Serum cystatin C is not a better marker of creatinine or digoxin clearance than serum creatinine

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Aims To assess whether cystatin C, a new serum marker of renal function, is a better index of creatinine or digoxin clearance than serum creatinine in older people.

Methods Twenty-two volunteers over the age of 65 years (mean 73 ± 5) were recruited from a healthy elderly volunteer database. None of the volunteers was taking digoxin or other medication known to interfere with digoxin kinetics or assay. Digoxin was infused at a dose of $7\text{--}10 \mu\text{g kg}^{-1}$ and blood samples were taken over the following 48 h and assayed for serum digoxin. Serum cystatin C, creatinine and creatinine clearance were measured and a calculated creatinine clearance was estimated using the Cockcroft Gault formula. Digoxin clearance was calculated using a pharmacokinetic software package. All values were log transformed to normalize their distribution.

Results Of the 22 volunteers enrolled into the study, 18 completed the study. Serum cystatin C ranged between 0.72 and 1.89 mg l^{-1} and serum creatinine ranged from 69.6 to $153.9 \mu\text{mol l}^{-1}$. Measured creatinine clearance ranged from 38 to 123 ml min^{-1} and calculated creatinine clearance from 29.5 to 88.0 ml min^{-1} . Digoxin clearance ranged from 51.0 to $103.5 \text{ ml min}^{-1}$. Cystatin C correlated extremely well with creatinine ($r=0.93$, $P<0.001$, 95% CI 0.82, 0.97) and with creatinine clearance ($r=0.67$, $P=0.002$, 95% CI 0.3, 0.87). Neither serum cystatin C nor serum creatinine correlated with digoxin clearance ($r=0.25$, $P=0.31$, 95% CI -0.25 , 0.64 and $r=0.44$, $P=0.068$, 95% CI -0.03 , 0.75, respectively). Measured creatinine clearance, however, did correlate well with digoxin clearance ($r=0.55$, $P=0.018$, 95% CI 0.11, 0.81).

Conclusions Serum cystatin C and serum creatinine show very similar correlations with creatinine and digoxin clearances. Serum cystatin C does not offer any advantages in this respect. It remains to be seen whether cystatin C offers any advantage over creatinine in elderly people in other respects.

Keywords: creatinine, cystatin C, digoxin, kidney function, older people

Introduction

The estimation of glomerular filtration rate (GFR) is commonly performed by measuring renal clearance of exogenous or endogenous substances and by measuring endogenous serum markers of glomerular filtration. Inulin

clearance, iothexol clearance and $^{51}\text{chromium}$ labelled EDTA are all considered 'gold standard tests' [1] but require the use of specialized personnel, take a period of several hours to measure, are costly and require specialized equipment. Creatinine is the most widely used measure of GFR but relies on the measurement of creatinine which reflects muscle mass [2] and is relatively nonsensitive since it may not increase out of the normal range until 50% of GFR has been lost [3]. Creatinine clearance also relies on the accurate collection of a timed urine collection. Research has therefore been directed towards the identification of a specific serum marker of GFR.

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Cystatin C is a nonglycosylated 13 kDa basic protein that is derived from the cystatin superfamily of cysteine protease inhibitors [4]. It is produced by all nucleated cells and its production rate is unaltered by inflammatory conditions [5]. Cystatin C is removed from the circulation by glomerular filtration and almost completely reabsorbed and catabolized by proximal tubular cells [6]. Cystatin C is thus a good candidate in the search for the ideal serum marker of GFR.

Several studies have shown that cystatin C correlates better with inulin or 51 chromium EDTA clearance than serum creatinine in adult patients with mild to moderately impaired renal function [6, 7]. Cystatin C has also been shown to be potentially better as a screening test to detect reduced GFR than creatinine [8]. Two groups have specifically investigated the use of cystatin C in paediatric populations [9, 10], and one group investigated cystatin C in relation to renal transplant patients [11], but to date there have been no published studies looking specifically at the use of cystatin C in the older population except to publish a reference range in this age group [12].

Digoxin is predominantly renally excreted and has a narrow therapeutic index leading to potential toxicity, especially in renal impairment [13]. GFR falls with age [14, 15] and therefore toxicity is more likely in the older population, as well as the greater likelihood of toxicity having important sequelae. Nomograms exist to predict steady state digoxin dosing using creatinine clearance as the marker of renal function [16]. If cystatin C correlates well with digoxin clearance then a simple serum marker of GFR could replace creatinine clearance in these nomograms. We have therefore compared cystatin C with creatinine and digoxin clearance.

