Technical Report

Iohexol clearance for GFR-determination in renal failure—single or multiple plasma sampling?

G. Sterner, B. Frennby, B. Hultberg and T. Almen

Division of Nephrology, Department of Vascular and Renal Diseases, Department of Diagnostic Radiology, University Hospital, Malmö and Department of Clinical Chemistry, University Hospital, Lund, Sweden

Abstract The present study examined the agreement between single and multiple sample plasma clearance of iohexol, a non-ionic contrast agent, in renal failure. Sixty-five patients with varying degree of renal insufficiency received 10 ml iohexol (300 mg I/ml) i.v. and plasma samples were collected four times during the following 3–24 h. Plasma-iodine concentrations were determined by X-ray fluorescence. Predicted creatinine clearance was used to choose one of the samples for determination of single sample clearance. A single plasma specimen collected at 4 h for GFR above 50 ml/min, at 7 h for GFR between 20 and 50 ml/min, and at 24 h for GFR below 20 ml/min gave values in good agreement with those based on a four sample slope clearance. No sign of nephrotoxicity was noted after administration of the contrast agent. It is concluded that single sample plasma clearance after single injection of iohexol gives a good estimate of GFR in renal failure and is advantageous in clinical practice.

Key words: glomerular filtration rate; iohexol; single sample clearance; NAG

Introduction

Glomerular filtration rate (GFR) can accurately be determined from plasma clearance of iohexol after a single bolus injection. Good agreement was observed between the plasma clearance of iohexol and other GFR-markers such as radiolabelled EDTA, DTPA, and inulin in adults [1–5] and in children [6]. Clearance based on a single plasma sample has recently also been recommended for GFR-determination either separately [5,7–10] or in connection with a radiological examination [11]. There are two prerequisites for the method: (i) there is knowledge about the distribution volume of the GFR-marker and (ii) the plasma sample is collected at the time-point when the size of the distribution volume has a minimal influence on the mathematical calculation of the GFR. As renal function declines this ideal time-point must gradually be postponed. Severe reduction of renal function demands prolongation of the sampling time for at least 24 h for both single sample clearance and slope clearance technique [12]. A problem arises when choosing the time-point for single sample clearance as renal function could be difficult to estimate, e.g. by serum creatinine.

We hypothesized that if we guessed the approximate level of GFR with aid of a predicted creatinine clearance [13] we might then be able to choose the appropriate sampling time necessary for an accurate determination of GFR with single sample clearance.

The aim of the present study was to study patients with reduced renal function and to compare iohexol clearance values based on single and multiple plasma samples. To evaluate if the proper sampling time for single sample clearance can be estimated in advance a predicted creatinine clearance [13] was used. Furthermore the toxic influence of iohexol on glomerular and tubular functions was studied.

Subjects and methods

Sixty-five patients (28 females) with varying degree of renal failure were included in the study. Mean age was 53 years (range 19–79 years). Forty-one patients had received a renal transplant more than 3 months earlier and had stable renal function. The remaining 24 patients had varying degree of chronic and stable renal failure. The diagnoses of the 65 patients are shown in Table 1. Mean serum-creatinine of the group was 207 μmol/l (range 63–810 μmol/l). The patients were examined in a non-fasting state. In the

Table 1. Diagnosis of the 65 patients studied

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patients</th>
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<tbody>
<tr>
<td>Chronic glomerulonephritis/vasculitis</td>
<td>19</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>13</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>11</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>8</td>
</tr>
<tr>
<td>Nephrosclerosis</td>
<td>7</td>
</tr>
<tr>
<td>Alport's syndrome or other hereditary diseases</td>
<td>6</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>1</td>
</tr>
</tbody>
</table>
morning they were given 10 ml iothixol (Omnipaque 300 mg I/ml) i.v. in one forearm through a venous cannula. All blood samples were collected from the contralateral arm. Predicted creatinine clearance using s-creatinine, age, sex and weight was calculated according to the Cockcroft–Gault formula [13].

