

Does adjustment of GFR to extracellular fluid volume improve the clinical utility of cystatin C?

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Abstract

Background—Cystatin C measurement has been proposed as a replacement for creatinine as a serum measure of glomerular filtration rate (GFR). It has also been suggested that GFR itself should be adjusted to the extracellular fluid volume (ECV) of a child rather than the body surface area (BSA).

Aims—To assess the potential of cystatin C compared to serum creatinine in assessing GFR and to establish whether adjustment of GFR to ECV rather than BSA affects the potential usefulness of cystatin C.

Methods—Cystatin C and plasma creatinine were measured in 64 paediatric patients undergoing 77 ⁵¹Cr-EDTA GFR measurements over a six month period.

Results—1/cystatin C concentrations were more closely related to GFR (median 98 ml/min/1.73 m², range 8–172) after adjustment for patient BSA ($r = 0.81$ versus $r = 0.44$). 1/Creatinine concentrations appeared to be an inferior estimate of BSA adjusted GFR ($r = 0.41$), even following the use of the Schwartz formula ($r = 0.37$). Bland Altman statistics showed cystatin C could still only predict 95% of GFR values to within ± 41 ml/min/1.73 m² of the ⁵¹Cr-EDTA method. The relation between GFR and 1/cystatin C was not improved by adjusting ⁵¹Cr-EDTA GFR to ECV rather than BSA ($r = 0.76$ versus $r = 0.81$).

Conclusions—Cystatin C appears superior to serum creatinine in paediatric subjects although its performance is unlikely to supplant ⁵¹Cr-EDTA GFR measurement. This performance is not being underestimated because of adjusting GFR to BSA rather than ECV.

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A reliable serum measure of glomerular filtration rate (GFR) would be especially useful in paediatric subjects because of the difficulty in collecting timed urine specimens and the need to adjust for the size of the child.

Cystatin C is a single chain polypeptide with 120 amino acid residues whose function is as a proteinase inhibitor to prevent connective tissue destruction.¹ The cystatin C gene is of the housekeeping type which is compatible with a stable production rate by most nucleated cells. Its low molecular weight, combined with a positive charge at physiological pH, allows it

to be freely filtered by the renal glomeruli, after which it is almost entirely reabsorbed and catabolised by proximal tubular cells.²

These features, together with its seemingly unaltered production rate during inflammatory conditions,³ has led to the suggestion that cystatin C could be used as an ideal endogenous marker of GFR in adults^{3–5} and children.^{6–7} Potentially it could also be a better marker than serum creatinine because, unlike creatinine, it is not secreted by the renal tubule, is not affected by muscle mass, and does not suffer the same problems with analytical interference.⁴

Therefore, the first aim of this study was to compare the clinical usefulness of cystatin C to serum creatinine as a measure of GFR in a group of paediatric patients.

When comparing GFR between adult and paediatric individuals of different sizes, adjustment is often made according to the person's body surface area (BSA). This usually entails applying an empirical formula following measurement of the subject's height and weight.⁸ Recently, good physiological arguments have been advanced to support the use of body fluid volumes rather than BSA for "normalising" GFR.^{9–10} Moreover, when assessing GFR using a single bolus injection technique such as ⁵¹Cr-EDTA, an estimate of extracellular fluid volume (ECV), as represented by the marker's volume of distribution (V_D), can be determined directly at the same time as the GFR measurement.⁹ This study therefore also attempted to establish the effect that adjustment of GFR to ECV rather than BSA had on comparisons with cystatin C and serum creatinine.

Patients and methods

Sixty four paediatric patients (34 boys, 30 girls; median age 5 years, range 3 months to 18 years) attending the Royal Manchester Children's Hospital participated in the study with agreement from the local ethical committee. Between them they had 77 ⁵¹Cr-EDTA assessments of GFR performed over a six month period. GFR measurements were requested for a variety of reasons: 46 were oncology patients either pre- or post-chemotherapy, 14 were inpatients with known renal disease, and a further four had miscellaneous conditions.

GFR was assessed by ⁵¹Cr-EDTA injection (1 μ Ci/kg) followed by sampling at two and four hours.¹¹ GFR was adjusted for body surface area using the Du Bois formula,⁸ and for V_D calculated using the terminal exponential of the plasma ⁵¹Cr-EDTA clearance curve to estimate the radioactivity count at time zero.

