance as reference methods for determination of GFR.

Patients and methods

Study groups

Venous blood samples were obtained from 381 children (aged 1.7–18 years, mean 12.1±4.8 years) with various renal pathology referred for determination of ^{51}Cr-EDTA clearance. The patients were attending the Pediatric Nephrology outpatient clinic. Mean height was 135±26 cm (range 52.0–190.1 cm), mean weight 36.7±18 kg (range 4.5–98.1 kg), and mean body surface area 1.16±0.38 m^2 (range 0.30–2.20 m^2).

Methods

^{51}-EDTA clearance studies were performed with a modification of the method originally described by Chantler and Barratt [8], and served as standard method for GFR assessment. The modifications were simultaneous application of 80 kBq ^{51}Cr-EDTA and 15 kBq I^{123}-hippurate per kilogram body weight. The clearance was determined by a single-point serum concentration measurement after 25 min and simultaneous determination of isotope activity count with two probes for detection of each tracer placed behind the child's thorax. The half-life of disappearance of each isotope in the body was calculated by a computer program from both the decay of the percutaneous measurement and the single-point serum concentration using the formula of Schreiter [9]. This method was previously described by Gellert et al. [10] from our unit and the only modification was a lower dose of I^{123}-hippurate. Of these 381 children, 216 with GFR values >90 ml/min per 1.73 m^2, defined as normal GFR, formed the reference group. The parents' and, in the case of adolescents, the patients' informed oral consent was obtained in each case, thus the study was in accordance with the ethical standards of the Helsinki declaration of 1975 (revised in 1983). Serum Cys-C was measured with the particle-enhanced turbidimetric assay (Dako, Glostrup, Denmark) in the Hitachi 717 analyzer. β2-MG was measured with the microparticle enzyme immunoassay (Abbott, Wiesbaden, Germany). Serum creatinine was determined enzymatically (PAP, Boehringer Mannheim, Germany).

Statistical analysis

Calculations were performed with the statistical package SPSS for Windows, version 7.5.2G (SPSS, Munich, Germany). The reference cohort with GFR values >90 ml/min per 1.73 m^2 was subdivided into female and male age groups. Differences between the groups were tested with the Kruskal-Wallis nonparametric analysis of variance and Mann-Whitney U-test. Associations between variables (e.g., age, creatinine, GFR) were assessed with Spearman's rank correlation coefficient. The central 95% intervals for Cys-C were calculated according to the recommendations of the International Federation of Clinical Chemistry [11]. $P<0.05$ was considered statistically significant. The diagnostic validity of Cys-C, β2-MG, creatinine, and the height/creatinine ratio for detecting reduced GFR in comparison with ^{51}Cr-EDTA clearance was evaluated by the receiver operation characteristic (ROC) curve analysis [12]. The GraphRoc for Windows software was used for calculations of the area under the curve [13].

Results

Reference intervals of Cys-C

The reference cohort included 104 female and 112 male children with GFR values between 90 and 150 ml/min per 1.73 m^2. Creatinine concentration was positively correlated with age (Fig. 1a), whereas Cys-C and β2-MG concentrations were neither age- (Fig. 1b, c) nor sex-dependent. Thus, for establishment of the reference limits of Cys-C, all male and female children could be combined into one group. The frequency distribution and the Kolmogorov-Smirnov test showed that the data were normally distributed. The mean Cys-C (±SD) was 0.94±0.27 mg/l. Therefore, we calculated the upper reference limit as mean ±1.96 SD, using the central 95% reference interval, according to the IFCC-recommended parametric procedure [11]. The upper cutoff value was thus 1.47 mg/l. The corresponding upper cutoff value calculated on the basis of the nonparametric approach was nearly identical at 1.38 mg/l.

