GFR measurement with iohexol and $^{51}$Cr-EDTA. A comparison of the two favoured GFR markers in Europe

Eva Brändström¹, Andrzej Grzegorczyk², Lars Jacobsson³, Peter Friberg¹, Anders Lindahl², and Mattias Aurell⁴

Departments of ¹Clinical Physiology, ²Clinical Chemistry, ³Radiation Physics and ⁴Nephrology, Sahlgrenska University Hospital, Göteborg, Sweden

Abstract

Background. The aim of the present study was to compare the most commonly used GFR markers for clearance measurements, $^{51}$Cr-EDTA and iohexol, using two different methods for iohexol analysis, HPLC and X-ray fluorescence, referring both to the multiple-sample and single-sample calculations, using $^{51}$Cr-EDTA as the reference method.

Methods. Forty-nine patients with an estimated GFR $>40$ ml/min were included. $^{51}$Cr-EDTA and iohexol were injected simultaneously and blood samples were taken 150, 195 and 240 min after injection of the respective marker.

Results. The multiple-point clearances, determined from HPLC and X-ray fluorescence, compared to $^{51}$Cr-EDTA correlated highly ($r=0.92$ and 0.95 respectively). The results from single-point clearance comparison, iohexol measured by HPLC vs $^{51}$Cr-EDTA, yielded a correlation of $r=0.91$, while single-point clearance from iohexol, analysed by X-ray fluorescence, obtained a correlation of 0.93 and an intercept statistically different from zero.

Conclusions. Iohexol and $^{51}$Cr-EDTA are comparable as GFR markers for multiple-point clearance measurements. The single-sample method for GFR $>40$ ml/min can be used with a high accuracy. The precision and accuracy of X-ray fluorescence analysis of low concentrations of iohexol were less than those of HPLC. Care should therefore be taken when using X-ray fluorescence that the injected dose of iohexol should result in a plasma concentration level of iodine of at least 0.06 mg/ml at the time of blood sampling.

Key words: Cr-EDTA clearance; iohexol; glomerular filtration rate

Introduction

In clinical practice, measurement of the glomerular filtration rate, GFR, is the major kidney function test.

The precise and reliable measurement of GFR has been studied and debated ever since it was introduced more than 70 years ago using creatinine as a marker [1]. Inulin was subsequently introduced by Shannon and Smith [2] in the 30s, and inulin is still used as the major reference substance for GFR measurements. However, inulin is expensive and tedious to use. In recent years, two major classes of substance have been introduced as alternatives to inulin for the measurement of GFR, namely the chelating agents EDTA and DTPA, and the radiological contrast media such as iotalamate, diatrizoate, and most recently iohexol [3]. Several studies have shown that these substances may well serve as GFR markers, and a consensus report has been published on GFR measurements using radiolabelled markers [4].

It is important, however, to develop methods for the non-radioactive measurement of GFR, as equipment for measurement of radioactivity or radiolabelled markers is not always available. In the US for example, the $^{51}$Cr-EDTA complex is not available for clearance studies, although this has been the most commonly used GFR marker in Europe for decades. Simple and reliable methods have now been developed for the measurement of the newly introduced non-ionic, low osmolar radiological contrast medium iohexol, which can be analysed by using high pressure liquid chromatography (HPLC) and X-ray fluorescence techniques [5,6]. This opens up the field for the use of iohexol as GFR marker and several studies have already indicated its usefulness [7–10]. Hitherto, there has been no study, however, on the simultaneous analysis of iohexol clearance comparing the two methods of iohexol analysis. Also, iohexol measured with the two techniques has not been compared with the GFR marker $^{51}$Cr-EDTA in simultaneous measurements using the plasma clearance method, i.e. calculations of the clearance value based on the plasma disappearance rate of the indicator after a single injection.

The aim of this investigation was to make a comparison of iohexol, assessed with two completely different techniques, and $^{51}$Cr-EDTA plasma clearance measurements—the latter used as the reference method, per-
Comparison of iohexol and $^{51}$Cr-EDTA clearances

Formed simultaneously, and the plasma clearance calculated according to different methods (single- and multiple-sample approach).

