GLOMERULAR FILTRATION RATE IN ADULTS

A single sample plasma clearance method based on the mean sojourn time

Margareta Gref
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Glomerular filtration rate (GFR) is a key parameter in evaluating kidney function. After a bolus injection of an exogenous GFR marker in plasma an accurate determination of GFR can be made by measuring the marker concentration in plasma during the excretion. Simplified methods have been developed to reduce the number of plasma samples needed and yet still maintain a high accuracy in the GFR determination.

Groth previously developed a single sample GFR method based on the mean sojourn time of a GFR marker in its distribution volume. This method applied in adults using the marker $^{99m}$Tc-DTPA is recommended for use when GFR is estimated to be $\geq 30$ mL/min.

The aim of the present study was to further develop the single plasma sample GFR method by Groth including patients with severely reduced renal function and different GFR markers.

Three different GFR markers $^{51}$Cr-EDTA, $^{99m}$Tc-DTPA and iohexol were investigated. Formulas were derived for the markers $^{51}$Cr-EDTA and iohexol when GFR is estimated to be $\geq 30$ mL/min. For patients with an estimated GFR $< 30$ mL/min a special low clearance formula with a single sample obtained about 24 h after marker injection was developed. The low clearance formula was proven valid for use with all three markers.

The sources of errors and their influence on the calculated single sample clearance were investigated. The estimated distribution volume is the major source of error but its influence can be reduced by choosing a suitable sampling time. The optimal time depends on the level of GFR; the lower GFR the later the single sample should be obtained. For practical purpose a 270 min sample is recommended when estimated GFR $\geq 30$ mL/min and a 24 h sample when estimated GFR $< 30$ mL/min. Sampling at 180 min after marker injection may be considered if GFR is estimated to be essentially normal.
The present thesis is based upon the following papers, referred to in the text by their Roman numerals:


<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>BSA</td>
<td>Body surface area</td>
</tr>
<tr>
<td>Cl</td>
<td>Clearance</td>
</tr>
<tr>
<td>Cl\textsubscript{BM}</td>
<td>Clearance according to Brøchner-Mortensen’s one-compartment model</td>
</tr>
<tr>
<td>Cl\textsubscript{SM}</td>
<td>Clearance according to Sapirstein’s two-compartment model, standard method</td>
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<tr>
<td>Cl\textsubscript{S}</td>
<td>Single sample clearance according to the mean sojourn time-based method</td>
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<td>Single sample clearance calculated with the low clearance formula</td>
</tr>
<tr>
<td>51\textsuperscript{Cr}-EDTA</td>
<td>Chromium-51-ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>ECV</td>
<td>Extracellular volume</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
</tr>
<tr>
<td>H</td>
<td>Height</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>I</td>
<td>Iodine</td>
</tr>
<tr>
<td>Q\textsubscript{0}</td>
<td>Total amount of the injected GFR marker</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>\bar{t}</td>
<td>Mean sojourn time of a GFR marker in the extra cellular volume</td>
</tr>
<tr>
<td>99m\textsuperscript{Tc}-DTPA</td>
<td>Technetium-99m-diethylenetriaminepentaacetic acid</td>
</tr>
<tr>
<td>W</td>
<td>Body weight</td>
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INTRODUCTION

The kidney has the primary function of the maintenance and composition of body fluids. It is one of the major excretory pathways of metabolic waste products. The first step in the excretory process takes place in the glomeruli and comprises a passive ultrafiltration of plasma. The volume of plasma filtered per minute is known as the glomerular filtration rate (GFR). The glomerular filtration rate is considered as the most valuable parameter in evaluating the entire kidney function. Therefore, it is necessary to have available an accurate, sensitive and reproducible method for the determination of GFR.

GFR is determined by measuring the clearance (Cl) from plasma of a GFR marker. Ideally the description of a clearance method should include the following information:

- the compartment that is cleared (concerning GFR, plasma is always cleared and the word plasma is sometimes omitted)
- the organs that participate e.g. renal clearance, total (all organs) clearance
- the name of the marker
- how the marker is administered e.g. continuous infusion, single injection
- the frequency and timing of sampling e.g. multiple sampling, single sample, late sampling

In clinical practice “renal clearance” indicates that the concentration of a GFR marker is measured both in the plasma and urine whereas “plasma clearance” indicates that the concentration is measured only in the plasma.

In this thesis total plasma clearance was investigated. No correction for extra renal clearance was made. Total plasma clearance of a GFR marker was termed plasma clearance. The marker was given as a single injection. The exogenous GFR markers used were $^{51}\text{Cr}$-EDTA, $^{99m}\text{Tc}$-DTPA and iohexol.

The value of plasma clearance was reported in absolute units of millilitres per minute, mL/min.
Marker substances for GFR

Both endogenous and exogenous markers have been used for the determination of glomerular filtration rate. An ideal marker for GFR would have these properties:
- free filtration through the glomerular membrane
- no reabsorption, secretion or metabolizing by the tubuli
- no effect on GFR
- non-toxic
- no binding to plasma proteins
- precise analysis available

Endogenous markers
The most common endogenous substance used is creatinine. The plasma-creatinine method is fast, cheap and has a low intra-individual variation. However several drawbacks exist with this marker. The plasma-creatinine concentration is dependant on the patient’s weight, muscular mass, age, gender and food intake. Creatinine has some tubular secretion and has a low sensitivity for detecting early and moderate renal dysfunction (1). Several formulas exist for calculating an estimated GFR from the plasma creatinine concentration (2, 3).

Cystatin C is an endogenous substance that is increasingly used. It has some advantages over creatinine being independent of muscular mass and food intake and having no tubular secretion (4, 5, 6). Cystatin C is rather good in estimating low GFR (7), but as creatinine is not so good in estimating high GFR. High doses of steroids increases the secretion of cystatin C and the estimated GFR will be underestimated (8). Thyroid dysfunction alters the cystatin C levels and thyroid function has to be considered when GFR is estimated from cystatin C (9, 10).

Exogenous markers
A more accurate determination of GFR demands the use of an exogenous GFR marker. In the 1930’s inulin, a fructose polymer, was introduced as a GFR marker. Inulin is generally accepted as a true GFR marker substance. The drawback of inulin is that the chemical analysis is time consuming and subject to interference. To avoid interfering substances an enzymatic method has been used (11). Attempts to label inulin with
radioactive iodine were also tried in order to improve the accuracy of the analysis. These attempts were not totally satisfactory due to instability of the radiolabelled substance.

Labelling of the chelates ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) with $^{51}$Cr and $^{99m}$Tc respectively was more successful and promised stable complexes. $^{51}$Cr-EDTA and $^{99m}$Tc-DTPA are the most used radiopharmaceuticals for the determination of GFR.

Furthermore, several contrast agents have been used as GFR markers. Today, iohexol is the one most adopted.

$^{51}$Cr-EDTA and iohexol clearance have shown good agreement with inulin clearance (12, 13). $^{99m}$Tc-DTPA clearance correlates well with $^{51}$Cr-EDTA clearance (14). However, plasma protein binding and radioinstability have been problems concerning $^{99m}$Tc-DTPA and the quality of different DTPA kits has varied (15, 16).

**Clearance techniques for determination of GFR**

The marker can be administered either by continuous infusion or as a single injection.

**Continuous infusion method**

The classical renal clearance method is to give an exogenous marker by continuous infusion. Having achieved steady state conditions, the renal clearance of the marker is given by the equation:

$$Cl = \frac{C_u \cdot F}{C_p}$$

Eq. 1

where $C_u$ is the urine concentration of the marker, $F$ is the urine flow and $C_p$ is the plasma concentration of the marker.

For clinical purposes, the technique using continuous infusion is cumbersome and impractical.

Difficulties obtaining accurate urine collections are the major source of error and catheterization may be required. A single injection of the GFR marker is more attractive with the possibility of getting the GFR calculated from plasma samples.
**Single injection method**

After a bolus injection is administrated the plasma clearance is given by the equation:

\[
Cl = \frac{Q_0}{\int_0^\infty C(t) dt}
\]  

Eq. 2

where \(Q_0\) is the amount of marker, \(C(t)\) is the plasma concentration at time \(t\) and \(\int_0^\infty C(t) dt\) is the area under the plasma time-concentration curve.

