Improvements in the use of plasma creatine as a marker of the glomerular filtration rate
Kemperman, F.A.W.

Citation for published version (APA):
Kemperman, F. A. W. (2001). Improvements in the use of plasma creatine as a marker of the glomerular filtration rate

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 5

GFR-estimation from plasma creatinine after inhibition of tubular secretion: relevance of the creatinine assay


Departments of Internal Medicine and Clinical Chemistry of the Hospital Onze Lieve Vrouwe Gasthuis, Clinical Chemistry of the Slotervaart Hospital and Clinical Chemistry and Internal Medicine of the Academic Medical Center, University of Amsterdam, The Netherlands

*Nephrol Dial Transplant* 1999;14:1247-1251
Abstract

Background: Estimation of GFR from plasma creatinine concentration after inhibition of tubular creatinine secretion with cimetidine provides a good assessment in patients with various nephropathies and with diabetes mellitus type 2. The aim of this study was to compare cimetidine-aided GFR-estimations using various creatinine assays.

Methods: In 30 outpatients with type 2 diabetes GFR was measured as the urinary clearance of continuously infused $^{125}$I-iothalamate. Plasma creatinine concentration was analyzed after oral cimetidine with an alkaline picrate (AP) method, with an enzymatic (PAP) assay and with HPLC. GFR-estimations were calculated with the Cockcroft-Gault formula (CG).

Results: AP-creatinine concentrations were significantly higher than PAP or HPLC values. GFR estimations by AP ($CG_{AP}$ 66 ± 19 ml/min/1.73m$^2$, mean ± SD) were significantly lower than GFR (89 ± 30), whereas $CG_{PAP}$ (85 ± 30) and $CG_{HPLC}$ (84 ± 34 ml/min/1.73m$^2$) were not. Bland and Altman analysis showed a difference between $CG_{AP}$ and GFR of -22.4 ± 17.7 ml/min/1.73m$^2$ for. This difference was larger the higher the GFR. The difference between $CG_{PAP}$ and GFR was only -3.8 ± 14.8 ml/min/1.73m$^2$ and between $CG_{HPLC}$ and GFR the difference was -4.4 ± 17.5 ml/min/1.73m$^2$ for HPLC, without any systematic difference.

Conclusion: A good assessment of the GFR from plasma creatinine after cimetidine administration is possible when creatinine is measured with an enzymatic assay or with the less convenient HPLC method. The more widespread and cheaper alkaline picrate assay is not suitable for GFR-estimation.
Introduction

Assessment of renal function is important for clinical management of patients and for intervention studies. The plasma creatinine concentration is not an accurate reflection of the glomerular filtration rate (GFR) [1]. Furthermore, accurate measurement of plasma creatinine is laborious [2]. The alkaline picrate (AP) method - described by Jaffé more than 100 years ago - is used most often, but is influenced by other chromogens than creatinine [3]. This causes overestimation of the plasma creatinine concentration, especially within the normal range in adults [2,4]. Several modifications of the AP method have not solved this problem [2]. The more recent enzymatic creatinine assays have a higher accuracy than the AP methods, but there is still an interference of bilirubin and various drug metabolites [5]. The coefficient of variation has been reported up to 6.4 % [5]. High performance liquid chromatography (HPLC) and gas chromatography with mass spectrometry (GC-MS) are the best methods for plasma creatinine analysis [6-8].

Creatinine clearance, used as a marker of GFR, is frequently incorrect because of errors in urine collection [9,10]. For this reason Cockcroft and Gault developed a formula to calculate creatinine clearance from plasma creatinine [11]. When plasma creatinine is overestimated by the AP method, an inappropriately low value of creatinine clearance will be the result, as plasma creatinine is in the denominator of the Cockcroft-Gault formula. On the other hand, creatinine clearance overestimates GFR because of tubular secretion of creatinine. This overestimation can be corrected using cimetidine as a competitive inhibitor of tubular creatinine secretion [12-15]. We have shown previously that a good estimation of GFR can be obtained by the Cockcroft-Gault formula in patients with normal or moderately reduced renal function, after administration of cimetidine 2400 mg during the 24 hours before a plasma creatinine sample is taken [16,17].