**Subjects and methods**

**Patients**

Fifty consecutive patients, referred to the Department of Clinical Physiology at the Sahlgrenska University Hospital for routine GFR measurement, were asked to volunteer, according to a protocol approved by the ethical committee. Twenty-five males, age range 19–74 years (mean age 48 years) and 25 females, age range 19–82 years (mean age 53 years), agreed to participate.

All patients had an estimated GFR $>40$ ml/min/1.73 m$^2$ BSA, as determined using creatinine, age, weight and gender of the patients [11]. The patients suffered from different renal diseases as shown in Table 1. They were all in stable conditions at the time of the investigation. Due to technical difficulties one patient was excluded from the calculations.

**Study protocol**

The investigation started at 8 a.m. and the patients were allowed a light breakfast but were asked to refrain from smoking. An indwelling venous Teflon catheter was inserted into an antecubital vein. A blood sample of 10 ml was obtained for background measurements. The markers were then given as single-injections. First $^{51}$Cr-EDTA was injected (3.7 MBq, Amersham, UK) and then, after having rinsed the cannula using 30 ml saline, 20 ml of iohexol (Omnipaque 300 mg/ml, Nycomed, Norway) was given within the next minute and the cannula was again flushed with saline. The exact time for the injection was registered and the syringes used for the $^{51}$Cr-EDTA and iohexol injections were carefully weighed before and after the injections.

Blood samples for analyses were then obtained, starting 2.5 h after the injections of the GFR markers. To define the plasma disappearance curve of the GFR markers, three blood samples (10 ml each) were obtained at 150, 195 and 240 min after the injections. After centrifugation (3000 g for 10 min), 3 ml plasma was used for scintillation counting of $^{51}$Cr-EDTA, 200 $\mu$l for HPLC measurements, and 3 ml for the X-ray fluorescence analysis.

During the waiting time between the start of the measurement and the first blood sample after 2.5 h, the patients were allowed to move around freely and also to have a light meal, but they were still asked to refrain from smoking. The blood samples were all taken from the same venous catheter in which the injection had been given.

**Table 1.** Diagnosis in 49 patients referred to examination for glomerular filtration rate

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urological disorders</td>
<td>13</td>
</tr>
<tr>
<td>Systemic disorders</td>
<td>12</td>
</tr>
<tr>
<td>Affective disease</td>
<td>4</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>4</td>
</tr>
<tr>
<td>Chronic glomerulonephritis</td>
<td>4</td>
</tr>
<tr>
<td>Kidney transplant patients</td>
<td>4</td>
</tr>
<tr>
<td>Hyperparathyroidism</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
</tr>
</tbody>
</table>

To check if the blood samples taken from the ‘injection’ arm affected the GFR results, we provided 14 patients with two venous catheters, one in each arm. In one of the catheters, the injection of GFR marker was given, followed by at least 30 ml of saline, and later the samples were taken from the catheter in the contralateral arm, as well as from the ‘injection’ arm. Plasma, obtained from both venous catheters was analysed separately and GFR calculated. No differences in GFR values could be observed. It must be stressed, however, that it is of great importance that the venous catheter is flushed with at least 30 ml of saline following injection, thereby avoiding ‘contamination’ of $^{51}$Cr-EDTA or iohexol.

**Clearance determinations (see Appendix)**

All clearances were calculated according to commonly used methods. Plasma clearance was calculated according to Brochner-Mortensen [12] with the modification that the plasma disappearance curve was determined by three measurements, 150, 195 and 240 min after injection. Plasma clearance was also calculated using the single-sample clearance according to the Jacobsson formula [13].

**Analysis techniques**

The radioactivity of $^{51}$Cr-EDTA in the plasma samples was measured together with a standard (3 ml) in a gamma counter (15 min for each sample). The standard was prepared from the same solution that was given to the patient. An exact amount of the $^{51}$Cr-EDTA solution was weighed carefully and added to 250 ml distilled water.

Iohexol was measured with two different techniques, the X-ray fluorescence technique and HPLC. For measuring the X-ray fluorescence the Renalyzer apparatus (PRX 90, Provalid, Sweden) was used. The plasma samples were inserted into the Renalyzer for a measuring time of 5 min and exposed to the radiation of 60 keV photons emitted from two sources of $^{241}$Am (11 GBq). The iodine in the iohexol molecules then emits characteristic X-rays which are registered by a 6-channel analyser. The X-ray radiation is proportional to the concentration of iohexol in the plasma samples. Repeated analyses of iohexol (iodine concentration range 30.7–245.3 mg/l), measured by X-ray fluorescence technique, were made for methodological testing, and yielded a coefficient of variation of 3%. The precision and accuracy of the X-ray fluorescence analysis of iodine for concentrations below 60 mg/l were less than those for the HPLC. Hence, it is important that the injected dose of iohexol is at least 20 ml, preventing concentration from being too close to the lower level of detection (Table 2).