Several models exist to describe the elimination of a GFR marker after a single injection and many simplified methods for clearance calculation have been presented with the aim of reducing the number of plasma samples needed yet still maintaining a high accuracy.

Nosslin used a multiple exponential model to describe the plasma concentration as a function of time (17). Many blood samples taken at short time intervals are needed to calculate clearance.

Sapirstein adopted a two compartment model to describe the plasma clearance: a vascular compartment into which the injection is made and a second peripheral compartment (18).

![Fig 1. The two compartment model.](image_url)

The GFR marker diffuses between the two compartments and the excretion takes place from the vascular compartment (Fig 1). Immediately after an intravenous injection of a GFR marker, this is diluted in the circulating plasma; the plasma concentration rapidly reaches a peak followed by an exponential fall as the marker diffuses into the extravascular
space. After some time, equilibrium is established between the intra- and extravascular flow. As the GFR marker continues to be excreted through the kidneys there is then a net flow from the extravascular space. In humans with normal renal function almost complete equilibration occurs approximately two hours after injection.

Mathematically, the elimination can be described biexponentially and plasma clearance calculated by the equation:

$$CL = \frac{Q_0}{\int_0^\infty C(t)dt} = \frac{Q_0}{\int_0^\infty (c_1e^{-b_1t} + c_2e^{-b_2t})dt} = \frac{Q_0}{c_1/b_1 + c_2/b_2}$$

Eq. 3

where $b_1$ and $b_2$ are disappearance rates of the marker and $c_1$ and $c_2$ are corresponding intercepts (Fig 2).

Plasma samples during the early fast fall of the GFR marker concentration as well as after equilibrium is established are needed for the calculation. When renal function is severely reduced equilibrium occurs late and sampling has to be prolonged up to 24 h (12).

**Fig 2.** The plasma concentration of a GFR marker ($C(t)$) as a function of time ($t$) after a single intravenous injection. A two compartment model with the disappearance rates $b_1$ and $b_2$ and the corresponding intercepts $c_1$ and $c_2$. 

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A one compartment model calculates clearance from a few samples obtained after equilibrium, after the final slope has been reached.

\[
Cl_i = \frac{Q_0}{\int_0^\infty C(t) dt} = \frac{Q_0}{\int_0^\infty c_i e^{-b_i t} dt} = \frac{Q_0}{c_1/b_1}
\]  
Eq. 4

The missing area due to the early fast fall of the disappearance curve is corrected for in different ways.

Chantler used the constant 0.93 to correct the calculated one compartment clearance (19).

\[
Cl = 0.93 \cdot Cl_i
\]

Brøchner-Mortensen found that the missing area expressed as a percentage of the total area was bigger in higher clearance and adopted a quadratic correction (20).

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

The Brøchner-Mortensen method (presented in 1972) is still the routine GFR method used in many clinical departments.

**Single injection, single sample methods**

Further simplifications have been made in the estimation of GFR following a single injection of the marker. Several methods for calculating the clearance from a single plasma sample have been presented (21-33).

In this thesis two different single sample methodologies have been used: the single sample method by Jacobsson (24) and the mean sojourn time-based method by Groth (25).

**Jacobsson’s method** is based on a one compartment model and includes two empirical correction factors by Brøchner-Mortensen: one for early, incomplete mixing of the marker in its distribution volume (20) and the other for non-uniform distribution after equilibrium has occurred (34). Single sample clearance according to Jacobsson is calculated as follows:

\[
Cl = \frac{1}{t/V^*+0.0016} \ln \left[ \frac{Q_0}{V \times C(t)} \right]
\]  
Eq. 5

\[
m = 0.991 - 0.00122 \times Cl
\]
\[ V' = \frac{V}{m} \]

\( V \) = distribution volume and is estimated from body weight (W)

\[ V_{\text{male}} = 166 \times W + 2490 \]

\[ V_{\text{female}} = 95 \times W + 6170 \]

(i) calculate an approximate \( Cl_1 \) with \( V \)

(ii) calculate \( m \) with \( Cl_1 \)

(iii) calculate \( Cl \) with \( V' = \frac{V}{m} \)

The distribution volume above (\( V_{\text{male}} \) and \( V_{\text{female}} \)) was determined for \( ^{51}\text{Cr-EDTA} \) by Granerus and Jacobsson (31). The same distribution volume has commonly been used for iohexol and was also used in the present studies. Jacobsson originally applied his method on \( ^{99m}\text{Tc-DTPA} \) clearance in adults (24) and determined the distribution volume as: \( V = 246 \times W \). The method has also been modified for children with adjusted distribution volumes (35, 36). Jacobsson found the optimal sampling time being \( t_{\text{opt}} = V'/Cl \). The method has also been used when renal function is severely reduced (37).

**The mean sojourn time-based methodology** was developed by Groth for calculating \( ^{51}\text{Cr-EDTA} \) clearance from a single plasma sample in children (25). The basis for the methodology is transformation of the assumed biexponential plasma disappearance curve of the GFR marker substance into an imaginary monoexponential curve with an identical mean sojourn time and an identical area under the curve when \( 0 < t < \infty \).

Clearance of a GFR marker can be described by the expression:

\[ Cl = \frac{ECV}{\bar{t}} \]

Eq. 6

where ECV is the extracellular volume defined as the distribution volume of the marker and \( \bar{t} \) is the mean sojourn time of the marker in ECV.

If, as an approximation, a complete and immediate distribution of a marker \( Q_0 \) in ECV is assumed the plasma time-concentration curve will be monoexponential.
Fig 3. An illustration of the transformation of the biexponential plasma time-concentration curve $C(t)$ into a monoexponential curve $"C(t)"$ with the disappearance rate $1/\bar{t}$ and the intercept $C_0 = Q_0/ECV$.

This imaginary curve $"C(t)"$ will have an intercept $= Q_0/ECV$ and a disappearance rate constant $= 1/\bar{t}$ and can be expressed as

$$ "C(t)" = \frac{Q_0}{ECV} e^{-\frac{t}{\bar{t}}} $$

Eq. 7

The curve will cross the factual plasma time-concentration curve at $t_u$ (Fig 3). If ECV and $t_u$ are known, $1/\bar{t}$ can be calculated as

$$ \frac{1}{\bar{t}} = \left( -\ln \frac{C(t_u)}{C_0} \right) / t_u = -\ln \left( \frac{C(t_u)}{Q_0} \cdot \frac{ECV}{Q_0} \right) / t_u $$

Eq. 8

In reality ECV and $t_u$ are not known. Groth found a good correlation between ECV and body surface area (BSA) in children and used an estimated ECV:

$$ ECV = f(\text{BSA}) $$

Eq. 9

and then defined a function $s(t)$:
\[ s(t) = -\ln\left(\frac{C(t) f(BSA)}{Q_0} \right) \frac{t}{t} \]  

Eq. 10

Even though \( s(t) \neq 1/t \) and varies with time, the relation \( s(t)/(1/t) \) could empirically be found as:

\[ g(t) = \frac{s(t)}{\left(\frac{1}{t}\right)} \]  

Eq. 11

The function \( g(t) \) corrects for the fact that the sample has not been obtained at \( t = t_u \) and that ECV has not been measured but estimated from BSA. In children \( g(t) \) was independent of GFR for \( t \) between 90 and 150 min. Combining equations 6, 9, 10 and 11 clearance can be calculated from a single plasma sample by the equation:

\[ Cl = -\ln\left(\frac{C(t) f(BSA)}{Q_0} \right) f(BSA) \frac{t}{t \cdot g(t)} \]  

Eq. 12

In 1986 Groth, together with Christensen, applied this method in adults using the tracer \(^{99m}\text{Tc-DTPA}\) (28). The correlation between ECV and BSA was not as good in adults as in children and \( g(t) \) was dependent on GFR. However, Christensen and Groth could use the relation between \( g(t) \) and clearance in developing an iterative method yielding accurate estimates of \(^{99m}\text{Tc-DTPA}\) clearance.

