Calculation of glomerular filtration rate expressed in mL/min from plasma cystatin C values in mg/L

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The Cockcroft – Gault formula is often used to calculate the glomerular filtration rate (GFR) from plasma creatinine results. In Sweden this calculation is not usually done in the laboratory, but locally in the wards. These manual calculations could cause erroneous results. In several studies plasma cystatin C has been shown to be superior to plasma creatinine for estimation of GFR. One limitation of using cystatin C as a GFR marker is that there is no conversion formula transforming cystatin C expressed as mg/L to GFR expressed as mL/min. In this study plasma creatinine and cystatin C were compared with iohexol clearance. A stronger correlation (p < 0.0001) was found between cystatin C and iohexol clearance (r² = 0.91) than between creatinine and iohexol clearance (r² = 0.84). From the correlation data a formula was calculated to convert cystatin C expressed as mg/L to GFR (mL/min). The formulas y = 77.24x⁻¹.2623 (Dade Behring cystatin C calibration) or y = 99.43x⁻¹.5837 (DakoCytomation cystatin C calibration) are used to calculate GFR expressed in mL/min from the cystatin C value in mg/L and both results are reported to the referral doctor. These formulas can provide the clinicians with reliable and readily available GFR data based on single measurements of cystatin C concentrations.

**Key words:** Creatinine; cystatin C; glomerular filtration ratio; iohexol; kidney

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**INTRODUCTION**

In the past few decades, serum or plasma creatinine has become the most commonly used marker of glomerular filtration rate (GFR) [1, 2]. Despite its common use, creatinine has limitations as a marker for renal function. GFR is often calculated from plasma creatinine using the Cockcroft–Gault formula [3]. Cockcroft–Gault (CCr) = (140 – age)/(serum creatinine) * (weight/72) * (0.85 for women) * BSA/1.73 m², where BSA is body surface area, estimated...
using the formula [4]: BSA (m$^2$) = 0.20247 × height (m)$^{0.725}$ × weight (kg)$^{0.425}$. This equation consists of several parts, which introduces several possibilities for erroneous results, especially if the calculation is performed manually. Creatinine is influenced by factors such as age, gender, muscle mass, physical activity and diet [5]. Owing to the non-linear relationship between plasma concentration and GFR [6], creatinine also lacks sensitivity in detecting small decreases in GFR, in the so-called creatinine-blind GFR area. Thus, better GFR markers are needed. Several markers such as β-trace protein, cystatin C and β2-microglobulin have been suggested as alternatives to creatinine [7–9]. The normal plasma level of cystatin C is <1.20 mg/L for patients under 50 years of age and <1.55 mg/L for patients over 50 years of age, while increasing levels are detected in the plasma of patients with reduced GFR. In our laboratory we have been using plasma cystatin C as a marker for GFR. Cystatin C is a polypeptide with a molecular mass of 13 kDa and a probable ellipsoid shape with axes of about 30 and 45 Å [10]. The amino acid sequence of human cystatin C was determined in 1981 [11]. Cystatin C functions as an inhibitor of cysteine proteases and can be found in all body fluids [12]. The cystatin C gene is of the housekeeping type, which indicates a stable production rate of cystatin C by most nucleated cell types [13]. The synthesis of cystatin C is not influenced by age, gender or inflammatory response. Plasma proteins with molecular masses below 15–20 kDa are generally almost freely filtered through the normal glomerular membrane. Studies of the handling of human cystatin C in the rat have shown that the plasma clearance of cystatin C is 94% of that of the generally used GFR marker $^{51}$Cr-EDTA [14].

The results of a recent meta-analysis indicate that cystatin C is superior to plasma creatinine [15] as a marker of renal function. One persisting problem with using cystatin C as a GFR marker is that there is no procedure to transform cystatin C concentrations in mg/L to GFR values in mL/min. The latter is often used to calculate, for example, the correct amount of antibiotics or cytotoxic drugs for individual patients. In order to provide clinicians with reliable and readily available GFR data based on single measurements of cystatin C, we have compared the results obtained by iohexol clearance measurements in mL/min and plasma cystatin C mass concentration values in mg/mL. The results were then used to calculate an equation converting cystatin C in mg/L to GFR in mL/min and compared with determinations of creatinine as a GFR marker.

**MATERIALS AND METHODS**

**Patient samples and assays**

Patients referred to the Department of Clinical Chemistry, University Hospital MAS, Malmö for routine iohexol clearance were included in the study (40 females and 60 males; age range 4–92 years).

GFR was determined by measuring the plasma clearance of iohexol, a radiocontrast agent, which is a reliable marker for GFR [16, 17]. Each patient was given 5 mL iohexol solution (Omnipaque, Nycomed Amersham) intravenously in an antecubital vein. Clearance was calculated from the iohexol concentration 4 h after the injection [18]. Serum iohexol levels were determined by high-pressure liquid chromatography [17, 19]. The total analytical imprecision of the method was 3.9% using a control sample with an assigned value of 32 mg/L and 4.3% for a control sample with an assigned value of 137 mg/L.

