GFR estimates using cystatin C are superior to serum creatinine in adult patients with cystic fibrosis

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Abstract

Background: Accurate assessment of renal function in patients with cystic fibrosis (CF) is vital for determining the appropriate dose of medications and for early detection of renal disease. Cystatin C (CysC) is a new marker of GFR with reportedly improved accuracy and precision compared to methods incorporating serum creatinine. The purpose of this study is to evaluate the predictive performance of cystatin C in estimating GFR in adult patients with CF.

Methods: Iothalamate was administered to enable measurement of GFR in 38 adult patients with CF and control subjects. Creatinine clearance (C&G) and GFR estimates (cystatin C clearance [Cys C] and abbreviated modified diet in renal disease [aMDRD]) were compared using Bland–Altman and receiver operating characteristic (ROC) analysis. GFR cutoff values of 80 and 90 mL/min−1.73 m2 were used in the analysis.

Results: The measured GFR was similar in both the CF and healthy volunteers 104 (32.2) and 105 (29.9), P=0.969 respectively. No significant difference in mean bias was noted between the predictive methods within the CF population. Cys C provided the most precise estimates of GFR in both populations. ROC curves demonstrated that CysC provided greater sensitivity and specificity compared to the aMDRD (AUC 0.93 vs. 0.54, P=0.003) and C&G (AUC 0.93 vs. 0.56, P=0.005) in CF at a cutoff GFR of 90 mL/min−1.73 m2.

Conclusion: Cystatin C clearance provides an improved marker of glomerular filtration rate in CF patients.

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Keywords: Glomerular filtration rate; Cystatin C; Creatinine clearance; MDRD

1. Introduction

An accurate assessment of renal function is vital to the dosing and monitoring of drugs and for the early detection of renal disease. This is of particular importance to patients with cystic fibrosis who receive repeated courses of potentially nephrotoxic antibiotics for the treatment of acute pulmonary exacerbations. Two recent reports from the United Kingdom showed that acute renal failure (ARF) associated with intravenous aminoglycosides is increasingly recognized in patients with CF [1,2].

The best index of renal function is glomerular filtration rate (GFR). Iothalamate is considered a gold standard measure for glomerular filtration rate (GFR) assessment, but requires intravenous infusion of the marker compound and timed urine collections over several hours, making this an impractical method for routine clinical use [3].

The most commonly employed methods for quantifying renal function in the clinical setting utilize the endogenous biomarker, serum creatinine, to estimate the patient’s glomerular filtration rate. Creatinine is formed as a byproduct of muscle catabolism at a relatively constant rate and is subsequently excreted by the kidney providing a clinically useful marker of
renal function. However, the use of formulas incorporating serum creatinine is known to result in inaccurate estimations of renal function in patients with malnutrition, liver disease, obesity, or significant third spaced fluid [4]. In addition, certain foods rich in creatine (e.g., red meat) and drugs that affect the tubular excretion of creatine (e.g., cimetidine) can alter serum creatinine concentrations independent of glomerular filtration [5]. Malnutrition, which occurs as a consequence of pancreatic insufficiency and the increased metabolic needs secondary to impaired respiratory function, is a frequent complication of cystic fibrosis. A reduced muscle mass in patients with CF may account for the overestimation of renal function using methods incorporating serum creatinine noted in a recent report [6].

An alternative endogenous biomarker to estimate GFR is cystatin C, a cysteine proteinase inhibitor involved in the intracellular catabolism of proteins. Structural analysis of the gene and its promoter demonstrate that Cys C is constitutively expressed by all nucleated cells exhibiting a stable production rate even in the presence of an acute inflammatory response [7]. It is freely filtrated in the renal glomeruli and almost completely reabsorbed and catabolized by the proximal tubular cells [8]. Studies performed in patients with various degrees of renal function, liver disease, and spinal cord injuries have shown a higher correlation and improved accuracy in predicting GFR when compared with methods incorporating serum creatinine. [9]. However, results in patients with diabetes, pediatric patients, and those with early renal impairment did not show a significant difference between Cys C and the formulas utilizing creatinine in predicting GFR, indicating the performance may be patient population specific [10–15]. Considering the malnutrition and associated loss of muscle mass often observed in patients with CF an evaluation of utility of cystatin C is warranted. Therefore, the aim of this study was to compare the predictive performance of Cys C clearance relative to existing methods for estimating GFR in patients with CF and age-matched controls.