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The V_D is then the total radioactivity injected/radioactivity count at time zero.¹¹ Plasma from the two hour sample was then stored at -20°C until analysis of cystatin C as a single batch by a PENIA method on the Behring BN 100 automated nephelometer (Dade Behring Ltd, Milton Keynes, UK). Within batch coefficients of variation (CV) were less than 5% across the assay range. Serum creatinine was measured on the same samples using a kinetic Jaffé method on a Beckman CX7 analyser, and the Schwartz formula ($48.6 \times \text{height (cm)}/\text{serum creatinine } (\mu\text{mol/l})$) was used to try and improve the relation between creatinine and GFR.¹²

1/Cystatin C and 1/creatinine concentrations were compared with ^{51}Cr -EDTA GFR by linear regression using the least squares method. The relation between 1/cystatin C and surface area adjusted ^{51}Cr -EDTA GFR was used to establish the Bland Altman "limits of agreement"

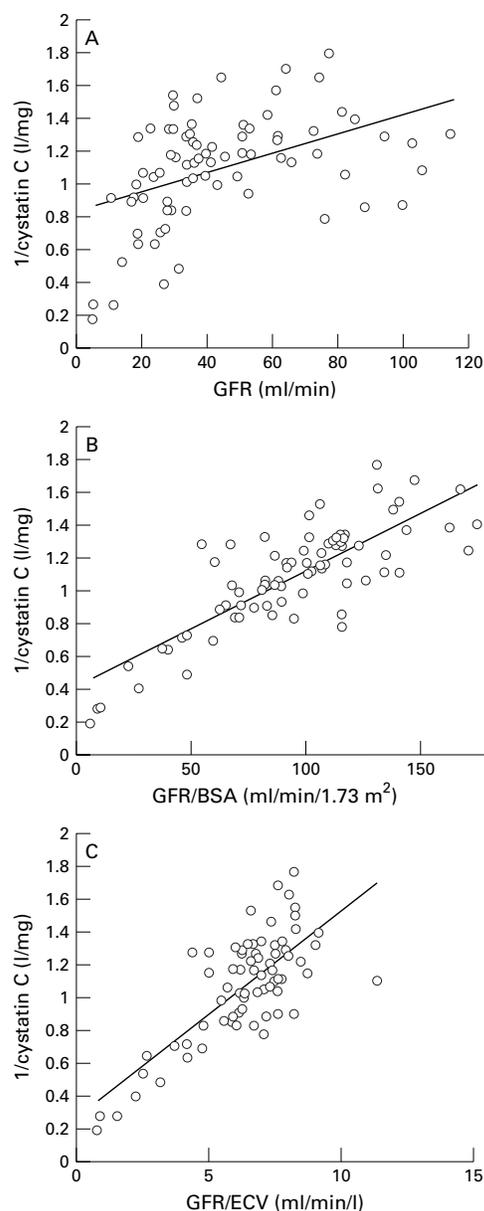


Figure 1 Relation between 1/cystatin C and (A) ^{51}Cr -EDTA GFR ($n=77$); (B) ^{51}Cr -EDTA GFR/BSA ($n=77$); and (C) ^{51}Cr -EDTA GFR/ECV ($n=72$).

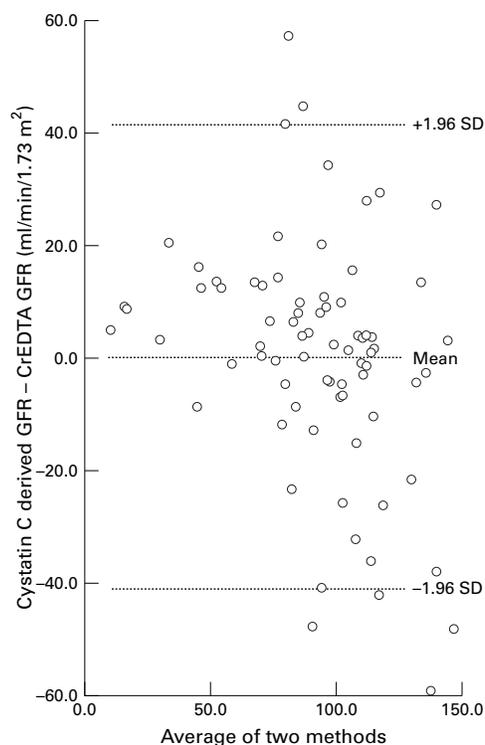


Figure 2 Bland Altman plot of agreement between cystatin C predicted GFR and ^{51}Cr -EDTA GFR/BSA.

between the cystatin C and ^{51}Cr -EDTA methods.¹³

Results

A wide range of ^{51}Cr -EDTA GFRs were present (median $98 \text{ ml/min}/1.73 \text{ m}^2$, range 8–172). 1/Cystatin C concentrations were more closely related to GFR measurements after the GFR was adjusted for patient surface area ($r = 0.44$ versus $r = 0.81$; fig 1A,B). This relation between 1/cystatin C and surface area adjusted GFR held true for lower GFRs ($<80 \text{ ml/min}/1.73 \text{ m}^2$, $r = 0.83$) more than for higher ones ($r = 0.56$). 1/Creatinine concentrations appeared to be an inferior estimate of GFR ($r = 0.41$), even following the use of the Schwartz formula ($r = 0.37$). However, Bland Altman statistics showed that cystatin C can still only predict 95% of GFR values to within $\pm 41 \text{ ml/min}/1.73 \text{ m}^2$ of the ^{51}Cr -EDTA method (fig 2).