The aim of the present study was to compare the accuracy of cimetidine-aided GFR-estimations by the Cockcroft-Gault formula using various assays for plasma creatinine. The HPLC method was compared with an AP method and an enzymatic creatinine assay. The study was done in patients with diabetes mellitus type 2, because correct evaluation of interventions to preserve renal function is important in this patient group.
Table 1  Demographic data of 30 patients with diabetes mellitus type 2

<table>
<thead>
<tr>
<th></th>
<th>median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (years)</td>
<td>55  (30-70)</td>
</tr>
<tr>
<td>gender (male:female)</td>
<td>18:12</td>
</tr>
<tr>
<td>weight (kg)</td>
<td>81  (53-115)</td>
</tr>
<tr>
<td>body mass index (kg/m$^2$)</td>
<td>28.3 (21.2-33.6)</td>
</tr>
<tr>
<td>body surface area (m$^2$)</td>
<td>1.89 (1.52-2.50)</td>
</tr>
<tr>
<td>race (White:Asian:Black)</td>
<td>15:11:4</td>
</tr>
</tbody>
</table>

Material and Methods

Study population

Thirty patients of the outpatient clinic with type 2 diabetes and various degrees of albuminuria were included [17]. Demographic data are described in Table 1. The most important exclusion criterium was a body mass index above 35 kg/m$^2$, because in these patients weight overestimates muscle mass. This will lead to an inappropriate overestimation of GFR [18]. Other exclusion criteria were plasma creatinine concentration exceeding 180 µmol/l, age above 70 years and regular use of cimetidine, trimethoprim or salicylates, since these drugs inhibit tubular creatinine secretion. The study protocol was approved by the committee of Medical Ethics of the Hospital Onze Lieve Vrouwe Gasthuis, Amsterdam.

Study protocol

The patients were instructed to take 800 mg of cimetidine at 7 a.m. and at 3 and 11 p.m. the day before a plasma creatinine sample was drawn. This was done at 8 a.m. This sample was analyzed by 3 methods: -a- an AP assay (two-point, fixed time kinetic Jaffé reaction, Hitachi 717, Boehringer Mannheim, Mannheim, Germany). The assay is based on the formation of an orange-red Janovsky complex between creatinine and picate under alkaline conditions; -b- an enzymatic Phenol/4-Aminoantipyrine (PAP) assay (Hitachi 747, Boehringer Mannheim, Mannheim, Germany). This assay consists of three enzymatic - creatininas e - steps and a final chromogen coupling reaction; -c- a HPLC assay, as described by Zwan and Blijenberg [7]. The formula of Cockcroft and Gault (CG) was used for the estimation of GFR from plasma creatinine concentration [11]:

84
GFR-estimation by various plasma creatinine assays

\[
\frac{(140\text{-age})^3\text{body weight}}{0.815^3\text{plasma creatinine}} \quad [\text{ml/min}]
\]

for women a correction factor of 0.85 was applied because of a different body composition; age is expressed as years, weight as kg and plasma creatinine concentration as \(\mu\text{mol/l}\).

Using the three plasma creatinine assays in this formula we obtained results of \(\text{CG}_{\text{AP}}\), \(\text{CG}_{\text{PAP}}\) and \(\text{CG}_{\text{HPLC}}\).

Immediately after taking the blood sample for creatinine determination, GFR and renal plasma flow (ERPF) were measured during continuous infusion of \(^{125}\text{I}-\text{iothalamate} \text{ and } ^{131}\text{I}-\text{hippurate} \text{ after a loading dose was given. GFR was calculated as the mean urinary clearance of } ^{125}\text{I}-\text{iothalamate of two 2-hour periods after a 2-hour equilibration period as described earlier [16,19]. GFR and the CG were corrected for body surface area according to the formula of DuBois and DuBois [20] and expressed as ml/min/1.73m}^2\).

Figure 1 Plasma creatinine concentrations analyzed by the alkaline picrate (AP) method, HPLC and an enzymatic PAP-assay. The data of each patient are connected with a drawn line.