Iohexol was also determined by the HPLC [14,15]. In brief, plasma samples were deproteinized by adding equal volume of 0.6 M perchloric acid to a 200-ml sample. Twenty-microlitre aliquot was injected onto the HPLC column. The separation of iohexol from endogenous substances was carried out on a reversed phase column Lichrospher 60 RP—Select B, 125 × 4 mm ID, 5 µm particle size (Merck, USA), with a mobile phase consisting of sodium dihydrogen phosphate solution, 60 mM, methanol 6% v/v and tetrahydrofuran 1% v/v, adjusted to pH 2.9 with concentrated phosphoric acid.

A baseline separation of exo- and endo-iohexol isomers was achieved with a resolution better than 1.5. Iohexol was detected at 244-nm wavelength by a UV absorbance detector (model 481, Lambda-Max, Waters). Iohexol quantitation
Table 2. Iohexol standard mixtures measured by HPLC and X-ray fluorescence

<table>
<thead>
<tr>
<th>Iodine concentration (mg/l)</th>
<th>Measured concentration (mg/l)</th>
<th>SD (mg/l)</th>
<th>% CV</th>
<th>Measured concentration (mg/l)</th>
<th>SD (mg/l)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.7</td>
<td>30.9</td>
<td>0.7</td>
<td>2.1</td>
<td>33.3</td>
<td>4.6</td>
<td>13.7</td>
</tr>
<tr>
<td>61.3</td>
<td>62.2</td>
<td>1.3</td>
<td>2.1</td>
<td>62.2</td>
<td>2.5</td>
<td>4.1</td>
</tr>
<tr>
<td>122.6</td>
<td>124.3</td>
<td>1.6</td>
<td>1.3</td>
<td>126.8</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>245.3</td>
<td>244.2</td>
<td>4.0</td>
<td>1.6</td>
<td>241.5</td>
<td>5.0</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Six mixtures were prepared individually for each level of concentration by a series dilution of iohexol stock standard, which in turn was prepared from a newly opened Omnipaque® vial (300 mg/ml).

was based on assessment of peak area. The method was validated within a concentration range from 3.8–529.0 mg/l. An excellent linearity was obtained. The detection limit for a signal to noise ratio of a factor 3 was 1.2 mg/l. Interassay coefficient of variation for iohexol in plasma control samples on two concentration levels, 44 and 106 mg/l, was 2.8 and 3.2% respectively.

Results

There were 49 complete sets of clearance measurements available for comparison. Each set consisted of GFR measured with the two GFR markers, $^{51}$Cr-EDTA and iohexol, both with the multiple-sample and the single-sample clearance for each marker.

In Figure 1 the data are presented for iohexol multiple-sample clearance estimation performed by HPLC and X-ray fluorescence. In both cases a statistically significant correlation was obtained. The slopes and the intercepts were not different from 1 and zero respectively, on the 5% significance level, indicating that both methods give GFR results equivalent to $^{51}$Cr-EDTA measurements.

Linear regression analysis was performed in order to compare the GFR results obtained from measurements with the two markers using the three-sample method and single-sample method. The multiple-sample method with $^{51}$Cr-EDTA was considered as a reference method for GFR measurement, to which one-sample $^{51}$Cr-EDTA clearance, one-sample iohexol clearance measured by HPLC, and one-sample iohexol clearance measured by X-ray fluorescence were compared. The individual results, equation of linear regression, correlation coefficient and regression graph are presented in Figure 2.

The slope parameter for one-sample clearance estimation with $^{51}$Cr-EDTA and HPLC with iohexol was 0.97 and 0.96 respectively, and it was not statistically different from 1 (5% significance level). The slope for one-sample iohexol clearance by X-ray fluorescence (0.83) was statistically different from the line of identity. The intercept differed from origo in the case of iohexol clearance measured by X-ray fluorescence, while for the HPLC and $^{51}$Cr-EDTA methods, the intercepts were not different from zero.