Based on several comparative studies, the single sample method by Christensen and Groth has been recommended by the Radionuclides in Nephrourology Committee on Renal Clearance for use in adults when GFR is estimated to be \( \geq 30 \) mL/min (38).
AIMS OF THE STUDY

General aims:
The mean sojourn time single sample method by Christensen and Groth was developed for $^{99m}$DTPA plasma clearance in adults. One aim of the study was to apply the method on GFR determination in adults using two other markers: $^{51}$Cr-EDTA and iohexol. Another aim was to apply the method on patients with advanced renal failure, estimated GFR < 30 mL/min.

Specific aims:
- to investigate if a single sample formula had the same accuracy whether it was derived from clearance calculated from the entire plasma time concentration curve or from clearance calculated according to the one compartment approach from a few samples on the final slope of the plasma time concentration curve.
- to investigate whether the single sample formula derived for a specific GFR marker could be used for calculating clearance of another GFR marker.
- to study the accuracy of the single sample method in the whole clearance range, when the single sample is obtained at different sampling times.
- to be able to predict a suitable sampling time.
**MATERIALS AND METHODS**

**Materials**

All patients included in the present study were adults and referred for determination of glomerular filtration rate. Sex, age, BSA and clearance distribution in the different patient groups are presented in Table 1.

**Paper I**

Paper I concerns $^{51}$Cr-EDTA plasma clearance. Two patient groups were included. *Group I* patients ($n = 46$) were examined at the Department of Clinical Physiology and Nuclear Medicine, Skejby University hospital, Aarhus, Denmark. Exclusion criteria were oedema, ascites and renovascular hypertension. Data from blood samples obtained between 2 and 300 min after marker injection were used to derive the single sample clearance formulas. The derived formulas were tested on *Group II* ($n = 1046$) containing consecutive patients referred for $^{51}$Cr-EDTA plasma clearance by the Brøchner-Mortensen method. *Group II* patients were examined at the Department of Clinical Physiology, Norrlands University hospital, Umeå, Sweden. Blood samples in *Group II* were obtained at 180, 210, 240 and 270 min after injection of the marker.

**Paper II**

Paper II concerns iohexol plasma clearance. Three patient groups were included. *Group I* and *II* were patients participating in a GFR study ($n = 95$) at the Department of Clinical Physiology, Norrlands University hospital, Umeå, Sweden. Prior approval of the study was obtained from the Ethical Committee at Umeå University. These patients were simultaneously investigated with three different GFR markers, $^{51}$Cr-EDTA, $^{99m}$Tc-DTPA and iohexol. Twenty-one patients had a GFR < 30 mL/min. Blood samples were obtained between 5 and 300 min with an additional 24 h sample from patients with a Cl < 30 mL/min. Patients with oedema and patients allergic to iodine were excluded. Patients were randomly divided into two groups. Data from *Group I* ($n = 48$) were used to derive a single-sample formula. This formula was then tested on *Group II* ($n = 47$). The formula was also applied to a third group, *Group III* ($n = 123$). This group contained patients referred for routine iohexol clearance by the Brøchner-Mortensen method. In *Group III*,
three to five blood samples were obtained between 180 and 300 min with an additional 24 h sample from patients with a s-creatinine > 200 μmol/L.

**Paper III**

*Paper III* concerns the determination of $^{99m}$Tc-DTPA plasma clearance in patients with advanced reduced renal failure. A subgroup of patients from the same GFR study as was used in *Paper II* was included. A low clearance, single sample formula was applied to the 21 patients having a Cl < 30 mL/min.

Table 1. Patient groups in *Paper I, II and III*. BSA = body surface area, Cl$_{SM}$ = clearance calculated according to the two compartment model of Sapirstein, Cl$_{BM}$ = clearance calculated according to Brøchner-Mortensen. Cl$_{SM+24h}$ and Cl$_{BM+24h}$ indicate that in low clearances a 24 h sample is included in the calculation.

<table>
<thead>
<tr>
<th></th>
<th>Paper I $^{51}$Cr-EDTA</th>
<th>Paper II Iohexol</th>
<th>Paper III $^{99m}$Tc-DTPA</th>
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<tbody>
<tr>
<td>Group</td>
<td></td>
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<tr>
<td>No of patients</td>
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<td>Mean</td>
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</table>

**$^{51}$Cr-EDTA and $^{99m}$Tc-DTPA analysis**

The $^{51}$Cr and $^{99m}$Tc activities were measured in a gamma counter with a 2 inch well type detector. 2 mL plasma samples, blanks and standard dilutions of the injection solutions were counted for up to 20 min ($^{51}$Cr) and 5 min ($^{99m}$Tc) respectively or to a statistical counting error below 1 %. All measurements were corrected for background activity and physical decay. When $^{51}$Cr-EDTA and $^{99m}$Tc-DTPA were used simultaneously the activity of $^{99m}$Tc was corrected for $^{51}$Cr overlap.
Iohexol analysis

Iohexol concentrations in deproteinized plasma samples were measured using high performance liquid chromatography (HPLC) according to the method of Krutzén et al (39). The separation was carried out on a reversed phase column, Nucleosil 5 C\textsubscript{18}, 200 x 4.6 mm (Machery-Nagel, Germany). Iohexol was detected at 253 nm by an UV absorbance detector. The mobile phase was a mixture of water/acetonitrile (97/3 by volume, adjusted to pH 2.5 with phosphoric acid). Plasma blank samples were always analyzed. In case of interfering compounds eluting close to iohexol, the acetonitrile concentration in the mobile phase was altered to achieve good separation.

The coefficient of variation in the iohexol analysis was 1.5 % when calculated from duplicates in the same series.

Derivation of a single sample plasma clearance formula by the mean sojourn time approach

The single sample formula by the mean sojourn time approach can be written as:

\[
Cl_s = \frac{-\ln \left( \frac{C(t)ECV}{Q_0} \right) ECV}{t \cdot g(t)_{corr}} \quad \text{Eq. 13}
\]

where \(Q_0\) is the injected amount of the GFR marker and \(C(t)\) is the marker concentration of the single plasma sample obtained \(t\) min after injection. ECV and \(g(t)_{corr}\) are unknown and have to be empirically determined. ECV, defined as the distribution volume of the used GFR marker, was related to body surface area and \(g(t)_{corr}\) was related to the sampling time \(t\) and to clearance.

Parameters needed to derive the single sample formula were calculated as follows:
Calculation of clearance (Cl) and extracellular volume (ECV)

Clearance and extracellular volume, used to derive the single sample formulas were calculated either according to a two-compartment model by Sapirstein et al (18), $Cl_{SM}$ and $ECV_{SM}$ or according to a one-compartment model by Brøchner-Mortensen, $Cl_{BM}$ (20) and $ECV_{BM}$ (34).

According to Sapirstein et al

$$Cl_{SM} = \frac{Q_0}{\int_0^\infty C(t)dt} = \frac{Q_0}{\int_0^\infty (c_1 e^{-b_1 t} + c_2 e^{-b_2 t}) dt} = \frac{Q_0}{b_1 + \frac{c_2}{b_2}} \quad \text{Eq. 14}$$

$$ECV_{SM} = \frac{Cl_{SM}^2}{Q_0} \int_0^\infty t \cdot C(t) dt = \frac{Cl_{SM}^2}{Q_0} \left( \frac{c_1}{b_1^2} + \frac{c_2}{b_2^2} \right) \quad \text{Eq. 15}$$

According to Brøchner-Mortensen

$$Cl_1 = \frac{Q_0}{\int_0^\infty C(t)dt} = \frac{Q_0}{\int_0^\infty c_1 e^{-b_1 t} dt} = \frac{Q_0}{b_1} \quad \text{Eq. 16}$$

$$Cl_{BM} = 0.990778 \cdot Cl_1 - 0.001218 \cdot Cl_1^2 \quad \text{Eq. 17}$$

$$ECV_{BM} = \frac{Cl_1}{b_1} \left[ \left( \frac{Cl_1}{Cl_i} \right)^2 - \frac{Cl_i}{Cl_1} + 1 \right] \quad \text{Eq. 18}$$

$$Cl_i = Cl_{BM} (0.00002512 \cdot PV + 0.9246) \quad \text{Eq. 19}$$

$b_1$ and $b_2$ are the disappearance rates of marker and $c_1$ and $c_2$ are the corresponding intercepts.