2. Methods

2.1. Study population and study design

Subjects were selected from two different open label randomized studies evaluating the pharmacokinetics of dicloxacillin and fexofenadine in cystic fibrosis (CF) patients compared to healthy volunteers (HV) [16,17]. During these studies glomerular filtration rate (GFR) was measured using iothalamate as a biomarker. Thirty-eight subjects, 19 cystic fibrosis (CF) patients and 19 age-matched healthy volunteers, were included in the present study. All CF patients had a confirmed diagnosis of cystic fibrosis (positive sweat chloride test and/or known CF genotype). None of the patients were currently pregnant or nursing an infant, post solid organ transplantation, or had significant anemia, renal or hepatic insufficiency. All subjects were older than 18 years and within 70–130% of their ideal body weight. The study protocol was approved by the Institutional Review Board, and each subject signed a written witnessed informed consent prior to participation in the study. After completion of the informed consent each subject participated in a screening visit, which included a review of medical history and physical examination (vitals, height, and weight). Laboratory analysis included a complete metabolic panel and complete blood count. All clinical work in both studies was performed at the General Clinical Research Center (GCRC) at LAC+USC Medical Center.

2.2. Study protocol

Subjects were admitted to the GCRC after an overnight fast. A single dose of iothalamate meglumine 456 mg (Conray 30; Mallinkrodt, St. Louis, MO) was administrated as an intravenous push dose 1 h after an oral fluid load of 600 ml of caffeine-free, sugar-free liquids. An oral fluid regimen was maintained at 150–200 ml/h for 6 h following the drug administration to ensure sufficient hydration during the urine collection period. Blood samples were drawn at times 0, 0.25, 0.5, 1, 2, and 3 h after dosing for all subjects in both studies. Samples were kept on ice until plasma was separated by centrifugation within 30 min of collection. Urine samples were obtained from spontaneous voiding every 30 min for the first 3 h after iothalamate administration. Both urine and plasma samples were stored at −70 °C until the time of analysis.

2.3. Determination of plasma and urinary iothalamate concentrations

Iothalamate urine and plasma samples were analyzed based on a previously published method [18]. Standard curves were created by linear regression of peak area ratio of iothalamate to theophylline (internal standard) versus known concentrations of iothalamate. The plasma and urine standard curves were linear in the range from 2–60 μg/ml and 10–250 μg/ml with correlation coefficients of at least 0.99 and 0.98 respectively in the first study and 0.94 and 0.99 respectively in the other. Unknown samples were estimated by applying the equation of the linear regression of the standard curve to the unknown sample peak area. The inter-day coefficients of variation (CV) for plasma and urine samples were less than 11% in both studies.

2.4. Pharmacokinetic analysis

Pharmacokinetic analysis was performed by applying a 1-compartment model with first order elimination to the measured plasma and urinary concentrations of iothalamate using the ADAPT II software (release 5, Biomedical Simulations Resource, University of Southern California, Los Angeles). Analysis was performed using the parametric expectation maximization algorithm. The dose, plasma and urine iothalamate concentrations, and the urine volumes were the inputs to the model. Iothalamate clearance was modeled with renal and nonrenal clearances. The primary output parameter of interest was the renal clearance, which was used as the measured GFR for each subject.

2.5. Data analysis

Creatinine clearance was estimated using the method of Cockcroft–Gault and normalized for body surface area. Measured GFR
was determined from the measured iothalamate clearance and was normalized for body surface area. GFR was predicted using the abbreviated MDRD equation and cystatin C clearance. Cystatin C clearance was estimated using the method of Tidman, where GFR=100/CysC – 14 [19].

2.6. Statistic analysis

Subject demographics and clinical characteristics were summarized using descriptive statistics. Differences between CF and healthy volunteers were determined using a two-tailed student t-test or chi square test where appropriate. A p-value < 0.05 was considered statistically significant. Methods described by Bland and Altman [20] were used to evaluate the bias and degree of agreement between the GFR estimates compared with the measured GFR. The precision of the predictive methods was determined from the standard deviation of the observed bias. Accuracy was reported as the percent of values within 30% and 50% of the measured GFR. Pearson’s correlation was used to evaluate the relationship between body mass index (BMI) and 50% of the measured GFR. Pearson’s correlation was used to provide the greatest precision in both populations as indicated by the least variability in bias (Table 2) and the most narrow 95% limit of agreement (LOA) in the Bland–Altman plots (Fig. 1). Accuracy within 30% of the measured GFR was greatest for the Cys C equation in the CF group, but no differences were noted within 50% of the measured GFR. No differences in accuracy of the predictive equations were apparent in the HV group.