In Figure 3 the difference between $^{51}$Cr-EDTA, as reference method, and multiple-point clearance measured by HPLC and X-ray fluorescence, are plotted against the mean value of the compared methods. The points are evenly distributed around the zero line over the whole clearance range. The calculated mean difference, their standard deviation, and clearance range for all methods are presented in Table 3.
Comparison of iohexol and $^{51}$Cr-EDTA clearances

![Graphs showing clearance comparisons](image)

Table 3. Clearance range, mean of differences and standard deviation for multiple-point clearance and single-point clearance measurements

<table>
<thead>
<tr>
<th>Clearance method</th>
<th>Clearance range (ml/min)</th>
<th>Difference (ml/min)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple-point clearance: 3 samples $^{51}$Cr-EDTA vs 3 samples iohexol</td>
<td>28–134</td>
<td>−0.16</td>
<td>6.17</td>
<td></td>
</tr>
<tr>
<td>$^{51}$Cr-EDTA vs HPLC</td>
<td>29–134</td>
<td>0.58</td>
<td>4.95</td>
<td></td>
</tr>
<tr>
<td>$^{51}$Cr-EDTA vs X-ray fluorescence</td>
<td>27–125</td>
<td>−1.7</td>
<td>5.94</td>
<td></td>
</tr>
<tr>
<td>$^{51}$Cr-EDTA vs X-ray fluorescence</td>
<td>32–116</td>
<td>−1.32</td>
<td>5.78</td>
<td></td>
</tr>
</tbody>
</table>

In Figure 4 the corresponding data of differences for single-sample clearance are presented. The distribution of data around the zero identity line for one sample iohexol clearance measured with X-ray fluorescence technique, substantiates the results of regression analysis, indicating a difference between these two methods. The GFR below 75 ml/min tends to be overestimated, whereas the GFR above 75 ml/min is underestimated, when using the X-ray fluorescence method for single-sample GFR measurement.
In this study we have compared GFR measurements using the recently introduced GFR marker iohexol with the well-known and reliable marker $^{51}$Cr-EDTA. We have shown that over the wide GFR range of 40–130 ml/min, iohexol clearance measurements correlated closely to those of $^{51}$Cr-EDTA. Furthermore, there was no difference between the clearance measurement irrespective of whether iohexol was analysed by means of the HPLC method or the fluorescence technique.

Non-radioactive contrast agents have come to play a more important role for determining GFR. Not only can GFR be determined in combination with a routine X-ray, using doses of 50–100 ml of e.g. iohexol [16] but the iohexol can also be used in a low dose, specifically aimed at assessing GFR [17,18]. Sensitive assay techniques have also evolved, such as the Renalyzer, utilizing X-ray fluorescence of iodine and the development of HPLC for measuring iohexol concentration [5,17,18]. Corroborating with the data of the present study, others have also found a good correlation with plasma clearance of iohexol, not only when compared to inulin but also to that of $^{51}$Cr-EDTA and $^{99m}$Tc-DTPA [5,7,17,19–23]. Furthermore, Brown and O'Reilly [7] made a detailed study utilizing bladder catheterization and the classical continuous infusion technique, and showed an excellent correlation between the renal clearances of iohexol and inulin.

In patients with severe impairment of renal function, contrast media are known to be nephrotoxic, especially in diabetic patients. However, slow intravenous injection of a small dose of contrast medium in a clearance procedure, as in the present study, is not nephrotoxic as is the case for a high-pressure injection, using several times more substance in an X-ray examination. Thus, one may preferentially use a low dose (<20 ml) of iohexol (300 mg I/ml). For example, in a large study of approximately 4000 iohexol clearance measurements, using a low dose of iohexol, no severe adverse reaction was noted [24]. Other adverse reactions such as allergic, hypotensive, and dyspeptic reactions are very rare as well, when iohexol is used for clearance measurements [25]. This is certainly due to the mode of administration and the low dose used.