The protocol was approved by the local ethics committee and every patient gave informed consent.

**Multiple sample plasma clearance (Cl slope)**

Four plasma samples were drawn 3, 4, 7, and 24 h after the i.v. injection of iohexol. Plasma-iodine concentrations were determined with X-ray fluorescence analysis (Renalyzer, Provalid AB, Lund, Sweden). Using a computerized program clearance was calculated according to the slope-intercept method with estimation of the area under the curve [14]. The area was corrected for the early distribution phase according to Brøchner-Mortensen [14]. The determination of the slope clearance was based on all four plasma samples except in some patients with nearly normal GFR, in whom the last sample was excluded. In these patients the last blood sample after 24 h contained iodine below the detection limit.

**Single sample plasma clearance (Clss)**

The method described by Jacobsson [7] was used with corrections for lack of complete uniform distribution and non-immediate mixing. The distribution volume was calculated as a function of the body weight [15]. The time for plasma sampling after injection of marker was chosen as follows: with a predicted clearance above 50 ml/min the plasma sample was taken after 4 h, with a predicted clearance of 20–50 ml/min after 7 h and below 20 ml/min after 24 h.

\[
\text{Cl}_{\text{ss}} = \frac{1}{t/V + 0.0016} \times \ln\left(\frac{Q_{\text{tot}}}{V \cdot [C_i]}\right)
\]

Where \(Q_{\text{tot}}\) is the total injected amount of iodine (mg), \(t\) is the time interval between injection and sampling (min), \(C_i\) is the iodine concentration in the plasma sample taken at the time \(t\) and \(V\) is the distribution volume (ml) of the patient.

The clearance values were adjusted to 1.73 m² body surface.

**Renal toxicity parameters**

Any adverse reaction that the patients reported after the contrast medium injection was registered.

The transplanted patients were examined on the morning before and on the two days following the injection of iohexol. S-creatinine was measured using a routine Jaffe method. Albumin/creatinine ratio was determined. The fractional excretion of albumin (bromcresol purple method) was calculated as the ratio between the albumin clearance and the creatinine clearance (normal value below 0.01 \(\times 10^{-3}\)). Urine albumin was analysed using electroimmunoassay with human albumin as standard.

\(N\)-acetyl-beta-glucosaminidase (NAG) was determined in urine according to Hultberg and Wieslander [16].

**Statistical analysis**

Linear regression analysis was used to examine the association between single and multiple plasma clearance.

Agreement between the methods was evaluated according to Bland and Altman [17]. Renal toxicity parameters were expressed as mean and range and differences between groups assessed by ANOVA, and paired and unpaired \(t\)-test.

**Results**

Correlation between iohexol clearance derived from a single plasma sample and from the traditional multiple sample slope was good \((r^2 = 0.96)\). The individual measurements for the whole material and for GFR below 40 ml/min are given in Figures 1 and 2. The difference between the four sample and the single sample plasma clearance against the mean of the two methods is shown in Figure 3a together with the 95% limits of agreement. Figure 3b shows the log-transformed data. The antilogs of the limits of agreement were 0.66 and 1.24 demonstrating that 95% of the single sample clearance fell within 0.66 and 1.24 times the four sample clearance values.

By choosing a too early plasma sample (on the basis of the predicted creatinine clearance) an overestimation of GFR was noted for the single sample method in some cases. When the sampling time for Clss was chosen retrospectively from the slope clearance, Clss...
Iohexol clearance in renal failure

and $C_{\text{slope}}$ agreed better than above (Fig. 4). As could be expected a very low degree of agreement was found when comparing predicted creatinine clearance directly with four sample clearance (Fig. 5).