Five of the CF patients had CFRD; however, no statistically significant difference was found between the predicted and measured GFR values in these patients. In addition, the median BMI for the patients with CFRD did not differ significantly from the other CF patients (18.9 vs. 20.95 respectively).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject demographics and clinical characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF (n=19)</td>
<td>HV (n=19)</td>
</tr>
<tr>
<td>Males/females</td>
<td>8/11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.3 (7.17)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>54.7 (6.24)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166 (8.06)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.3 (2.16)</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>65.4 (8.84)</td>
</tr>
<tr>
<td>Serum cystatin C (mg/L)</td>
<td>0.81 (0.15)</td>
</tr>
<tr>
<td>GFR (mL/min–1.73 m²)</td>
<td>104 (32.2)</td>
</tr>
</tbody>
</table>

Values are mean (standard deviation).

Table 2 | Bias, precision, and accuracy of GFR predictive equations |
<table>
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</thead>
<tbody>
<tr>
<td>Cystic fibrosis</td>
<td>Mean (SD), mL/min–1.73 m²</td>
<td>Bias</td>
<td>Precision</td>
<td>P</td>
<td>Accuracy % (within 30%)</td>
<td>Accuracy % (within 50%)</td>
<td>Relative difference (%)</td>
</tr>
<tr>
<td>CLcr (C&amp;G)</td>
<td>115 (23.1)</td>
<td>11.3</td>
<td>39.1</td>
<td>0.225</td>
<td>47.4</td>
<td>84.2</td>
<td>21.1 (42.1)</td>
</tr>
<tr>
<td>GFR (aMDRD)</td>
<td>115 (22.5)</td>
<td>10.8</td>
<td>40.7</td>
<td>0.262</td>
<td>42.1</td>
<td>89.5</td>
<td>21.6 (48.8)</td>
</tr>
<tr>
<td>GFR (CysC)</td>
<td>114 (20.4)</td>
<td>9.6</td>
<td>27.1</td>
<td>0.140</td>
<td>78.9</td>
<td>84.2</td>
<td>16.1 (30.8)</td>
</tr>
</tbody>
</table>

Healthy volunteers

| Mean (SD), mL/min–1.73 m² | Bias | Precision | P | Accuracy % (within 30%) | Accuracy % (within 50%) | Relative difference (%) |
|-----------------|-----------------|------------|---------|-------------|-----------------|-------------|------------|
| CLcr (C&G) | 112 (17.0) | 7.3 | 35.0 | 0.373 | 63.2 | 79.0 | 16.3 (42.6) |
| GFR (aMDRD) | 100 (16.3) | – 4.3 | 31.7 | 0.561 | 79.0 | 89.5 | 3.2 (33.9) |
| GFR (CysC) | 124 (18.8) | 19.4 | 27.5 | 0.007 | 68.4 | 79.0 | 25.6 (31.7) |

Values are mean (standard deviation).
Receiver operating characteristic (ROC) curves for determining the diagnostic accuracy for detection of an abnormal GFR with the different formulas are shown in Fig. 2. Cutoff levels set at 90 and 80 mL/min-1.73 m² were chosen to define how sensitive and specific the estimates of GFR were in identifying mild reductions in GFR. ROC analysis was performed for CF patients and healthy volunteers separately and is summarized in Table 3. The AUC for Cys C was significantly greater than the value for the C&G or MDRD in CF patients with a GFR cutoff of 90 mL/min-1.73 m². The AUC for Cys C was also greater in CF patients with a GFR cutoff of 80 mL/min-1.73 m² but this did not reach statistical significance. No significant difference in the predictive equations was noted in the healthy volunteer group at either GFR cutoff value.

4. Discussion

In this study, we demonstrate that cystatin C clearance may be an alternative marker of GFR in patients with CF when compared with estimates incorporating serum creatinine such as C&G and an improved marker of GFR compared to the aMDRD equation. Specifically, CysC demonstrated greater sensitivity and specificity in identifying reduced GFRs (GFR cutoff value of 90 mL/min-1.73 m²) when compared with the equations incorporating serum creatinine in the cystic fibrosis population.

