Measured GFR using a population pharmacokinetic model for iohexol

Kwame Boateng

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Department of Pharmaceutical BioSciences
School of Pharmacy
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IV
Abstract

Introduction: Determining renal function (i.e glomerular filtration rate) is in many ways essential in clinical practice, in terms of diagnosis and drug therapy. Exogenous markers, such as iohexol, are mainly used for a more accurate determination of GFR. A low dosage of iohexol is injected to the patient and GFR is determined by using an algorithm and plasma concentrations measured at 2 and 5 hours after administration. Non-parametric population pharmacokinetic models are invaluable tools for describing the pharmacokinetic properties of different drug and are suitable for identifying subpopulations deviating pharmacokinetic properties. Population models, unlike algorithms are not dependent on specific sampling times.

Method: A standardized dose of iohexol is injected and up to 12 plasma concentrations of iohexol are measured with HPLC-UV. The pharmacokinetic properties are investigated using non-parametric population modeling (Pmetrics®). With the help of the MMopt function in Pmetrics®, the most informative sampling times for determining the AUC of iohexol are identified. The developed model is then utilized to investigate optimal sampling times to determine the AUC for iohexol, which is then used to determine individual GFR. The model is assessed against AUC determined with the trapezoidal method and also the standard method where GFR is determined using plasma concentrations 2 and 5 hours after administration.

Results: 13 renal transplant recipients were included in the study. A 2-compartment model with primary parameters allometrically scaled with centralized body weight described the pharmacokinetic properties of iohexol best. Individual observation-prediction plot showed r²-values at 0.996. With the optimal sampling times determined with the help of the MMopt function, GFR was determined with both 2-and 3-point measurements. Predicted GFR showed close values to the reference for most but was massively underpredicted for certain patients.

Conclusion: The massive underprediction was most likely due to the limited number of test subjects included in this study. Future work on building a better model will require the inclusion of more patients; especially more data in the later post administration phase in patients with low renal function.
Sammendrag

Introduksjon: Bestemmelse av pasienters nyrefunksjon (i.e. glomerulær filtrasjonsrate, GFR) er i mange situasjoner nødvendig i klinikken med tanke på diagnose og dosering av legemidler. For en mer nøyaktig bestemmelse av GFR bruker man for tiden hovedsakelig ulike eksogene stoffer så som iohexol. En lav dose iohexol injiseres og ved bruk av en algoritme og konsentrasjonene målt 2- og 5 timer etter administrering, kan GFR bestemmes. Ikke-parametrisk populasjonsmodellering av farmakokinetiske data er et kraftfullt verktøy for å beskrive ulike legemidlers farmakokinetikk og spesielt godt egnet for å identifisere subpopulasjoner som med avvikende farmakokinetikk. En fordel med å bruke populasjonsmodeller for å beskrive kinetikken til forskjell fra algoritmer, så er modeller ikke avhengig av prøvetaking på førbestemte tidspunkter.


Resultater: 13 nyretransplanterte pasienter ble inkludert. En 2-kompartement modell med primære parametre allometrisk skalert til sentralisert kroppsvækt ga en god beskrivelse av farmakokinetikken til iohexol i pasientene. Individuelle observasjon vs. prediksions plott viste r²-verdier opp mot 0.996. Med optimale prøvetakingstider bestemt ved hjelp av MMopt, ble GFR beregnet for både 2-og 3-punktsmålinger. Sammenlignet med referansen, stemte GFR for de fleste pasientene men underpredikerte grovt hos enkelte pasienter.

Konklusjon: Den grove underpredikasjonen er mest sannsynlig pga mangel på testsubjekter under modellbygningen. Forsterkning av modellen vil kreve inkludering av flere pasienter og spesielt pasientdata i den postadministrative fasen hos pasienter med lav nyrefunksjon.
Acknowledgements

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Foremost, I would like to thank my main supervisors, Professor Anders Åsberg and Professor Stein Bergan. Thank you for your guidance and close supervision during my research. I have greatly valued your encouragement, advice, funny remarks and constructive comments. I would also like to express my gratitude to the department of nephrology. I am grateful for your patience and knowledge.

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A great thanks to my family and friends for the support, especially my mom and dad for always believing and supporting me in everything I do.