$PV$ is the estimated plasma volume in millilitres calculated from body weight ($W$) in kilograms, in females as $41^* W$ and in males as $45^* W$. 
**Calculation of body surface area (BSA)**

Body surface area (in m$^2$) was calculated from body weight and height ($H$) according to Haycock et al (40)

$$BSA = 0.024265 \times W^{0.5378} \times H^{0.3964}$$  \hspace{1cm} \text{Eq. 20}

$W$ in kg and $H$ in cm.

BSA was used to establish an empirical relationship between ECV (ECV$_{SM}$ or ECV$_{BM}$) and BSA.

**Calculation of mean sojourn time ($\tilde{t}$)**

The mean sojourn time of a GFR marker in ECV, $\tilde{t}$, was calculated from Eq. 6 as

$$\tilde{t} = \frac{ECV_{SM}}{Cl_{SM}}$$  \hspace{1cm} \text{Eq. 21}

**Calculation of the fractions**  \hspace{1cm} $g = s(t) / (1/\tilde{t})$

The fractions were calculated for $t = 180, 210, 240, 270, 300 \text{ min and } 24 \text{ h}$ with

$$s(t) = \frac{-\ln \left[ C(t) \frac{ECV_{SM}}{Q_0} \right]}{t}$$  \hspace{1cm} \text{Eq. 22}

and $\tilde{t}$ calculated according to Eq. 21.

Regression analysis of $s(t) / (1/\tilde{t})$ on $Cl_{SM}$ for different $t$ was performed. The fraction $g = s(t) / (1/\tilde{t})$ was found to be dependant both on $t$ and on $Cl_{SM}$. Multiple regression analysis was performed resulting in a $g(t)_{cor}$ function to be used in the single sample formula, Eq. 13.

The parameters above are given with clearance and extracellular volume calculated according to Sapirstein, $Cl_{SM}$ and $ECV_{SM}$. When the single sample formula was derived using the one-compartment model by Brøchner-Mortensen, $Cl_{BM}$ and $ECV_{BM}$ had to be used instead.
In *Paper I* both models were used and two single sample $^{51}$Cr-EDTA clearance formulas were derived, $\text{Cl}_{S\text{-SM}}$ and $\text{Cl}_{S\text{-BM}}$. In *Paper II* the two-compartment model by Sapirstein was used to derive the iohexol single sample clearance formula.

**Statistics**

Linear and multiple regression analyses were performed in deriving the formulas. The Wilcoxon matched-pairs test was used in *Paper I*, when comparing the different single sample formulas. When comparing the derived single sample formulas with a multi-sample formula, linear regression analysis and calculated differences with their means and standard deviations were used.
RESULTS

Clearance according to Brøchner-Mortensen’s one-compartment model compared to Sapirstein’s two-compartment model

Clearance calculated according to Brøchner-Mortensen, $Cl_{BM}$ was compared to clearance calculated according to Sapirstein, $Cl_{SM}$. The comparison was done for $^{51}$Cr-EDTA clearance in *Group I, Paper I* ($n = 46$). Further comparisons were done for patients included in *Paper II* for $^{51}$Cr-EDTA ($n = 96$) and for iohexol clearance ($n = 95$) and for $^{99m}$Tc-DTPA clearance ($Cl < 30$ ml/min) in *Paper III* (21 patients, 29 examinations). The differences $Cl_{SM} - Cl_{BM}$ are plotted against $Cl_{SM}$ in Fig 3.

![Graphs showing clearance comparisons](image)

**Fig 3.** The differences $Cl_{SM} - Cl_{BM}$ plotted against $Cl_{SM}$. Upper left: $^{51}$Cr-EDTA (*Group I, Paper I*), upper right: $^{51}$Cr-EDTA (patients from *Paper II*), bottom left: iohexol (*Paper II*) and bottom right: $^{99m}$Tc-DTPA ($Cl < 30$ ml/min) (*Paper III*).
Regression lines and differences are presented in Table 2. Lower clearance values are obtained with the method of Brøchner-Mortensen. However, the differences are small and at lower clearances negligible.

**Table 2.** The comparison between Cl\textsubscript{BM} and Cl\textsubscript{SM}. Regression lines (x = Cl\textsubscript{SM}, y = Cl\textsubscript{BM}) and correlation coefficients r are given together with the mean and standard deviation (SD) of the differences Cl\textsubscript{SM} - Cl\textsubscript{BM}.

<table>
<thead>
<tr>
<th>Linear regression</th>
<th>Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regr.line</td>
<td>r</td>
</tr>
<tr>
<td>51Cr-EDTA clearance</td>
<td></td>
</tr>
<tr>
<td>n = 46</td>
<td>y = 0.959x + 0.70</td>
</tr>
<tr>
<td>n = 96</td>
<td>y = 0.954x + 1.37</td>
</tr>
<tr>
<td>Iohexol clearance</td>
<td></td>
</tr>
<tr>
<td>n = 95</td>
<td>y = 0.972x + 0.90</td>
</tr>
<tr>
<td>99mTc-DTPA clearance</td>
<td></td>
</tr>
<tr>
<td>n = 29 (Cl&lt;30ml/min)</td>
<td>y = 0.993x - 0.04</td>
</tr>
</tbody>
</table>

**Derived single sample formulas by the mean sojourn time approach**

**51Cr-EDTA clearance (Paper I)**

Regression analysis using the entire plasma time-concentration curve, as defined by all the plasma samples obtained, together with Sapirsteins two-compartment model gave:

\[
ECV_{SM} = 10800 \times BSA - 5579 \quad \text{Eq. 23}
\]

\[
g_{SM}(t)_{corr} = \left( -4.18 \times 10^{-6} \times t + 6.43 \times 10^{-4} \right) Cl + 1.60 \times 10^{-6} \times t^2 - 0.00103 \times t + 1.25 \quad \text{Eq. 24}
\]

\( g_{SM}(t)_{corr} \) is dependant of \( Cl \) and to calculate clearance an iterative method was used in *Paper I*. According to Watson (41) iteration is not necessary, since equation 13 can be rewritten as:
\[ C_l \times t \times g(t)_{\text{corr}} + \ln \left[ C(t) \frac{ECV}{Q_0} \right] ECV = 0 \]  
Eq. 25

and inserting \( g_{SM(t)} \text{corr} \), a quadratic equation in \( C_l \) is received:

\[ a \times C_l^2 + b \times C_l + c = 0 \]  
Eq. 26

with the solution

\[ C_l = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \]  
Eq. 27

\[ a = \left( -4.18 \times 10^{-6} \times t + 6.43 \times 10^{-4} \right) \times t \]

\[ b = \left( 1.60 \times 10^{-6} \times t^2 - 0.00103 \times t + 1.25 \right) \times t \]

\[ c = \ln \left[ C(t) \frac{ECV}{Q_0} \right] ECV \]  
Eq. 28

The values \( a \) and \( b \) are constants for a given time \( t \), while \( c \) is calculated from the measured concentration of the marker in the plasma sample at time \( t \), \( C(t) \), the injected amount of marker, \( Q_0 \), and \( ECV \) estimated as \( ECV_{SM} \) from Eq. 23.

The term \( -b/2a \) is positive and > 800 in the time interval 180 – 300 min. Therefore, as \( a \) is negative, only the positive value of the square root in Eq. 27 has to be considered.