**Statistical analysis**

The results are given as means ± standard deviation (SD). Comparisons were made using two way ANOVA and the paired Wilcoxon test in case of non-Gaussian distribution. Agreements between the various plasma creatinine assays or between GFR and its estimated value were analyzed by the method proposed by Bland and Altman [21]. The accuracy and precision are shown in this type of analysis by relating the difference between two methods in each patient to the mean of the same two methods in the same patient. Whether a trendwise change in the difference is present for increasing mean values can also be analyzed. The limits of agreement can
be expressed as mean + 2SD and mean - 2SD, in which 95% of the values are situated. This method is preferred to correlation coefficients as in a perfect agreement the points lie along the line of identity instead of any straight (regression) line.

![Figure 1](image1.png)

**Figure 1** shows the results of the various assays for plasma creatinine concentration. The difference between creatinine results of AP and HPLC was highly significant \((P < 0.001)\). The difference between creatinine results of PAP and HPLC was much smaller, but the PAP value was lower in 24 of 30 patients and therefore statistically significant \((P < 0.005)\). In Figure 2 the Bland and Altman analysis shows a mean difference between plasma creatinine analyzed by AP and by HPLC of 19.0 \(\mu\text{mol/l}\). The limits of agreement were 3.4 and 34.6 \(\mu\text{mol/l}\). Especially in the lower range of creatinine values the (percentual) difference was large. More agreement was found between the enzymatic PAP-assay and HPLC (mean difference -3.1 \(\mu\text{mol/l}\), limits of agreement -12.7 and 6.5 \(\mu\text{mol/l}\)). PAP-creatinine values were lower than HPLC the higher the creatinine concentration.

**Results**

**Figure 2** Bland and Altman analysis of all 30 patients. In this analysis the difference between two methods is plotted against their mean for each individual patient. This was done for the alkaline picrate (AP) and HPLC-creatinine concentrations (left panel) and for the enzymatic PAP and HPLC-creatinine concentrations (right panel), after cimetidine administration. Mean difference is indicated by a drawn line, the limits of agreement (mean - 2SD and mean +2SD) are indicated by the dashed lines.
Fig. 3 Glomerular filtration rate (GFR) and its estimated value by the Cockcroft-Gault formula (CG) after cimetidine of all 30 patients in relation to the line of identity. Left panel: CG-estimation using the alkaline picrate (AP) creatinine assay; middle panel: using the enzymatic PAP-analysis; right panel: using the HPLC-method.

The data of GFR and its estimated value are shown in Figure 3 in relation to the line of identity. GFR-estimations by CG<sub>AP</sub> (66 ± 19 ml/min/1.73 m<sup>2</sup>) were significantly different from GFR (89 ± 30 ml/min/1.73 m<sup>2</sup>, P < 0.001), whereas there were no differences between CG<sub>PAP</sub> (85 ± 30) or CG<sub>HPLC</sub> (84 ± 34) and GFR. The Bland and Altman analysis of GFR-estimations is shown in Figure 4. The difference between CG<sub>AP</sub> and GFR was larger the higher the GFR. Some agreement between the two methods existed only in the lowest range. The mean difference was -22.8, the limits of agreement were -58.8 and 12.6 ml/min/1.73 m<sup>2</sup>. A better agreement over the whole range of measurements without any systematic difference was seen between either CG<sub>PAP</sub> or CG<sub>HPLC</sub> and GFR. The differences were -3.8 ml/min/1.73 m<sup>2</sup> for CG<sub>PAP</sub> and GFR and -4.4 ml/min/1.73 m<sup>2</sup> for CG<sub>HPLC</sub> and GFR. Moreover, the limits of agreement were similar for CG<sub>PAP</sub> (-33.4 and 25.8 ml/min/1.73 m<sup>2</sup>) and CG<sub>HPLC</sub> (-39.4 and 30.6 ml/min/1.73 m<sup>2</sup>). This means that estimation of GFR by the enzymatic PAP-assay was as good as by HPLC.