Iodine could be measured not only by the sensitive HPLC method but also by using the Renalyzer technique (Provalid, Lund, Sweden), which is based on X-ray fluorescence. This is a simple and sensitive technique, provided that the iodine concentration exceeds approximately 0.06 mg iodine per ml. The technique is reported to yield a sufficiently linear relationship in the region of clinical interest, including concentrations in the range of 0.05 mg iodine per ml to 7 mg iodine per ml. Thus care has to be taken when iodine concentrations tend to be low, as in high clearance intervals, the variation of the results between repeated analysis of the same sample is about ±4% in the interval of 0.1–4 mg iodine per ml [9]. When low
concentrations are surmised, the HPLC technique has a higher accuracy and presents with only 2% coefficient of variation, as evidenced from the present study. In addition it should be noted that the HPLC technique requires a dose of only 5 ml iohexol (300 mg iodine per ml) in reaching a high sensitivity, whereas the Renalyzer technique needs about 20 ml. Taken together, we found a high degree of concordance both in the two methods used for measuring iodine concentration, and in each of these clearance measurements showing a high degree of correlation with multiple-points $^{51}$Cr-EDTA clearance.

The present study showed excellent correlation between single-point and multiple-point clearance measurements for both $^{51}$Cr-EDTA and the iohexol, as measured either with HPLC or by X-ray fluorescence. This means that the clearance determinations can be simplified rather substantially, which is important from the point of view of cost and availability of the measurements. From a theoretical point of view, it does suggest that the approximations involved in the calculations (see Appendix) are acceptable and do not distort the actual GFR calculation. Interestingly, we noted that when one point clearance method of iohexol was determined by X-ray fluorescence, it demonstrated an intercept on the Y axis significantly above zero. This was not the case when iohexol was determined by HPLC and calculated accordingly, and compared with multiple sampling $^{51}$Cr-EDTA clearance estimation. Since there could be no difference in volume of distribution, this error must be related to the precision of the Renalyzer instrument, having difficulties in correctly assessing lower concentrations of iodine. This is a problem only in patients with rather better preserved renal function.

In summary, the present study provides data in patients with various degrees of renal function impairment, showing good correlation between $^{51}$Cr-EDTA and iohexol clearance measurements. Moreover iodine concentration measured by X-ray fluorescence and HPLC yielded clearance values in good agreement with $^{51}$Cr-EDTA multiple-point clearance estimation. The HPLC technique showed high accuracy and sensitivity for low iodine concentrations, thus being superior to that of X-ray fluorescence technique in this respect.

Furthermore a high correlation was established between clearance measurements, calculated from single plasma sample to those obtained by multiple samples using both GFR markers. The single-point plasma method for determining GFR can be recommended in clinical practice for both markers, keeping in mind that the X-ray fluorescence method may have problems with correctly detecting low iodine concentrations, a situation which may arise when measuring single-sample clearance in patients with high GFR. This error can, however, be considered as being rather small and be obviated by administering a higher dose of iohexol in patients in whom a more normal GFR may be anticipated.

**References**

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tion rate from low-dose injection of iohexol and a single blood sample. Invest Radiol 1991; 26: 332–336


Appendix

Calculations

Multiple sample clearance, according to Brøchner-Mortensen, is the classical way of determining the GFR [12].

Clearance (Cl) of the GFR marker is expressed as:

\[ Cl = \frac{Q}{b/c_1} \text{ (ml/min)} \]

where \( Q \) = amount of injected marker, \( b \) = disappearance rate of marker (min\(^{-1}\)), \( c_1 \) = intercept on y axis.

Clearance correction for non-immediate mixing of the tracer substance is expressed as

\[ Cl = 0.990778 \cdot Cl_1 - 0.001218 \cdot Cl_1^2 \]

Single-sample clearance:

\[ Cl_1 = 1/[\left(\frac{t \cdot m}{V} + 0.0016\right) \cdot \ln \left(\frac{Q}{V \cdot C(t)}\right)] \]

where \( C(t) \) = plasma concentration of marker, \( t \) = time after injection, \( V \) = distribution volume, \( Q \) = amount of injected marker, \( m = 0.991 - 0.00122 \cdot Cl_1 \)

After calculation of \( m \) the equation becomes:

\[ Cl = 1/\left[\left(\frac{t \cdot m}{V} + 0.0016\right) \cdot \ln \left(\frac{Q}{m \cdot V \cdot C(t)}\right)\right] \]

where \( V_{\text{male}} = 166 \times \text{body-weight in kg} + 2490 \) (ml), \( V_{\text{female}} = 95 \times \text{body-weight in kg} + 6170 \) (ml).

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