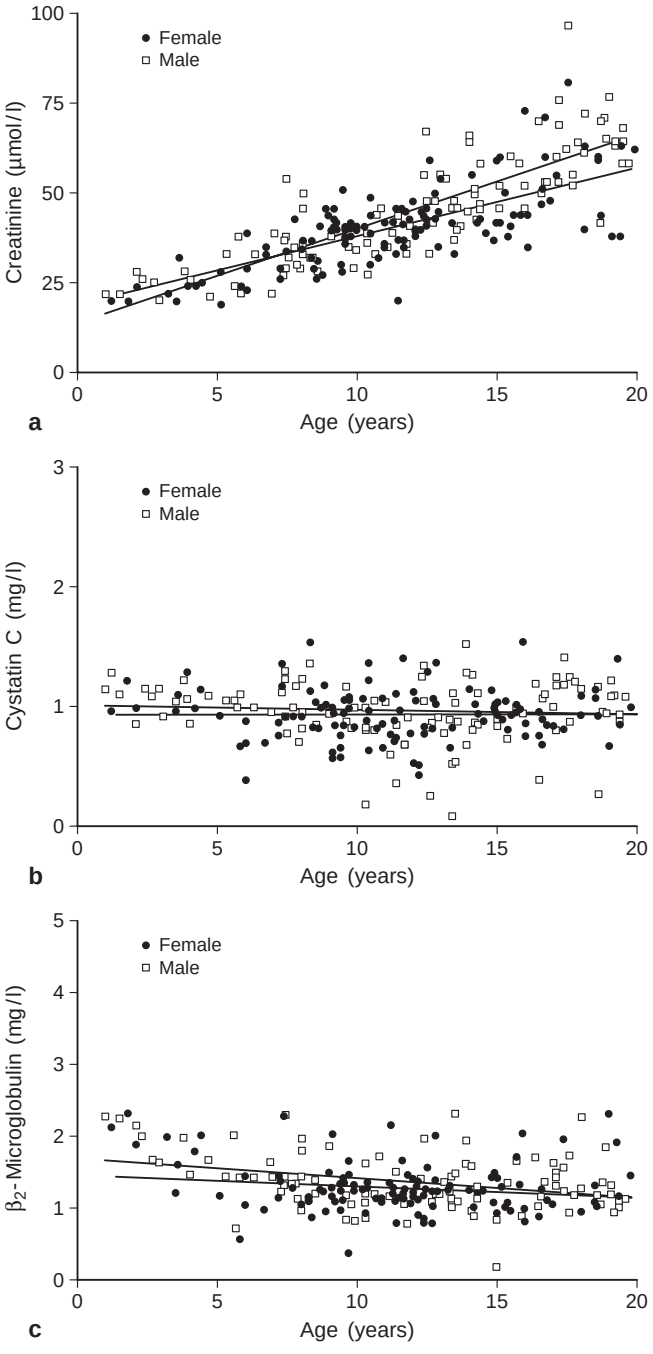

Fig. 1 **a** Serum creatinine (enzymatically determined) in 216 children with normal glomerular filtration rate (GFR) according to age. The slope of the regression lines were 1.926±0.1660 for females and 2.598±0.1685 for males. **b** Cystatin C (determined by particle-enhanced turbidimetric assay, Dako) in 216 children with normal GFR according to age. The slopes of the regression lines were not significantly different, and also not significantly different from zero. **c** β_2-Microglobulin (determined by microparticle immunoassay, Abbott) in 216 children with normal GFR according to age. The slopes of the regression lines were not significantly different

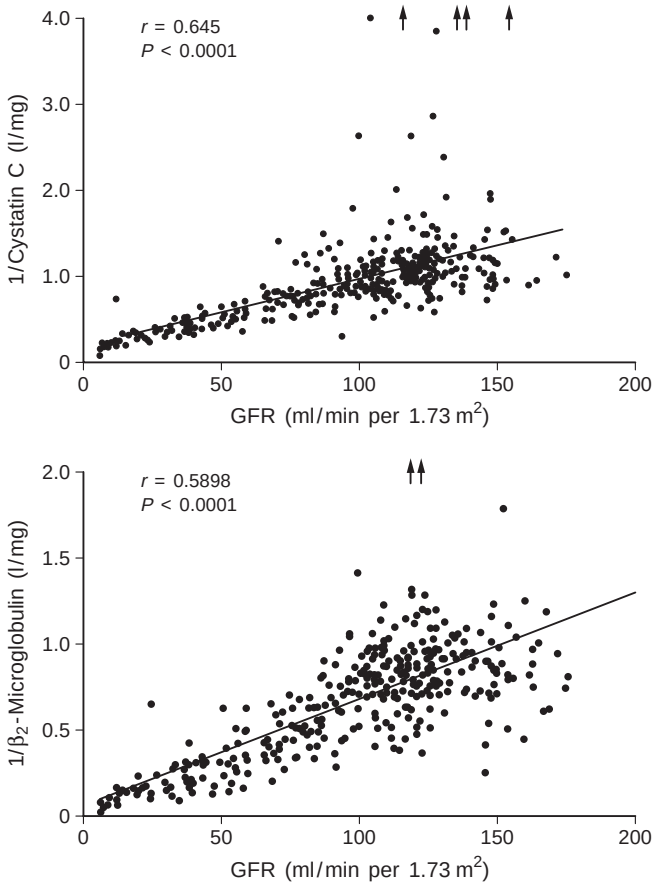

Fig. 2 Reciprocal of cystatin C and β_2-microglobulin versus GFR. A few values were above the top of the *y* axis scale (individual positions indicated as *arrows*) which were taken into calculation of linear regression but not shown

Cys-C and β2-MG and their relation to GFR and creatinine

Mean ^{51}Cr-EDTA clearance of the 381 children was 68±33 ml/min per 1.73 m^2 (range 12–211). There was a positive correlation between ^{51}Cr-EDTA clearance and the GFR estimate using the Schwartz formula [2] (r=0.78, P<0.0001). Similar results were obtained with Cys-C and serum creatinine when plotted against GFR and it was possible to draw a significant nonlinear regression line (exponential decay) between Cys-C and GFR. There also was a positive correlation between the ^{51}Cr-EDTA clearance and the reciprocal of Cys-C (r=0.64, P<0.0001, Fig. 2) and β2-MG (r=0.59, P<0.0001, Fig. 2).