May 2016
Abbreviations

RAS  Renin-angiotensin system
GFR  Glomerular filtration rate
CKD  Chronic kidney disease
UTI  Urinary tract infection
CKD-EPI  Chronic Kidney Disease Epidemiology Collaboration
MDRD  Modification of diet in renal disease
SCR  Serum creatinine
Cr-EDTA  Chromium Ethylenediaminetetraacetic acid
Tc-DTPA  Diethylene-triamine-pentaacetate
HPLC-UV  High performance liquid chromatography – Ultra violet
OUS  Oslo Universitetssykehus
AUC  Area under the curve
AIC  Akaike information criterion
IT2B  Iterative 2-stage Bayesian
NPAG  Non-parametric Adaptive Grid software
CL  Clearance
V  Volume of distribution
Vp  Peripheral volume
Q  Intercompartmental clearance
BMI  Body mass index
MMopt  Multi model optimal sampling
SD  Standard deviation
PK  Pharmacokinetics
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1 Introduction

1.1 Renal function

1.1.1 Pharmacokinetics

The fundamentals of pharmacokinetics are in many ways a cornerstone for good prescribing and drug development [1]. The pharmacokinetics of a drug refers to how a drug is handled by the body. Processes like absorption, distribution, metabolism and elimination describe the drug's lifespan in the body[2]. These processes are described by mathematical models, which in many ways have been used in other fields such as biological chemistry and nuclear physics[1]. Clearance, a term used to describe elimination, is a fundamental concept in pharmacokinetics. Clearance of a drug is hardly measured directly but calculated with formulas that sometimes include the other processes or terms used to describe them[2].

Pharmacokinetics can now be studied in populations of patients who are taking a drug. The advantage of studying a population is the ability to analyze variability in pharmacokinetics discovered not only within patients but also between patients. An example would be the variations in drug concentration, which will occur with renal impairment when the patient is taking a drug excreted in the urine[1].

1.1.2 The Kidney

The human body’s main method of waste product elimination is through the kidneys and is the primary function of the kidneys. They also regulate the volume and osmolality of the extracellular fluid by altering the amount of sodium and water excreted[3]. These functions are executed with ease despite the variation in dietary intake and other environmental demands. They are functions that contribute to maintaining balance within the human body’s internal environment, also known as homeostasis[4].

The kidney performs other tasks in the human body, such as:

- The acid-base homeostasis. The kidney secretes acid, which is loaded into the body by daily food intake. It then supplies bicarbonate to compensate for the consumed bicarbonate for buffering the acid[5].
- Gluconeogenesis. A process by which the kidneys and liver generate glucose from substances, other than carbohydrates resulting in the reduction of blood glucose levels during starvation[4].
- Transforming vitamin D to its active form. Vitamin D is activated in a 2-step process, involving first 25-hydroxylation in the liver to 25-(OH) vitamin D and then 1-hydroxylation in the kidneys[6].
- Production of renin, an aspartyl protease that catalyzes the first steps in the activation of the renin-angiotensin-system (RAS). RAS is one of the major control systems for blood pressure and fluid balance.[7]
- Production of erythropoietin. A fall in tissue oxygen triggers the secretion of erythropoietin, resulting in increased production of the red blood cells [8].

The kidneys ability to execute its functions is due to the nephron, the functional unit. The nephron consists of five parts known as the glomerulus, proximal tubule, loop of Henle, distal convoluted tubule and the collecting duct. Each component of the nephron contributes to the kidney’s process of waste product elimination[9]. Filtration, secretion and reabsorption are known as the kidneys process of work, enabling it to execute its duties. Fluids from the capillaries are filtered, leaving behind high-molecular-weighted molecules. Important molecules and nutrients that passed through the filtration process are reabsorbed and transported back to the blood. The rest of the fluid is then excreted as urine[4].

In clinical practice, a reliable measurement for renal excretory function is of great importance. The kidney’s several functions depend on the glomerular filtration rate (GFR). GFR is considered in clinical practice as a unit of measurement for renal function[10].

1.1.3 Glomerular filtration rate
The rate of filtration in the glomerulus for a healthy adult is approximately 140 mL/min/1.73m². The rate is low at birth but approaches adult levels at the end of age two and is maintained at this level until the age of 40[11]. This rate of filtration, also known as glomerular filtration rate, is generally accepted as an index of renal function in clinical practice and is therefore an important marker for renal disease[12]. It describes the flow rate of filtered plasma through the kidney[12]. As previously mentioned, a healthy adult has a GFR of 140mL/min/1.73m². After the age of 40, GFR declines with about 8mL/min/1.73m² every 10 years. Population-based data estimation suggests that decline may even begin as early as the ending of the second decade in an adult’s life. The progressive structural and functional deterioration of the kidney that comes with aging is a cause of the progressive
decrease in GFR[11]. GFR levels below 60mL/min/1.73m² causes increase of prevalence of complications of chronic kidney disease. This represents a loss of half or more of an adult’s kidney function level. Further decline of GFR leads to more severe cases, as the definition of kidney failure is GFR less than 15mL/min/1.73m² accompanied by signs and symptoms of uremia[13]. Stages of chronic disease and GFR levels are displayed in table 1.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GFR (mL/min/1.73m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kidney damage with normal kidney function</td>
<td>&gt;90 ± kidney damage</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage with mild loss of kidney function</td>
<td>89-60 ± kidney damage</td>
</tr>
<tr>
<td>3</td>
<td>Moderate loss of kidney function</td>
<td>59-30 ± kidney damage</td>
</tr>
<tr>
<td>4</td>
<td>Severe loss of kidney function</td>
<td>29-15 ± kidney damage</td>
</tr>
<tr>
<td>5</td>
<td>Kidney failure</td>
<td>&lt;15 ± kidney damage</td>
</tr>
</tbody>
</table>