When the single sample formula was derived from samples obtained between 180 and 300 min after marker injection and Brøchner-Mortensens one-compartment model the found \( ECV \) and \( g(t)_{\text{corr}} \) functions were:

\[ ECV_{BM} = 11476 \times BSA - 7321 \]  
Eq. 29

\[ g_{BM(t)}_{\text{corr}} = \left( -1.30 \times 10^{-6} \times t - 1.19 \times 10^{-3} \right) Cl + 3.00 \times 10^{-6} \times t^2 - 0.00206 \times t + 1.49 \]  
Eq. 30

The single sample clearance, \( C_l_{BM} \) can be calculated as above from Eq. 27 with

\[ a = \left( -1.30 \times 10^{-6} \times t - 1.19 \times 10^{-3} \right) \times t \]
\[ b = \left( 3.00 \times 10^{-6} \times t^2 - 0.00206 \times t + 1.49 \right) \times t \]

and \( c \) from Eq. 28 using ECV estimated as ECV_{BM} from Eq. 29

**Iohexol clearance (Paper II)**

The iohexol formula was derived from a patient group including patients with Cl < 30 mL/min. Using a two-compartment model the derived ECV and \( g(t)_{corr} \) formulas were:

\[ ECV = 9985 \times BSA - 3431 \quad \text{Eq. 31} \]

\[ g(t)_{corr} = \left( -6.49 \times 10^{-6} \times t + 8.85 \times 10^{-4} \right) CI + 1.143 \quad \text{Eq. 32} \]

The single sample clearance can then be calculated from Eq. 27 with

\[ a = (-6.49 \times 10^{-6} \times t + 8.85 \times 10^{-4}) \times t \]
\[ b = 1.143 \times t \]

and \( c \) from Eq. 28 with ECV estimated according to Eq. 31

**Low clearance formula (Paper II and III)**

When deriving the single sample clearance formulas, the calculated g-values varied between 0.9 and 1.3 in the time interval 180 to 300 min. In low clearances, a late sample was obtained about 24 h after marker injection. The received g-values calculated at \( t \sim 24 \) h were all close to 1 (Fig. 4).
Fig 4. The fractions $g = s(t)/(1/t)$ calculated for iohexol and samples obtained between 3 and 5 h (left) and if Cl < 30 mL/min for a late sample obtained between 22 and 26 h (right).

This means, that in low clearances, $g(t)_{corr}$ in Eq. 13 can be set to 1 and single sample clearance calculated from a 24 h sample using the formula

$$\text{Cl}_{S(24h)} = \frac{-\ln \left( C(t) \frac{ECV}{Q_0} \right) ECV}{t}$$

Eq. 33

(t in minutes)

Test of the derived single sample formulas

$^{51}$Cr-EDTA clearance (Paper I)

The derived single sample formulas $\text{Cl}_{S-SM}$ and $\text{Cl}_{S-BM}$ were, together with the $^{99m}$Tc-DTPA-formula by Christensen and Groth, applied to Group II, Paper I ($n = 1046$). Reference clearance was calculated according to Brøchner-Mortensen, $\text{Cl}_{BM}$. Regression lines and differences at $t = 180$ min and $t = 270$ min are presented in Table 3. The regression analysis of $\text{Cl}_{S-SM}$ on $\text{Cl}_{BM}$ gave regression lines close to the line of identity. Both the $\text{Cl}_{S-BM}$ formula and the DTPA-formula slightly overestimated GFR.
For lower clearance values ($\text{Cl} < 80 \text{ ml/min}$) the differences $\text{Cl}_{BM} - \text{Cl}_S$ were smaller if $\text{Cl}_S$ was calculated using the 270 min sample. At higher clearances the use of a 180 min sample resulted in smaller differences, Fig 5.

Table 3a. Comparison between single sample $^{51}$Cr-EDTA clearance calculated at $t= 180$ min and $t = 270$ min and $\text{Cl}_{BM}$. Regression lines and correlation coefficients are presented.

<table>
<thead>
<tr>
<th>Single sample formula</th>
<th>$t = 180$ min, $x = \text{Cl}_{BM}$</th>
<th>$t = 270$ min, $x = \text{Cl}_{BM}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regr. line</td>
<td>$r$</td>
</tr>
<tr>
<td>$\text{Cl}_S$-SM</td>
<td>$y = 1.00x + 1.30$</td>
<td>0.982</td>
</tr>
<tr>
<td>$\text{Cl}_S$-BM</td>
<td>$y = 1.11x - 1.86$</td>
<td>0.976</td>
</tr>
<tr>
<td>$\text{Cl}_S$, DTPA-formula</td>
<td>$y = 1.12x - 5.51$</td>
<td>0.985</td>
</tr>
</tbody>
</table>

Table 3b. Differences $\text{Cl}_{BM} - \text{Cl}_S$. Mean and standard deviation in mL/min when single sample clearance is calculated at $t = 180$ min and $t = 270$ min and the combination $t = 270$ min if $\text{Cl}_{BM} < 80 \text{ mL/min}$ and $t = 180$ min if $\text{Cl}_{BM} > 80 \text{ mL/min}$.

<table>
<thead>
<tr>
<th>Single sample formula</th>
<th>$t = 180$ min</th>
<th>$t = 270$ min</th>
<th>$t = 270$ if $\text{Cl}_{BM} &lt; 80$</th>
<th>$t = 180$ if $\text{Cl}_{BM} &gt; 80$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>$\text{Cl}_S$-SM</td>
<td>-1.5</td>
<td>5.8</td>
<td>-1.2</td>
<td>6.3</td>
</tr>
<tr>
<td>$\text{Cl}_S$-BM</td>
<td>-3.5</td>
<td>8.2</td>
<td>-3.7</td>
<td>9.1</td>
</tr>
<tr>
<td>$\text{Cl}_S$, DTPA-formula</td>
<td>-3.2</td>
<td>6.9</td>
<td>-3.4</td>
<td>6.1</td>
</tr>
</tbody>
</table>
Fig 5 Differences $\text{Cl}_{BM} - \text{Cl}_{S\text{-SM}}$ plotted against $\text{Cl}_{BM}$ at $t = 180$ min (left) and at $t = 270$ min (right).

$^{51}\text{Cr-EDTA clearance, } \text{Cl} < 30 \text{ mL/min (Unpublished data)}$

The low clearance formula, $\text{Cl}_{S(24h)}$ (Eq. 33 with ECV from Eq. 29) was applied to $^{51}\text{Cr-EDTA}$ clearance for patients with $\text{Cl} < 30 \text{ mL/min}$ (21 patients, 29 examinations).

The differences $\text{Cl}_{BM} - \text{Cl}_{S(24h)}$ are plotted in Fig 6. The mean of the differences was 0.25 mL/min and the SD was 1.0 mL/min.

Fig 6. $^{51}\text{Cr-EDTA}$ clearance, $\text{Cl} < 30 \text{ ml/min}$. Differences $\text{Cl}_{BM} - \text{Cl}_{S(24h)}$ plotted against $\text{Cl}_{BM}$.
Iohexol clearance (Paper II)

The derived iohexol clearance formulas, $\text{Cl}_S$ and $\text{Cl}_{S(24h)}$ were applied to Group II ($n = 47$) and to Group III ($n = 123$) in Paper II. Jacobsson’s formula Eq. 5 was also used. Unpublished data.

In Fig 7a the differences $\text{Cl}_{SM} - \text{Cl}_S$ are plotted against $\text{Cl}_{SM}$ (Group II). $\text{Cl}_S$ is calculated for $t = 180, 210, 240, 270, 300, 24h$. In Fig 7b Jacobsson’s formula is used. The same pattern can be seen: a greater scatter at lower clearance values, when early samples are used. When $\text{Cl} < 30 \text{ mL/min}$ an accurate result is only obtained with the 24 h sample.

Fig 7a. The Differences, $\text{Cl}_{SM}-\text{Cl}_S$ in mL/min when $\text{Cl}_S$ (Eq. 27) is calculated at different t.
When the general formula $Cl_S$ (Eq. 27) was applied to low clearances in Group III, using the 24 h sample, the two highest values (27 and 35 mL/min) were overestimated while the low clearance formula $Cl_S(24h)$ (Eq.33) yielded accurate results. The combination of $Cl_S$ calculated using a 270 min sample if s-creatinine < 200 µmol/L and clearance calculated using the low clearance formula with a 24 h sample if s-creatinine > 200 µmol/L yielded good results. Jacobsson’s formula is used with the same approach: a 270 min sample unless s-creatinine > 200 µmol/L, when a 24 h sample is used. The differences are plotted in Fig 8. In Table 4 the results are summarized.