Prior studies comparing different methods of estimating GFR in CF are few and none have been formally validated in this population. Two studies are found in the literature comparing GFR methods in CF patients [6,22]. Touw et al. [22] compared predicted and measured creatinine clearance in adult patients [18] with CF. Results of this study showed that all three predictors of creatinine clearance tended to over predict the measured creatinine clearance. In a more recent study Al-Aloul et al. compared measured 24-hour creatinine clearance with ten different formulas used to predict GFR, including MDRD and C&G in 74 adult CF patients and 29 matched normal controls [6]. Seven of the formulas, including C&G and MDRD, were found to be equally applicable in CF patients, and none demonstrated superiority to the other in terms of precision of the estimates or correlation with the measured creatinine clearance. The authors concluded that the accuracy of these formulas is limited and not as robust as the reference method (i.e. measured creatinine clearance). The results of our study are in agreement with these two studies demonstrating that the available predictive methods appear to overestimate GFR in patients with CF. Our results extend these findings to show that CysC provides a more precise measure of GFR. In addition, CysC provides greater sensitivity and specificity for identifying reduced GFR.

To our knowledge, this is the first study to evaluate the new biomarker Cys C in estimating GFR in patients with CF. In a recent meta-analysis [9] serum cystatin C was determined to be superior to serum creatinine (SCr) as a marker of kidney function by both superior correlation coefficients and greater ROC-plot AUC values in patients with various degrees of kidney function. Additional studies performed in patients with various disease states with clinical characteristics shared by patients with CF (i.e. malnutrition, mild to moderate impaired kidney function, diabetes) [5,23,24] are in agreement with these results suggesting that the use of Cys C in CF patients may be favorable to the formulas using serum creatinine. The results of the present investigation suggest that Cys C is a suitable alternative marker
of GFR compared to the C&G and aMDRD equations. There are several possible reasons for the apparent improved precision and sensitivity/specificity of GFR estimates based on Cys C rather than serum creatinine utilized in the aMDRD equation. In contrast to serum creatinine, Cys C levels in the serum appear to be unaffected by age, gender, weight, muscle mass, diet, or physical activity [25]. A significantly higher correlation was found between 1/Cys C and 51Cr-EDTA than with 1/Cr in spinal cord injury patients [24]. In patients with liver cirrhosis, a significantly greater correlation was found between 1/Cys C and inulin compared to 1/Cr [26]. A poor correlation between GFR and creatinine in individuals with muscle atrophy is likely a result of reduced serum creatinine concentrations leading to a falsely higher predicted GFR. Similarly, use of the aMDRD equation for estimation of GFR in patients with malnutrition resulted in significant overestimation of the measured GFR [4]. CF patients may also exhibit a reduced muscle mass due to malnutrition as indicated by the significantly reduced BMI noted in this population. While no significant correlation was noted between the bias of C&G or MDRD and the BMI, the overall precision and in particular the sensitivity and specificity in CF patients with GFR values 90 mL/min-1.73 m² or above were much improved with Cys C. Coll et al. [10], demonstrated that serum creatinine levels only started to become abnormal when the GFR was ~75 mL/min–1.73 m². In contrast, elevated values of Cys C were observed at a GFR of 88 mL/min–1.73 m². These results are supported by other studies [23,27] in which ROC analysis revealed significantly larger AUCs for Cys C when compared with creatinine in subjects with normal to mild renal insufficiency. These results suggest that Cys C has an improved diagnostic accuracy for identifying mild renal disease and may perhaps provide earlier detection of renal impairment in CF patients. This is of particular importance in patients receiving repeated intravenous aminoglycoside or colistimethate courses for treatment of acute pulmonary exacerbations. In addition, routine monitoring of Cys C may provide a more sensitive marker of mild renal disease in patients with CFRD allowing early intervention.