As blood sampling was extended for 24 h the patients were under close supervision. There were no reported adverse effects (as urticaria, flushing, and itching) in conjunction with the injected contrast medium in any of the 65 subjects. U-NAG levels were significantly raised before injection of contrast medium in the group of transplanted patients compared with a reference population (0.57, 0.06–1.77 versus 0.21, 0.04–0.65; mean, range, $P<0.001$). Table 2 shows the effect of a low dose iohexol administration to patients with a renal graft. There was no statistically significant change in neither the U-NAG concentrations nor in s-creatinine and the albumin-creatinine clearance ratio.

**Discussion**

GFR is frequently calculated from the dose given and the plasma slope curve after single injection of a suitable marker [14]. Recently reports have described a simplification using only one plasma sample [18,19]. Jacobsson [7] introduced correction factors for lack of uniform distribution of the GFR-marker and for the non-immediate mixing. Several other substances have been utilised for determination of single sample clearance [8,20–22].

The single sample method has been recommended down to a GFR of 30 ml/min [23]. According to theoretical estimations later plasma samples are necessary for accurate determination of single sample clearance when renal function declines further. Jacobsson [7] calculated the optimal sampling time at a GFR of 30 ml/min to be 8–9 h.

We have reported excellent agreement between single and multiple plasma sample iohexol clearances in patients with low GFR [12]. Prolongation of the sampling time up to 24 h is necessary in either clearance method if reliable GFR values are sought. In clinical practice it is, however, sometimes difficult in advance to estimate renal function and thus to choose the
In the renal toxicity study in patients with stable renal allograft after the administration of iohexol, Values are given as mean and range. Analysis of variance was performed and revealed no changes between the groups.

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>1 day after</th>
<th>2 days after</th>
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<tbody>
<tr>
<td>S-creatinine (µmol/l) n=40</td>
<td>140, 69-400</td>
<td>142, 71-386</td>
<td>142, 74-399</td>
</tr>
<tr>
<td>Albumin/creat. clearance ratio n=40</td>
<td>0.15, 0.001-2.21</td>
<td>0.14, 0.01-1.83</td>
<td>0.15, 0.01-2.06</td>
</tr>
<tr>
<td>U-NAG (U/mmol creat.) n=38</td>
<td>0.57, 0.06-1.77</td>
<td>0.64, 0.06-2.0</td>
<td>0.72, 0.08-2.42</td>
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</table>

The single sample clearance has shown slightly larger variation than based on four plasma samples when compared with a clearance where a "true" estimation of the area under the curve was calculated from 14 blood samples [9]. Differences in determining the extracellular volume in which the GFR-marker is distributed has been reported for single sample clearance [14,23]. Knowledge of the distribution volume is mandatory when calculating single sample clearance. Severe oedema, sometimes found in patients with renal or hepatic failure, could interfere with the calculation of distribution volume for the GFR-marker. In these occasions single sample but also multiple sample clearance could give erroneous values for GFR [5,25]. We noted identical values for iohexol when comparing single and multiple sample clearance in three patients with ascites (non-published observations). Renal clearance of e.g. radio contrast media is the only way to eliminate the problem with capricious estimations of the extracellular volume.

A prerequisite for using the GFR-marker in renal failure is that it is non-toxic. The safety of the contrast agent used in this study was therefore paid special interest. We found no signs of contrast nephrotoxicity as no statistically significant increase could be found of urinary NAG after the iohexol administration. This enzyme is known to be a very sensitive test and is considered to detect even a slight renal injury [26,27]. This confirms the results from an earlier iohexol study [12]. The contrast dose used in the present study was well tolerated by the patients. In a larger material of approximately 4000 iohexol clearance measurements no severe adverse reaction was noted [28].

In conclusion clearance based upon multiple blood samples can be substituted by a single blood specimen clearance in patients with reduced renal function. The accuracy of the method is, however, dependent on proper sampling time and no universal point of time can be used for all GFR-levels. Single sample clearance gives for clinical purpose useful estimation of GFR and is clearly advantageous for the patients.

**References**

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Iohexol clearance in renal failure


Received for publication: 13.3.95
Accepted in revised form: 17.11.95