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Fig 7b. The Differences, $Cl_{SM}-Cl_{S-Jac}$ in mL/min when Jacobsson’s formula is used at different t.
Fig 8. Differences $\text{Cl}_{BM} - \text{Cl}_S$ in Group III calculated from a 270 min sample (filled circle) and from a 24 h (open circle). The mean sojourn time formula ($\text{Cl}_S$) is used left and Jacobsson’s formula ($\text{Cl}_{S-Jac}$), Eq. 5 right.

**Table 4a.** Linear regression. Single sample clearance compared to multi-sample clearance. Single sample Cl calculated from a 270 min sample or a 24 h (low clearance). Jacobsson’s formula is calculated both with ECV estimated for EDTA according to Eq. 5 and with ECV estimated for iohexol according to Eq. 31.

<table>
<thead>
<tr>
<th>Single sample formula</th>
<th>Group II, $x = \text{Cl}_{SM}$</th>
<th>Group III, $x = \text{Cl}_{BM}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regr.line</td>
<td>$r$</td>
</tr>
<tr>
<td>$\text{Cl}<em>S$, Eq. 27; $\text{Cl}</em>{S(24h)}$, Eq. 33</td>
<td>$y = 0.990x - 0.31$</td>
<td>0.997</td>
</tr>
<tr>
<td>$\text{Cl}_S$-Jac, Eq. 5</td>
<td>$y = 0.961x + 0.55$</td>
<td>0.997</td>
</tr>
<tr>
<td>$\text{Cl}<em>S$-Jac, ECV$</em>{ioh}$ (Eq. 31)</td>
<td>$y = 1.008x - 1.28$</td>
<td>0.996</td>
</tr>
</tbody>
</table>

**Table 4b.** Differences, $\text{Cl}_{SM}-\text{Cl}_S$ in Group II and $\text{Cl}_{BM}-\text{Cl}_S$ in Group III. Mean and SD in mL/min.

<table>
<thead>
<tr>
<th>Single sample formula</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>$\text{Cl}<em>S$, Eq. 27; $\text{Cl}</em>{S(24h)}$, Eq. 33</td>
<td>0.9</td>
<td>2.7</td>
</tr>
<tr>
<td>$\text{Cl}_S$-Jac, Eq. 5</td>
<td>1.9</td>
<td>2.9</td>
</tr>
<tr>
<td>$\text{Cl}<em>S$-Jac, ECV$</em>{ioh}$ (Eq. 31)</td>
<td>0.8</td>
<td>3.2</td>
</tr>
</tbody>
</table>
**99mTc-DTPA clearance (Paper III)**

The low clearance formula, $\text{Cl}_{S(24h)}$ was applied to $^{99m}\text{Tc}$-DTPA clearance < 30 mL/min (21 patients, 29 examinations).

ECV was calculated as the distribution volume of $^{99m}\text{Tc}$-DTPA, determined by Christensen and Groth (28):

$$ECV = 8116.6 \times BSA - 28.2 \quad \text{Eq. 34}$$

The mean of the differences $\text{Cl}_{BM} - \text{Cl}_{S(24h)}$ was -0.5 mL/min and the standard deviation was 1.0 mL/min.

To demonstrate the necessity of a prolonged sampling time in a multi-sample method when GFR is low, a Brøchner-Mortensen clearance was calculated from two early samples. Not including the 24 h sample resulted in an overestimation of 2.5 mL/min.

The results are presented in table 5.

**Table 5.** Differences in $^{99m}\text{Tc}$-DTPA clearance < 30 mL/min. Simplified clearances are calculated from a 24 h sample ($\text{Cl}_{S(24h)}$) and from two early samples at 3 and 5 h ($\text{Cl}_{BM(3 \text{ and } 5 \text{ h})}$) and at 3 and 4 h ($\text{Cl}_{BM(3 \text{ and } 4 \text{ h})}$) respectively and compared to Brøchner-Mortensen clearance calculated from samples between 3 and 24 h. Mean and standard deviation in mL/min.

<table>
<thead>
<tr>
<th>Clearance formula</th>
<th>Differences</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Cl}_{S(24h)}$</td>
<td>-0.5</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>$\text{Cl}_{BM(3 \text{ and } 5 \text{ h})}$</td>
<td>-2.5</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>$\text{Cl}_{BM(3 \text{ and } 4 \text{ h})}$</td>
<td>-2.5</td>
<td>2.6</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Reference clearance
In the single sample GFR method by the mean sojourn time approach, Groth (21) assumed that the plasma time-concentration curve was biexponential - a two-compartment model according to Sapirstein (18). In the present study, Brøchner-Mortensen’s one-compartment model (20) was also used. Comparing the two models, clearance according to Brøchner-Mortensen, $\text{Cl}_{\text{BM}}$ slightly underestimates clearance according to Sapirstein, $\text{Cl}_{\text{SM}}$. This was the case for all three GFR markers used (Table 2, Fig 3).

Brøchner-Mortensen empirically determined a correction by adapting three to four exponential functions to the plasma time-concentration curve. A calculated concentration $C(0)$ of the marker at time $t = 0$ was obtained assuming an immediate mixing of the injected marker in the plasma volume and calculated as the injected amount of the marker divided by the plasma volume. The plasma volume was determined using Evans blue. In reality, $C(0)$ will be lower since the diffusion of the marker from the plasma into the extravascular space will start immediately. There is also an arterial-venous difference and using venous sampling, a third compartment often can not be distinguished (42). Brøchner-Mortensen’s way of determining the plasma time-concentration curve results in a greater area under the curve and thus a lower clearance. This explains the observed difference between the two methods. The Brøchner-Mortensen correction has greater influence at higher clearance and a greater scatter is then observed. The Brøchner-Mortensen correction was determined for clearance lower than 140 mL/min.

The differences using Sapirstein and Brøchner-Mortensen are small and the use of $\text{Cl}_{\text{BM}}$ as a reference method was considered justified, when testing the derived formulas. With $\text{Cl}_{\text{BM}}$ as the reference method it was possible to apply the derived formulas to a larger material, since $\text{Cl}_{\text{BM}}$ has been the routine method for many years in our department.

The recommended formulas below are derived using a two compartment model according to Sapirstein.

$^{51}\text{Cr}-\text{EDTA clearance}$
Many single sample GFR formulas are derived using already simplified methods. One of the aims in Paper I was to investigate whether a more accurate formula was obtained using the entire plasma time concentration curve than using Brøchner-Mortensen’s simplified
Another aim was to see if the $^{99m}$Tc-DTPA formula by Christensen and Groth could be applied to patients investigated with $^{51}$Cr-EDTA as the GFR marker. The single sample formula derived from $C_{\text{BM}}$ resulted in a small overestimation of GFR. The $^{99m}$Tc-DTPA formula by Christensen and Groth also slightly overestimated $^{51}$Cr-EDTA reference clearance.

Using $C_{\text{SM}}$ to derive the single sample $^{51}$Cr-EDTA formula with the mean sojourn time approach resulted in a formula giving good agreement with the reference method. Higher accuracy was obtained using a 180 min sample compared to a 270 min in clearance values > 80 mL/min. The coefficient of variation (CV %) was 6.2 % in Group II with the single sample at $t = 270$ min if $C_l < 80$ mL/min and at $t = 180$ if $C_l > 80$ mL/min. The coefficient of variation was expressed as 1 SD$_{\text{diff}}$ in percent of the mean clearance value. However, the material used to derive the single sample $^{51}$Cr-EDTA formula did not include low clearances and late sampling and the formula should not be used when a low GFR has been estimated.

**Iohexol clearance**

The patients in *Paper II* were included to allow deriving formulas also applicable to lower clearance values. Late sampling is necessary in low clearance and an additional 24 h sample was obtained when clearance was < 30 mL/min. When testing the derived general iohexol formula, $C_{\text{ls}}$, in the time interval 3 to 24 h (Fig 7a), samples between 4 and 5 h yielded acceptable results if $C_l > 30$ mL/min. In lower clearances, only the 24 h sample gave good results. In deriving the general iohexol formula, the highest clearance was 25 mL/min, where a 24 h sample had been obtained. When the general formula was applied to $C_l > 25$ mL/min in Group III with $t = 24$ h, an overestimation occurred (Fig 3B in *Paper II*). The special low clearance formula, $C_{\text{ls}(24\text{h})}$ (Eq. 33), yielded accurate results with all 24 h samples obtained. To avoid overestimation the special low clearance formula is recommended for use with a 24 h sample in patients with an estimated low GFR.