Fig. 2. ROC curve analysis of diagnostic accuracy of GFR estimated from cystatin C, MDRD, and C&G formulas in patients with CF (top panel) and healthy volunteers (bottom panel). The GFR determined with iothalamate was used as the gold standard and the discrimination point was set at (A) GFR <90 mL/min-1.73 m², and (B) GFR <80 mL/min-1.73 m².
Table 3

ROC analysis for creatinine clearance (G&G), and GFR estimated cystatin C clearance (CysC) and the abbreviated MDRD equation in CF and healthy volunteers

<table>
<thead>
<tr>
<th>GFR cutoff</th>
<th>GFR estimate</th>
<th>AUC</th>
<th>95% C.I.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF (n=19)</td>
<td>80 (n=14)</td>
<td>C&amp;G</td>
<td>0.543</td>
<td>0.303–0.769</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cystatin C</td>
<td>0.800</td>
<td>0.556–0.944</td>
</tr>
<tr>
<td></td>
<td></td>
<td>aMDRD</td>
<td>0.500</td>
<td>0.267–0.733</td>
</tr>
<tr>
<td>90 (n=10)</td>
<td></td>
<td>C&amp;G</td>
<td>0.556</td>
<td>0.314–0.779</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cystatin C</td>
<td>0.928</td>
<td>0.712–0.991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>aMDRD</td>
<td>0.539</td>
<td>0.300–0.765</td>
</tr>
<tr>
<td>HV (n=19)</td>
<td>80 (n=16)</td>
<td>C&amp;G</td>
<td>0.646</td>
<td>0.397–0.847</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cystatin C</td>
<td>0.771</td>
<td>0.524–0.928</td>
</tr>
<tr>
<td></td>
<td></td>
<td>aMDRD</td>
<td>0.719</td>
<td>0.469–0.896</td>
</tr>
<tr>
<td>90 (n=14)</td>
<td></td>
<td>C&amp;G</td>
<td>0.686</td>
<td>0.436–0.874</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cystatin C</td>
<td>0.657</td>
<td>0.408–0.855</td>
</tr>
<tr>
<td></td>
<td></td>
<td>aMDRD</td>
<td>0.607</td>
<td>0.361–0.818</td>
</tr>
</tbody>
</table>

a CG vs. CysC.

b CysC vs. aMDRD.

c CG vs. aMDRD.

Many studies have been performed to determine whether GFR is altered in patients with CF demonstrating conflicting results. Some showed a significantly increased GFR in the CF population compared to controls [28–30], while others demonstrate no significant differences [6,31–35]. A number of the early studies conducted in the 1980s indicate GFR is higher in patients with CF. In contrast, more recent studies starting in the 1990s demonstrate no significant differences in GFR. In particular, a study conducted by Strandvik et al. did not find any significant difference in GFR between CF and healthy volunteers. They suggested that the change from a fat-restricted diet to a high fat diet in the late 1980s may be responsible for normalization of the GFR in patients with CF and may account for the apparent discrepancy in the studies [34]. The investigators tested their hypothesis in a clinical study in which they provided fatty acid supplementation over a 3 year period. The mean GFR decreased significantly from 133±19 mL/min–1.73 m² at the start of the study to 111±14 mL/min–1.73 m² (P<0.05) which was within normal values after 1-year [34].

Other possible reasons for the discrepancy in the published studies of GFR may be related to differences in age or disease severity. Our study results are consistent with those of recent investigations and indicate no significant difference in GFR between patients with CF and age-matched control subjects.

There are several limitations to our data. Firstly, the number of subjects, although larger than most published studies with CF to date, is small. In addition, the age range included adults between the age of 20 and 44 years in HV and 24 to 48 years in CF patients, but no adolescents or children. Age is one of the determining factors for GFR, as GFR decreases significantly when you get older. Taking this into account, subjects in our study were age-matched and the GFR of iothalamate were not significantly different between healthy volunteers and CF, but additional studies in pediatric CF patients are needed. Finally, subjects representing a full range of renal function were not included in this study. Our primary interest was to characterize GFR and renal clearance of drugs in representative patients with CF and controls. Additional studies enrolling CF subjects with a range of renal function would help to determine the validity of Cys C in patients with various degrees of renal function. Future studies evaluating specific subgroups that have a higher risk for renal disease, such as those with CFRD and those using multiple intravenous aminoglycoside courses would also be beneficial in establishing the role of Cys C in CF.

In conclusion, we found Cys C clearance provides improved sensitivity, specificity, and precision in estimating GFR in adult patients with cystic fibrosis when compared with equations incorporating serum creatinine (e.g., C&G and aMDRD). The use of Cys C should be considered for clinical monitoring in the CF population, particularly in those patients at higher risk for renal disease, such as those with CFRD and those using multiple intravenous aminoglycoside or colistimethate therapies.

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References