Methods

This study was approved by the local Research Ethics Committee at Kings College Hospital.

Subjects

Twenty-two healthy volunteers were recruited from the Clinical Age Research Unit database between July 1998 and January 1999. All volunteers signed a written consent form after the study had been explained to them fully and were free to leave the study at any time. The ages ranged from 67 to 86 years (mean 73 ± 5) and 15 were female. The weights of the volunteers ranged from 52 to 107 kg (mean 69 ± 16 kg). The volunteers were not taking digoxin or any drugs known to interfere with digoxin metabolism or assays. They all underwent a screening history, examination and simple blood tests were taken (urea, creatinine and electrolytes, glucose and calcium) 1–2 weeks before the study day.

Protocol

Digoxin at a dose of $7\text{--}10 \mu\text{g kg}^{-1}$ (LanoxinTM injection $250 \mu\text{g ml}^{-1}$ Wellcome lot Z6378A) was infused into the left antecubital vein in 30–40 ml normal saline over 1 h. Blood samples were taken from the right arm at baseline and then at 10, 20, 30, 40, 50, 60, 90 min and at 2, 3, 4, 6, 8, 24, 30 and 48 h after starting the infusion and assayed for serum digoxin. All volunteers collected a 24 h urine sample for creatinine clearance estimation between the second and third day of the study and additional blood was taken at 24 h for serum creatinine and cystatin C.

Laboratory analytical methods

Serum digoxin was measured using the Emit^R 2000 Digoxin Assay (Syva^R Company, Dade Behring Inc, Cupertino, CA 95014) on a Cobas Mira S Analyser (Roche Diagnostic Systems). The between run coefficient of variation (CV) for concentrations above $1.2 \mu\text{g l}^{-1}$ was $<8\%$ and for those below this level, $<12\%$. Serum cystatin C was measured by particle enhanced nephelometric immunoassay (PENIA) on a Behring Nephelometer Analyser (Dade Behring, Marburg, Germany) and the between run CV was 5%. Serum creatinine was measured by fixed interval Jaffe on the IL Monarch 2000 (Instrumentation Laboratory, Warrington, UK) and the between run CV was 2.8%.

Data analysis

Calculated creatinine clearance was calculated according to the Cockcroft Gault formula [17]. Pharmacokinetic analysis was performed using the SIMP computer package [18]. The area under the curve ($\text{AUC}(0,\infty)$) was calculated from the equation $\text{AUC}(0,\infty) = \text{AUC}(0,48 \text{ h}) + C_{48}/\lambda_z$ where C_{48} = digoxin concentration at 48 h and λ_z = elimination rate constant and was modelled using least squares regression analysis optimized with a nonlinear regression analysis. From this, clearance was calculated as $\text{dose}/\text{AUC}(0,\infty)$. Digoxin clearance was also calculated using $\text{AUC}(0,48 \text{ h})$ corrected for dose.

Statistical analysis

Since cystatin C, creatinine, creatinine clearance and digoxin clearance were log normally distributed, all values were log transformed before correlations were undertaken. Astute Statistics Package (1993–5) add-in for Microsoft Excel (DDU software, University of Leeds, Old Medical School LS2 9JT, UK) was used for all statistical calculations. The relationship between measured creatinine clearance and calculated creatinine clearance was assessed using an Altman Bland plot.

Results

Twenty-two volunteers were enrolled into the study. Four volunteers were excluded from the final calculations because of one failure to finish the study due to problems with venous access, one failure of the pump infusing the digoxin and two sets of samples were diluted incorrectly in the laboratory. The results presented are therefore from 18 volunteers.

Serum cystatin C concentrations ranged from 0.7–1.9 mg l⁻¹ (reference range >50 years 0.84–1.55 mg l⁻¹) [19] and serum creatinine concentrations ranged from 70–154 µmol l⁻¹ (reference range >50 years, women 51–85 µmol l⁻¹) [19]. All patients collected a 24 h urine sample and the volumes ranged from 0.96–2.63 l. Three volunteers who admitted to missing one urine sample in the 24 h, had 24 h urine volumes of 1.14 l, 1.23 l and 1.15 l. The measured creatinine clearances ranged from 38–123 ml min⁻¹ (mean 74 ± 21), and calculated creatinine clearance from 30–88 ml min⁻¹ (mean 56 ± 17).