Jacobsson’s single sample formula, Eq. 5, is commonly used with the distribution volume for $^{51}$Cr-EDTA to calculate iohexol clearance. When applied to the iohexol material in *Paper II*, very similar results were obtained with Jacobsson’s method as with the mean sojourn time based iohexol formula. A minor underestimation could be observed with Jacobsson’s formula. If the distribution volume for iohexol, Eq. 31, was used in Jacobsson’s formula, clearance values closer to the reference were obtained (Table 4).
The single sample iohexol clearance with the mean sojourn time approach resulted in a CV of 4.4% in Group II and 5.0% in Group III when calculated from a 24 h sample in estimated GFR < 30 mL/min and from a 270 min sample in estimated GFR > 30 mL/min.

**Low clearance formula**

Empirically, the $g(t)_{corr}$ in Eq. 13 was found to give a value = 1 for low Cl and t between 22 and 26 h (Fig 4) yielding a simplified low clearance formula, Eq. 33. Using the low clearance formula, accurate results were achieved for all three GFR markers. The formula is recommended when GFR is estimated to be less than 30 mL/min.

Calculating $g(t)_{corr}$ in the DTPA formula (28) and in the EDTA formula, Eq. 24 for $t = 24$ h and Cl < 30 mL/min will give $g(t)_{corr}$ values far from one: ~0.2 and ~3 respectively, which would result in GFR values greatly over- and underestimated respectively. These formulas were not derived for low clearance and late sampling and should, then, not be used.

Using the low clearance formula the CV was 5.0% in Cl < 30 mL/min both for $^{51}$Cr - EDTA and $^{99m}$Tc-DTPA clearance.

**Sources of errors in calculating plasma clearance**

In general, single sample GFR methods are considered slightly less accurate than multi-sample methods, e.g. Brøchner-Mortensen’s method (43, 44).

The sources of error and their influence differ between single and multi-sample methods.

**Estimated ECV**

The estimation of extracellular volume, defined as the distribution volume of the used GFR marker, is the major source of error when GFR is determined using single sample methods. The distribution volume differs slightly between different markers. ECV has been estimated from bodyweight, bodyweight and gender, body surface area and from lean body mass (24, 31, 28, 45). In children, Groth (25) found a high correlation between ECV and BSA, $r = 0.97$. The correlation in adults was not as good. For $^{99m}$Tc-DTPA a correlation coefficient, $r = 0.35$ was found (28). For $^{51}$Cr-EDTA and iohexol the correlation was higher, $r = 0.81$, $SD_{y/x} = 1.9$ litre (Paper I) and $r = 0.74$, $SD_{y/x} = 2.0$ litre (Paper II) respectively. An attempt to estimate ECV from lean body mass was done (Paper II). The
correlation in the used material was not improved compared with estimation based on BSA.

Estimating ECV from anthropometric data means that the error is related to the patient. The repeatability in single sample clearance may be high, though the accuracy in some patients may be low. A single sample method should not be used in subjects where ECV cannot be well estimated from anthropometric data, e.g., in pregnant women and in patients with severe derangements in fluid balance (46).

A more accurate estimation of ECV, not using anthropometric data, would improve the single sample method.

Using Brøchner-Mortensen’s method, ECV is not needed in the calculation and the error in estimated ECV thus does not influence $Cl_{BM}$.

**Sampling time**

The influence of the error in ECV on the single sample clearance depends on the sampling time. The optimal sampling time, $t_{-opt}$, where the inaccuracy in ECV has least importance (Fig 9) is given by the equation:

$$t_{-opt} = \frac{t}{E} = \frac{ECV}{Cl}$$

![Fig 9](image.png)

**Fig 9.** The optimum time for drawing the single blood sample plotted against clearance and calculated from $ECV_{SM}$ and $Cl_{SM}$ for iohexol (Paper II, Group I and II)
The function $g(t)_{\text{corr}}$ will partly compensate for the single sample not being obtained at an optimal time. In *Paper II, Group III* the derived iohexol formula was used with a 270 min sample if s-creatinine < 200 μmol/L and a 24 h sample if s-creatinine > 200 μmol/L. The influence on the calculated single sample clearance when ECV is increased with 2 L (1 SD error in estimated ECV) is shown in Fig 10.

![Graph showing the change in Cl\text{S} (Paper II, Group III) if estimated ECV is increased with 2 L (1 SD, error in estimated ECV). Single sample at 270 min (filled circle) and at 24 h (open circle).](image)

**Fig 10.** Change in Cl\text{S} (*Paper II, Group III*) if estimated ECV is increased with 2 L (1 SD, error in estimated ECV). Single sample at 270 min (filled circle) and at 24 h (open circle).

In *Paper II* a single sample between 4 and 5 h is recommended for estimated GFR ≥ 30 mL/min and at about 24 h for estimated GFR < 30 mL/min. GFR can be estimated from the concentration in plasma of creatinine or cystatin C.

With estimated clearance around 30 mL/min, acceptable single sample clearance values are mostly obtained both with a 24 h sample and a sample between 4 and 5 h. However, in these cases two samples should be considered since a clearance higher than the estimated 30 mL/min may result in a very low marker concentration in a 24 h sample and a clearance considerably below 30 mL/min needs a late sample in order to obtain an accurate value.

If a higher accuracy is required with clearance values > 80 mL/min, a 180 min sample is preferable according to t-opt (Fig 9). The calculated single sample clearance also correlated better with the reference clearance if a 180 min sample instead of a 270 min sample was used when clearance was higher than 80 mL/min (Fig 5).

Using Jacobsson’s single sample method Gaspari et al.(47) investigated the optimal time of the single sample in a group of 686 patients with a wide range of renal function and
proposed 10 h when estimated GFR < 40 mL/min, 4 h between 40 and 100 mL/min and 3 h when GFR is estimated > 100 mL/min. Sterner et al. (37) proposed 24 h when GFR < 20 mL/min, 7 h between 20 and 50 mL/min and 4 h with estimated GFR > 50 mL/min. In very low estimated GFR (< 5 mL/min) a sampling time between 48 - 72 h was proposed by Nilsson-Ehle and Grubb (48). For practical purpose we recommend sampling at 4.5 h when estimated GFR > 30 mL/min and at 24 h if estimated GFR < 30 mL/min. Sampling at 3 h may be considered if GFR is estimated to be essentially normal.

Accuracy in C(t)
The accuracy in the concentration measurement of the marker will have a greater influence on a multi-sample method than on a single sample method (44, 49).
A 2 % error in C(t) in the single sample formula, Eq. 13 will give an error in ClS of 1.3 mL/min (t = 270 min, ECV = 18000 mL).
Using Brøchner-Mortensen’s method, an error in C(t) causes a change in the final slope of the plasma time-concentration curve, b1 in Fig. 2. A small error in final slope may introduce a great change in the area under the curve and thus also in ClBM. The error in ClBM depends on clearance, number of blood samples and sampling times. In Fig. 11 the error (1 SD) in ClBM is calculated, when normally distributed random errors, 0 – 2 %, were introduced in each concentration measurement. The simulation was repeated 100 times. ClBM was calculated from 4 samples obtained 180, 210, 240 and 270 min after marker injection. Adding a late sample will decrease the uncertainty in low clearance measurement. An earlier sampling period, 150 – 240 min will decrease the uncertainty in high ClBM measurement (50).

Errors in injected amount of marker and in registered time of injection and of sampling
These errors are usually small and their influence is of the same magnitude in single and multi-sample methods (44, 49). However, in decentralized GFR measurements some of these preanalytical errors might be considerable and a cause for the observed poor repeatability (51).
According to Brøchner-Mortensen (12) the total day-to-day variation of clearance in patients with stable renal function is in the order of 5 %, when a multi sample method is used.

Bird et al (52) in healthy volunteers observed no difference in day-to-day reproducibility with a single and a multi sample method.