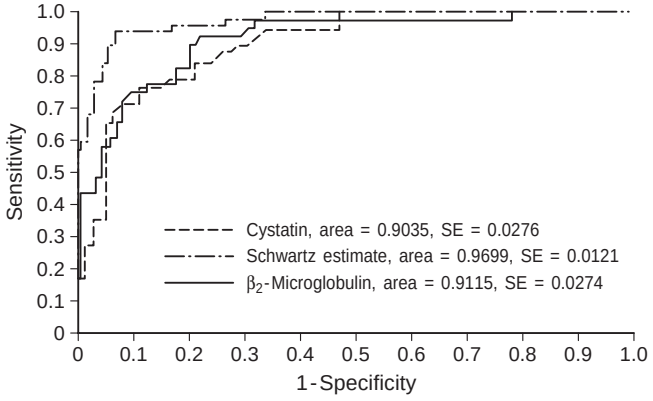

Fig. 3 Receiver operation characteristic plots for the diagnosis of impaired GFR, comparing the GFR estimate according to Schwartz, cystatin C, and β_2-microglobulin. *SE*, Standard error

ROC analysis

To evaluate the diagnostic validity of Cys-C, β2-MG, creatinine, and height/creatinine ratio, the ROC analysis was performed in 216 children with normal GFR (>90 ml/min per 1.73 m^2) and 165 children with reduced GFR (<90 ml/min per 1.73 m^2). ROC plot results are summarized in Fig. 3. For β2-MG, the area was 0.9115 and standard error (SE) 0.0274; for Cys-C, the area was 0.9035 and SE 0.0276, not significantly different from β2-MG (P=0.8378). For the GFR estimate according to the Schwartz formula, the ROC plot area was 0.9699 and SE 0.0121, not significantly different from either β2-MG (P=0.1877) or Cys-C (P=0.1196), although there was a tendency towards the best area for the GFR estimate according to Schwartz. However, the curve of that estimate runs significantly above that of serum creatinine (data not shown, area=0.883, SE=0.032). It follows that Cys-C is not better than the conventional parameters β2-MG, creatinine and height/creatinine ratio for discriminating between patients with normal and reduced GFR.

Discussion

The identification of patients with mildly impaired GFR in the so-called creatinine blind area remains a challenge for pediatric nephrologists. To date, inulin clearance remains the gold standard [14]. However, a full inulin and *p*-amminohippurate clearance is cumbersome in children, and the necessity of timed urinary sampling makes the method unreliable in children [15]. Therefore, in our

Table 1 Diagnostic sensitivity, specificity, and efficiency at the cutoff levels of parameters investigated for detection of impaired glomerular filtration rate

Parameter	Cutoff level	Sensitivity	Specificity	Efficiency
Cystatin C	1.38 (mg/l)	0.67	0.95	0.86
β_2-Microglobulin	2.27 (mg/l)	0.59	0.94	0.83
Schwartz formula	90 (ml/min per 1.73 m^2)	0.84	0.91	0.89

center we use a modified single-shot ^{51}Cr-EDTA clearance, according to the method of Chantler and Barratt [10].

Serum creatinine, which is the most commonly used marker for estimation of GFR, is age and muscle mass dependent, and reference values also depend on the biochemical method used for determination. In order to compensate for the age dependency of serum creatinine, the use of height/creatinine ratios has become well established in routine clinical practice [2, 15]. In our study, using enzymatically determined serum creatinine (measured in mg/dl) and the factor 0.55 for children above 2 years of age (estimate according to Schwartz), there was a reasonably good correlation between the ^{51}Cr-EDTA clearance and the estimated GFR (r=0.78, P<0.0001). It is appreciated that the original factor 0.55 applied to the Jaffe method, and the factor should be only 80% of the original factor when measuring creatinine enzymatically, and each laboratory should define their own factor. However, serum creatinine is muscle mass dependent, and it remains difficult to detect children with a mildly impaired GFR.