Discussion

This study has shown that a good estimation of the GFR by the Cockcroft-Gault formula in patients with diabetes mellitus type 2 and a body mass index up to 35 kg/m<sup>2</sup> is difficult, but if the
plasma creatinine concentration was analyzed after cimetidine administration by an enzymatic or HPLC-assay the best possible estimation was obtained. We chose patients with plasma creatinine concentrations less than 180 μmol/l, because the GFR in these patients can range between normal and markedly reduced. Also, in this range the period after administration of cimetidine needed to reach a new steady state of plasma creatinine - without tubular secretion - is less than 24 hours [16].

Figure 4 Bland and Altman analysis of all 30 patients. In this analysis the difference between the CG-estimation of GFR after cimetidine and the 125I-iothalamate GFR is plotted against the mean value of CG and GFR for each individual patient. Left panel: CG-estimation using the alkaline pikeate (AP) creatinine assay; middle panel: using the enzymatic PAP-analysis; right panel: using the HPLC method. Mean difference is indicated by a drawn line, the limits of agreement (mean - 2SD and mean +2SD) are indicated by the dashed lines. See Figure 5 for abbreviations.

A marked underestimation of GFR occured in the range above 60 ml/min/1.73m², when AP-creatinine values were used in the CG-formula. Consequently, estimation of GFR with this procedure might erroneously suggest a decrease in renal function. This can be an important factor in the clinical management of these patients, for instance, when a renal biopsy is considered. Other authors have also reported underestimation of GFR by the CG-formula using an AP assay in diabetic patients [22,23]. The mean difference of AP-creatinine and HPLC-creatinine values in the present study was 19.0 μmol/l, but the relative difference could increase
GFR-estimation by various plasma creatinine assays

to 94% when the creatinine concentration was lower. This caused a considerable difference of \( \text{CG}_{\text{AP}} \) and \( \text{CG}_{\text{HPLC}} \), the latter of which represented GFR more accurately. \( \text{CG}_{\text{PAP}} \) and \( \text{CG}_{\text{HPLC}} \) were similar and the mean difference with GFR was small.

However, the scatter of the values reflected by the limits of agreement was quite large. Such a scatter is typically shown in a Bland and Altman analysis, where moderate differences with the "line of identity" (zero difference) become more apparent than in a correlation plot. Nevertheless \( \text{CG}_{\text{PAP}} \) and \( \text{CG}_{\text{HPLC}} \) are useful for estimation of GFR, as we have previously shown that estimations based on 24-hour creatinine clearance have an even wider scatter [17]. Because the HPLC-assay is too elaborate for clinical practice, the enzymatic PAP-assay is the preferred creatinine analysis for GFR-estimation.

In the literature many studies dealt with the question of GFR-prediction and many formulas have been advocated as being the most accurate [13-17,22-24]. None of these studies mentioned the importance of the creatinine assay employed. The alkaline picrate or (kinetic) Jaffé method is still widely used, but is inaccurate despite its modifications [2,3]. This is especially so in the creatinine concentration range up to 110 \( \mu \text{mol/l} \), where impairment of renal function is usually not expected. In this range significant changes in renal function can be reflected by rather small changes in plasma creatinine. Yet, the main benefits can be expected from interventions aimed to preserve renal function in this group of patients. Especially in diabetic nephropathy, where hyperfiltration is present in the initial phase, early treatment with ACE-inhibitors and tight control of blood pressure can slow down deterioration of renal function, both in type 1 and in type 2 diabetes mellitus [25-27].

In conclusion, GFR-estimation after cimetidine administration using an enzymatic plasma creatinine assay and the formula of Cockcroft and Gault leads to good results compared to HPLC, clearly superior to estimations obtained with an alkaline picrate analysis.

Acknowledgements

We especially thank J.C. Kennedy for his dedicated radioisotope determinations and GFR-calculations performed in the laboratory of Dr. M.A. Prins, E. Schipper for laboratory support, Dr. W. Lameijer for cimetidine analysis in urine, A.A.M. Hart and D.G. Struijk for statistical advice and the nurses of the outpatient clinic for much assistance. Part of the data obtained with the enzymatic PAP-assay have already been published previously [17].
References