Hansen et al (53) studied the rate of decline in GFR over several years in patients with diabetic nephropathy and found corresponding rate of decline in single and multi-sample GFR.

In the large study (686 patients) by Gaspari et al (47) good accuracy was found in 75 % of the patients (a difference within 5 %) between single and multi-sample GFR. In some patients a considerably higher difference was noted but could not be explained.

In the present studies CV between 4 and 6 % were found. Higher discrepancies were found in some patients in group II, Paper I and in group III, Paper II. Several of these patients (Paper I) had oedema or severe electrolyte derangement at the time of examination.

Plasma clearance overestimates renal clearance in patients with oedema (54). Groth & Aasted (55) found that calculation of plasma clearance from a single sample overestimated renal clearance more than a multi-sample method in these patients.

Gaspari et al considered that “the real advantage of the single-point GFR calculation should be accurately evaluated because neither an improvement of precision in GFR
calculation nor a reduced time of patient observation after filtration marker injection is achieved.”

The total number of samples to be drawn and analysed is not reduced in proportion to the reduction in plasma samples switching from a multi to a single sample method, since a plasma blank is still needed. Also standard solutions should be analyzed anyhow. The total number of samples to be analysed may be approximately halved. Patient time for injection and sampling will though be more than halved with a single sample method.

**Quality control**

Brøchner-Mortensen’s method offers a good internal quality control of the individual plasma samples (concentration measurements and registered times of sampling) by checking the goodness-of-fit to a single exponential curve by using a graphical plot or the value of the correlation coefficient (44, 56). If the points are not on a single exponential curve the outlier may be identified by applying a single sample formula (56) to the different points. This quality control is missing in the single sample method.

De Sadeleer proposed a post-test quality control for the single sample GFR method in children (57). De Sadeleer investigated a possible error in the injected dose or in the plasma sample. By using an estimated distribution volume, the initial plasma concentration was calculated and together with the available single sample an artificial slope intercept clearance was calculated and compared to the single sample clearance. If a difference > 10 mL/(min·1.73m²) was found, the presence of an error in the single sample method was highly probable. However, a smaller difference does not exclude erroneous data. This quality check for the single sample GFR method should also be applicable to adults.

**Decentralized GFR**

The use of iohexol as a GFR marker has made decentralized GFR measurement possible. However, poor repeatability in decentralized iohexol GFR determinations in Sweden has been reported (51). In Sweden, iohexol clearance is often performed using a single sample method. The iohexol concentration is measured by HPLC. As explanation to the large
discrepancies between repeated GFR measurements the authors suggest preanalytical problems: erroneous registered times for injection or blood sampling or erroneous registered amounts of iohexol administered. It is also suggested that stop-watches are provided to the wards in order to get all time registrations by one watch (51).

Some other aspects should also be considered when using iohexol.

Brøchner-Mortensen et al found that the same intravenous catheter could be used both for injection of the marker and for blood sampling without any problems of contamination (58). Contamination has not been a problem with $^{51}$Cr-EDTA and $^{99m}$Tc-DTPA, but the iohexol solution has a high viscosity and contamination may occur. In multi-sample methods contamination is recognized as a too high concentration in the first sample. Unless the concentration is very high, contamination in a single sample cannot be recognized and GFR will be underestimated. Preferably the intravenous catheter used for injection of iohexol should not be used for blood sampling. The contralateral arm should be used for blood sampling if possible.

The HPLC technique is based on separation by reversed phase chromatography and on quantification by UV absorption. Several endogenous and exogenous substances may interfere with the quantification of iohexol. Some late eluting substances may also interfere with the subsequent sample. Therefore, plasma blanks and samples should always be analysed in duplicate to detect any interfering substance requiring modification of the chromatographic method. A washing procedure between samples has also been proposed to eliminate late interfering substances (46).

The administrated iohexol may be a weight amount or a measured volume. The specific weight of the iohexol solution is high (1.345 g/cm$^3$ at 20 ºC, Omnipaque 300 mg I / mL) and the different ways of quantifying the administered dose of iohexol must be handled properly by the analysing laboratory.

If single sample decentralized iohexol clearance is used it should be limited to a few wards with a minimum of staff involved. In order to obtain accurate results the staff should be well educated and familiar with the procedure. If the preanalytical procedures are in doubt a multi sample method is recommended providing good quality control.
Limitations

A single sample method should not be used in the presence of severe oedema, ascites or other expanded body volume (46). Single sample plasma clearance will overestimate GFR in the presence of an enlarged distribution volume. Renal clearance should be performed.
RECOMMENDED FORMULAS

A general single sample formula is recommended for use in adults when GFR is estimated to be $\geq 30$ mL/min. The plasma sample is recommended to be obtained between 4 and 5 h after marker injection. If a more accurate determination of GFR is needed for patients with an estimated GFR $> 80$ mL/min, a sample obtained at 3 h yields a somewhat better result. Different regression coefficients are to be used for each marker.

When estimated GFR is $< 30$ mL/min a special low clearance formula with a 24 h sample is recommended.

**General formula** (for estimated GFR $\geq 30$ mL/min)

\[
Cl_s = \frac{-b + \sqrt{b^2 - 4ac}}{2a}
\]

with $c$ calculated from the equation

\[
c = \ln \left[ C(t) \frac{ECV}{Q_0} \right] ECV
\]

$C(t)$ is the measured concentration of the marker in the plasma sample at time $t$ ($t$ in min), $Q_0$ is the injected amount of marker. ECV in mL and estimated from BSA in m$^2$.

$a$, $b$ and ECV are dependant on the used marker and are calculated as follows:

**$^{99m}$Tc-DTPA clearance**

\[
a = (1.70 \times 10^{-6} \times t - 1.20 \times 10^{-3}) \times t
\]

\[
b = (-7.75 \times 10^{-4} \times t + 1.31) \times t
\]

\[
ECV = 8116.6 \times BSA - 28.2
\]

The $^{99m}$Tc-DTPA formula is the Christensen and Groth formula (28) rewritten according to Watson (41).
\[ {^{51}}\text{Cr-EDTA clearance} \]

\[
a = \left(-4.18 \times 10^{-6} \times t + 6.43 \times 10^{-4}\right) \times t
\]

\[
b = \left(1.60 \times 10^{-6} \times t^2 - 0.00103 \times t + 1.25\right) \times t
\]

\[ ECV = 10800 \times BSA - 5578.6 \]

\[ \text{Iohexol clearance} \]

\[
a = (-6.49 \times 10^{-6} \times t + 8.85 \times 10^{-4}) \times t
\]

\[
b = 1.143 \times t
\]

\[ ECV = 9985 \times BSA - 3431 \]

\[ \text{Low clearance formula (for estimated GFR < 30 mL/min)} \]

\[
Cl_{51}^{[24h]} = \frac{-\ln \left[C(t) \frac{ECV}{Q_0} \right] ECV}{t}
\]

(t in min)

ECV as above for \(^{99m}\text{Tc-DTPA}, {^{51}}\text{Cr-EDTA} \) and iohexol respectively.
CONCLUSIONS

The single sample plasma clearance method based on the mean sojourn time of a GFR marker in its distribution volume had previously been validated for determination of $^{99m}$Tc-DTPA clearance in adults with an estimated GFR $\geq 30$ mL/min. The method has now been further developed and formulas derived resulting in accurate results for two other GFR markers, $^{51}$Cr-EDTA and iohexol.

A special low clearance formula with a single sample obtained about 24 h after marker injection has been derived and proven valid for use with all three markers if GFR is estimated to be lesser than 30 mL/min.

To obtain the best possible results separate formulas should be used for each marker.

For practical purpose we recommend sampling at 4.5 h when estimated GFR $> 30$ mL/min and at 24 h if estimated GFR $< 30$ mL/min. Sampling at 3 h may be considered if GFR is estimated to be essentially normal.
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REFERENCES


7. Jonsson A-S; Flodin M; Hansson L-O; Larsson A. Estimated glomerular filtration rate (eGFR/CystC) from serum cystatin C shows strong agreement with iohexol clearance in patients with low GFR. *Scand J Clin Lab Invest* 2007; 67: 801-809