Both β2-MG [16] and Cys-C [17] are reliable markers for GFR. The advantages include age and muscle mass independence [8, 18]. Both parameters have been claimed to have a better diagnostic sensitivity than for the serum creatinine detection of impaired GFR [17, 19, 20]. β2-MG has the disadvantage of being increased in patients with several malignancies and infectious diseases, particularly lymphoproliferative disorders [21]. However, because of its low molecular weight, the serum concentration in most children will primarily be determined by GFR [22]. Because of the age independence and promising reports in adults, it was reasonable to perform similar studies in children.

The main advantage of Cys-C and β2-MG in children is their independence of the patient's age [23]. This only applies to children over 2 years of age. In our cohort, there were no infants. Bökenkamp et al. [24] recently published reference intervals for Cys-C for infants using the same Dako kit, and showed the highest concentrations in the first days of life, with a rapid decrease during the first 4 months. Beyond the 2nd year of life, the findings of these authors are in accordance with our findings, and this age independence makes Cys-C a new tool for detecting impaired GFR in children.

Similar to the findings in adults, in our study the ROC plot area for serum creatinine using age-dependent normal values was slightly worse when compared with both Cys-C and β2-MG, although this did not reach statistical significance. Surprisingly, there was only a barely significant difference in the diagnostic efficiency of the height/creatinine ratio compared with creatinine using age-dependent normal values. Although it is well established that the Schwartz formula overestimates the GFR in patients with a GFR <15 ml/min per 1.73 m^2, the overestimation in patients with a GFR >90 ml/min per 1.73 m^2 is negligible, and was 10.3%±3% when the GFR was >50 ml/min per 1.73 m^2 [25]. In our study very few patients had a GFR between 40 and 50 ml/min per 1.73 m^2. We feel that using a height/creatinine ratio provides a much better way of interpreting GFR in patients with normal and mildly impaired renal function than age-dependent creatinine reference values. Compared with the Schwartz formula, the diagnostic efficiency for detection of impaired GFR for both Cys-C and β2-MG were rather disappointing. The ROC plot areas for Cys-C and β2-MG were not significantly different from the height /creatinine ratio clearance estimate for the detection of impaired GFR. These findings are in accordance with a similar study undertaken in 60 children using inulin clearance [26].

Cys-C may be useful for the identification of moderate impaired GFR without the need to correct for age dependency as has to be done with serum creatinine. As a further potential advantage, its muscle mass independence needs to be evaluated in children. However, this study shows that for the detection of mildly impaired GFR in patients with nephropathy, a full clearance study at a specialized center cannot be replaced by measurement of serum concentrations of Cys-C and β2-MG.

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LITERATURE ABSTRACTS

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Proteinuria, hypertension and chronic renal failure in X-linked Kallmann's syndrome, a defined genetic cause of solitary functioning kidney

Nephrol Dial Transplant (1998) 13:1998–2003

Background Anosmia and hypogonadotrophic hypogonadism are the classic features of X-linked Kallmann's syndrome, a disorder caused by mutations of KAL, a gene expressed during kidney and brain development. About a third of patients have a solitary functioning kidney, but little is known about their renal morbidity.
Methods We studied seven patients aged 22–35 years with X-linked Kallmann's syndrome and a solitary functioning kidney.
Results Two patients developed significant proteinuria associated with mild to moderate arterial hypertension in the second to third decades of life. In one, proteinuria and renal impairment preceded the appearance of hypertension, and the disorder progressed to chronic renal failure. The remaining five patients had normal plasma creatinine concentrations and no significant proteinuria although four had borderline systolic and/or diastolic hypertension. In two sets of patients from the same kindreds, there was a striking discordance for the occurrence of renal morbidity.
Conclusions All patients with X-linked Kallmann's syndrome should be screened for renal malformations, and those with solitary kidneys require life-long follow-up to detect hypertension, proteinuria and renal failure.

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Efficacy and safety of low molecular weight heparin in renal transplantation

Transplantation (1998) 66:533–534

Background Deep venous thrombosis (DVT) is a common problem with potentially devastating results in patients undergoing major surgical procedures. Certain renal transplant recipients are particularly at risk for allograft loss as a consequence of renal vein and artery thrombosis. Over the past few years, low molecular weight heparin has been well established as an accepted modality of treatment and prophylaxis of DVT. The efficacy and safety of low molecular weight heparin in the prophylaxis of DVT following renal transplantation in adults has not previously been reported.
Methods Dalteparin was administered to 120 adult renal transplant recipients postoperatively at the Oregon Health Sciences University.
Results No patient developed allograft arterial or venous thrombosis. One patient developed subclavian vein thrombosis. No bleeding complications were encountered, and side effects were very minimal.
Conclusion Prophylaxis with dalteparin is an effective and safe modality for the prevention of thrombosis in adult patients undergoing renal transplantation.