Plasma cystatin C measurements were done with a latex enhanced reagent (N Latex Cystatin C, Dade Behring, Deerfield, IL, USA) using a Behring BN ProSpec analyzer (Dade Behring). The total analytical imprecision of the method was 4.8% at 0.56 mg/L and 3.7% at 2.85 mg/L. The results were converted to DakoCytomation (Glostrup, Denmark) calibration with the formula: DakoCytomation result = 0.85 * ProSpec result + 0.35. This conversion was made in order to obtain comparable results between different laboratories in Sweden. Plasma creatinine measurements were performed with the modified kinetic Jaffe reaction using an Advia 1650 analyzer (Bayer Corp., Tarrytown, NY, USA) and reported using SI units (µmol/L). The total analytical imprecision of the method was 2.6% at 170 µmol/L and 2.4% at 740 µmol/L. Both these assays were carried out at the Department of Clinical Chemistry, University Hospital, Uppsala. All assays were performed independently without...
prior knowledge of other test results. The study was approved by the local ethics board at Uppsala University (01-167).

Statistical calculations

Statistical analysis was performed with Excel 2000 (Microsoft Corporation, Seattle, WA, USA). Equations for the correlation curves were calculated by using “best fit” in Excel (Microsoft Corporation).

RESULTS

Correlation between cystatin C and iohexol clearance

Cystatin C values (mg/L) obtained with Dade Behring calibration (Fig. 1) and DakoCytomation calibration (Fig. 2) both showed a strong correlation with iohexol clearance (r^2 \approx 0.91).

Equations for the two correlation curves were calculated.

We used Dade Behring calibration of the cystatin C method (mg/L) to obtain the formula: 

\[ y = 77.24x^{-1.2623} \]

and DakoCytomation calibration of the cystatin C method (mg/L) to obtain the formula: 

\[ y = 99.43x^{-1.5837} \]

Correlation between creatinine and iohexol clearance

There was a significantly stronger correlation (p < 0.0001) between cystatin C (mg/L) and iohexol clearance (r^2 = 0.91) than between creatinine (µmol/L) and iohexol clearance (r^2 = 0.84) (Figs 1–3).

DISCUSSION

Glomerular filtration rate is generally accepted as the best overall index of renal function. Reduced GFR is the most important complication of renal disease. Reduced GFR influences the metabolism and clearance of many pharmaceuticals used today. Thus, in many cases the recommended dose has to be adjusted.
Fig. 2. Correlation between cystatin C (Dako calibration) and iohexol clearance for individual patients.

\[ y = 99.434x^{-1.5837} \]

\[ R^2 = 0.9124 \]

Fig. 3. Correlation between creatinine and iohexol clearance for individual patients.

\[ y = 12310x^{-1.1564} \]

\[ R^2 = 0.8362 \]
depending on the patient’s GFR. For instance, because antibiotics and cytotoxic drugs are usually prescribed according to GFR, there is a need for GFR markers. Inulin, iohexol and $^{51}$Cr-EDTA clearances are considered the gold standards for GFR measurements. The disadvantage with these assays is that they are cumbersome, costly and slow, which could delay the start of treatment. Assays such as plasma creatinine and cystatin C can provide rapid test results. Creatinine in combination with the Cockcroft–Gault equation is often used to estimate GFR. Using actual body weight in the Cockcroft–Gault equation overestimates the GFR for obese patients. An alternative could be to use lean body mass, but this is not commonly available. Creatinine often overestimates GFR in patients with slightly reduced GFR. This may cause the doctor to treat the patient with unnecessarily high drug doses, which will increase the cost and possibly cause side effects. In Sweden, calculation of GFR using the Cockcroft–Gault equation is generally performed locally in the wards. The equation contains height and weight that may be difficult to measure correctly in all cases. To increase the quality of GFR measurements we are currently in the process of replacing plasma creatinine measurements with plasma cystatin C, which in our opinion and experience is a better marker for GFR than plasma creatinine. Cystatin C results are given in the unit mg/L and a constant problem is that the doctor has to convert a mass concentration value in mg/L to a GFR value in mL/min. With the ubiquitous use of computers in the clinical chemistry laboratory, no human intervention for such a calculation should be required and the present study suggests two suitable formulas for transforming a cystatin C result in mg/mL to a GFR result in mL/min.

In the present investigation, a cluster of iohexol results of around 10–20 mL/min can be observed, while the corresponding cystatin C results vary between 2.5 and 4.5 mg/L. This could indicate that cystatin C has a better discriminatory capability than iohexol clearance in patients with GFR in the 10–20 mL/min range.

We have measured cystatin C levels in samples from patients whose GFRs were determined using the iohexol clearance method. The results have then been used to calculate an equation to convert cystatin C levels in mg/mL to GFR in mL/min. This has been greatly appreciated by our clinical colleagues and has contributed to the rapid increase in the use of cystatin C as a GFR marker. The assay is available as stat. sample at all hours in our laboratory.

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REFERENCES


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