The V_D of ^{51}Cr -EDTA (expressed as a percentage of body weight) showed a median of 33.4% (interquartile range 29.0–37.6%). There was a moderate correlation between GFR adjusted for ECV (as measured by V_D) and BSA ($r = 0.83$). The relation between GFR and 1/cystatin C was not improved by adjusting ^{51}Cr -EDTA GFR to ECV rather than BSA ($r = 0.76$ versus $r = 0.81$; fig 1C). The effect on the relation between GFR and 1/creatinine differed slightly, depending on whether the Schwartz formula was used ($r = 0.35$ versus $r = 0.37$) or not ($r = 0.52$ versus $r = 0.41$).

Discussion

The assessment of GFR in paediatric subjects is necessarily more complex than in adults, in

part because of the difficulty in collecting timed urine specimens, the need to adjust GFR to the size of the child, and the especially poor predictive value of traditional markers such as serum creatinine in this age group.¹⁴ This has led to the widespread use of isotopic methods, such as ⁵¹Cr-EDTA, for accurate GFR assessment in children. However, such tests are labour intensive (and therefore costly), involve the use of radioactive substances, and often require the child to undergo multiple blood tests.

This study has confirmed the findings of others^{6,7} that as a single serum/plasma measure of GFR, cystatin C appears to reflect ⁵¹Cr-EDTA clearance more closely than creatinine in paediatric patients. Moreover, cystatin C appears to benefit from an apparent ability to take account of a subject's body surface area when assessing GFR. Even when the Schwartz formula was used to take account of subject height, this did not improve the serum creatinine estimate of GFR further.

Despite the impressive correlation found between 1/cystatin C concentrations and BSA adjusted GFR ($r = 0.81$), Bland Altman analysis showed that cystatin C is still only reliable enough to predict GFR to within approximately ± 40 ml/min/1.73 m² of the ⁵¹Cr-EDTA result. Indeed, because the cystatin C derived GFR data have been determined on the same patients as the ⁵¹Cr-EDTA GFR, the figure of ± 40 ml/min/1.73 m² is likely to be as close an agreement as can possibly be achieved. This performance, which is consistent with previous data,⁷ is unlikely to persuade clinicians to completely supplant ⁵¹Cr-EDTA measurement with that of cystatin C.

The second part of our study therefore aimed to establish whether the relation between cystatin C and ⁵¹Cr-EDTA GFR could be further improved by adjustment of GFR to ECV rather than BSA.

The rationale for always adjusting GFR to body surface area apparently dates back to the nineteenth century when the "law of surface area" was used in physiology and medicine to calculate fluid and drug requirements.⁹ In the early twentieth century, the application of BSA formulae to urea clearance seemed to give children and adults comparable numerical values,¹⁵ and since then the utilisation of this adjustment has fallen, largely unquestioned, into common use.

The use of "normalising" GFR to body fluid volumes rather than BSA was first suggested nearly half a century ago.¹⁵ Recently there has been a resurgence of interest because routinely performed single injection techniques for assessing GFR, such as ⁵¹Cr-EDTA or ^{99m}Tc-DTPA, can simultaneously give an indication of the dilution space (V_D) of the particular marker.^{9,10} The V_D values of these markers have, in turn, been found to be linearly related to a subject's extracellular fluid volume.¹⁶

This study has shown the same moderate relation between BSA and ECV ($r = 0.83$) as in other studies,¹⁰ but theoretically it has been argued that adjustment to a marker's V_D should be superior because it gives a fundamental

indication of the average time an individual molecule of the marker has to wait, after equilibration within its distribution volume, before it is filtered at the glomerulus.¹⁰

In practice, we found adjustment to ECV did not improve the ability of cystatin C to predict ⁵¹Cr-EDTA assessed GFR. While we cannot make any inferences from this about the relative merits of ECV and BSA adjustment, we can be confident that we are not underestimating the clinical utility of cystatin C because of using BSA rather than ECV.

Before we dismiss cystatin C measurement as a replacement for single injection techniques, it must be borne in mind that even before adjustment we are assuming that the ⁵¹Cr-EDTA measure of GFR is the "gold standard". However, GFR measurements by this technique are subject to variation because of errors involved in the counting of radioactivity, the measurement and injection of the radioactive substance, weighing of materials, timing of sampling, and as mentioned, the formula used in the estimation of body surface area. In addition, true GFR is known to vary rapidly (by up to 70%) because of physiological changes induced by, for example, the effect of dietary protein loads.¹⁷ Thus, because of the different timescales involved in measurement it is conceivable that although cystatin C is giving a slightly different indication of GFR compared to ⁵¹Cr-EDTA, they may both be equally accurate. Nevertheless, proving or disproving this suggestion will be extremely difficult.

In summary, this study has confirmed the superiority of cystatin C over serum creatinine as a measure of GFR in paediatric subjects. Adjustment of GFR to ECV rather than BSA does not improve the clinical utility of cystatin C measurement.

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