After infusion of 7–10 µg kg⁻¹ (mean infusion of 520 µg) digoxin intravenously over 1 h the maximum digoxin concentration was 14.1 µg l⁻¹ with a mean 11.7 ± 1.5 µg l⁻¹. By 48 h, at the end of the sampling time, the mean concentration of digoxin was 0.4 µg l⁻¹. Digoxin clearance ranged from 51–104 ml min⁻¹ (mean 75.7 ± 16 ml min⁻¹).

There was a very strong correlation between serum cystatin C concentration and serum creatinine concentration ($r=0.93$, $P<0.001$, 95% CI 0.82, 0.97) (Figure 1a). Cystatin C concentration also correlated well with measured creatinine clearance ($r=-0.67$, $P=0.002$, 95% CI 0.30, 0.87) (Figure 1b). The agreement between calculated creatinine clearance and measured creatinine clearance was investigated using an Altman Bland plot. This showed a mean difference of 19 ml min⁻¹ (95% confidence limit of 14–23 ml min⁻¹) with calculated values being lower than measured values. These correlations did not change when the data from the patients who admitted to missing a urine sample were excluded.

Neither serum cystatin C concentration ($r=-0.3$, $P=0.31$, 95% CI -0.25, 0.64) nor serum creatinine concentration ($r=-0.4$, $P=0.07$, 95% CI -0.03, 0.75) correlated significantly with digoxin clearance. However measured creatinine clearance was weakly correlated with digoxin clearance ($r=0.55$, $P=0.018$, 95% CI 0.11, 0.81) (Figure 2).

The mean percentage of AUC(0,∞) accounted for by AUC(0,48 h) was 66% (95% CI 60, 71%). The analyses involving digoxin clearance were repeated using AUC(0,48 h) corrected for dose. The findings were almost identical including the statistical conclusions.

Figure 1 a) Log cystatin C concentration *vs* log creatinine concentration and b) log cystatin C concentration *vs* log creatinine clearance.

Figure 2 Log creatinine clearance *vs* log digoxin clearance.

Discussion

Two reference ranges for cystatin C in older people have been published. The first Finch *et al.* [12] was derived from 401 samples from healthy home living subjects between 65 and 101 years. The reference ranges were age related: 60–79 years = 0.93–2.68 mg l⁻¹, >80 years = 1.07–3.35 mg l⁻¹. The second, Norland *et al.* [19] was not performed specifically in the older population and cystatin C was analysed on a DAKO analyser, which gives higher results than the Behring analyser, used by Finch *et al.* [12] and in the present study. (Behring cystatin C = 0.15 + 0.76 × DAKO cystatin C) [20]. Two men and two women were randomly selected from each 1 year birth cohort above 20 years of age with the oldest person being was 89 years old. The reference range published by Norland *et al.* [19] is also age related and for >50 years = 0.84–1.55 mg l⁻¹. Thus, these reference ranges do not agree with each other, especially in the older age groups. Data from the present study was much more in keeping with the ranges published by Norland *et al.* [19]. Our volunteers showed a threefold variation in creatinine clearance but none had severe renal impairment.

In keeping with others, our work has shown the serum cystatin C concentration to be very similar to serum creatinine concentration and creatinine clearance as a marker of renal function. The findings support the hypothesis that creatinine concentration and cystatin C concentration have similar properties as plasma markers of GFR [21].

The Cockcroft and Gault formula [17] consistently produced lower values of creatinine clearance than the measured creatinine clearance. This has been shown previously in this age group and may reflect inaccuracies in the measured creatinine clearances [22].

Neither cystatin C concentration nor creatinine concentration showed a good correlation with digoxin clearance. The latter in the present study was similar to that quoted in young patients by Ewy *et al.* [15] but lower than that quoted for young patients by Cusack *et al.* [14]. The digoxin clearances quoted in these two studies for older people were lower than our values. The older patients in the study by Ewy *et al.* [15] were healthy elderly volunteers living at home but in the study by Cusack *et al.* [14] they were in-patients receiving digoxin for its positive inotropic effect.

Inaccuracies may have been introduced when calculating the AUC for digoxin clearance because of the use of low concentrations of the drug, which were close to or at the limit of quantification. The use of such low concentrations at the end of the sampling period is an inevitable consequence of using single dose methodology. Using AUC(0,48 h) corrected for dose did not,

however, give better correlations with cystatin C concentration, creatinine concentration or creatinine clearance. Cystatin C concentration could not be used to predict steady state digoxin concentration, and nor did it correlate better than creatinine concentration with creatinine clearance.

In conclusion, serum cystatin C concentration is no better than serum creatinine concentration at predicting digoxin clearance and it remains to be seen whether the former will have any advantages over the latter as a serum marker of GFR in elderly patients with normal or impaired renal function.

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