Age-associated loss of kidney function has been recognized for decades. It varies vastly amongst individuals and although it is considered as a natural process, decreased GFR in elderly is an independent predictor of adverse outcomes, such as cardiovascular disease and death. Age-associated loss of kidney function is just one of many other risk factors of chronic kidney disease[13]. Other risk factors are displayed in table 2:

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Definition</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Susceptibility</strong></td>
<td>Increase susceptibility to kidney damage</td>
<td>Older age, family history of CKD*, reduction in kidney mass, low birth weight.</td>
</tr>
<tr>
<td><strong>Initiation factors</strong></td>
<td>Directly initiate kidney damage</td>
<td>Diabetes, high blood pressure, autoimmune diseases, systemic infections, UTI*, urinary tract obstruction, drug toxicity.</td>
</tr>
<tr>
<td><strong>Progression factors</strong></td>
<td>Cause worsening kidney damage and faster decline in kidney function after initiation of kidney damage</td>
<td>Higher level of proteinuria, higher blood pressure, poor glycemic control in diabetes, smoking</td>
</tr>
<tr>
<td><strong>End-stage factors</strong></td>
<td>Increase morbidity and mortality in kidney failure</td>
<td>Lower dialysis dose, temporary vascular access, anemia, low serum albumin level, late referral</td>
</tr>
</tbody>
</table>

*CKD – Chronic kidney disease. *UTI – Urinary tract infection
Due to the adverse outcomes of decline in renal function mentioned earlier, assessing GFR is of great importance in clinical practice. GFR can either be estimated based on endogenous markers, using validated algorithms, or accurately measured by exogenous probes. Clearance techniques used to measure GFR could involve endogenous (creatinine, urea) or exogenous (inulin, iohexol, iothalamate) filtration markers. GFR estimations are, most often, sufficient in clinical practice but the accurately measured GFR is required in certain cases[14]. Renal transplant recipients, potential kidney donors or research studies use a more accurate assessment of renal function, thus requiring a formal measurement of GFR[10]. Infusion of an exogenous agent is required to measure GFR due to the non-existence of an ideal endogenous substance for GFR measurement.

Certain strict physiological characteristics are required for a substance in order for it to be useful as a marker or a reference method for GFR. These include production and plasma concentration of the marker being constant if GFR does not change, exclusive excretion by kidneys and mainly via filtration through the glomerulus, non-secretion nor absorption by renal tubules. The marker should also be easily measured in plasma and also urine and non-toxic[14]. It does not matter if the renal function markers are endogenous or exogenous, but for accurate assessment of GFR only exogenous markers are currently used.

1.1.4 eGFR

Acquiring the “true” GFR by measurement of clearance of a marker can be cumbersome and costly, thus making GFR estimations more attractive in clinical practice[15]. Estimated GFR is often good enough for monitoring changes in renal function in an individual patient over time. It also forms the basis for classification of chronic kidney disease.

Endogenous markers (Creatinine)

The most widely used endogenous method of reference is serum creatinine concentration. Creatinine is a breakdown product of creatinephosphate in muscle tissue, produced at relatively constant rate, depending on muscle mass. It is inexpensive and generally accessible to measure. Serum creatinine and urinary creatinine clearance are methods of estimating GFR with creatinine. Serum creatinine measurements are easy and fairly precise, making it an attractive alternative in clinical practice[16]. Creatinine levels in the blood increase with decreasing renal function and serum creatinine levels can be used to calculate GFR[17].
Urinary creatinine clearance is highly dependent on the accuracy of urine collection[16]. Urine is collected for 24 hours and creatinine levels measured in the urine determines the clearance of creatinine, which is supposed to be correlated to GFR. Secretion of creatinine by the renal tubules causes this method of measurement to overestimate GFR[18].

The result of using creatinine as a marker is estimation and not an accurate measure of GFR. Several factors such as sex, age, race, muscle mass and dietary protein intake contributes to the reduction of creatinine’s accuracy as an indicator of GFR[18]. Due to these influences, several equations have been formulated to correct the inaccuracy. The formulas have been developed using data from patients with chronic kidney disease[16].

An approximate amount of over 40 different prediction equations are available for determining GFR using creatinine as a marker but only three of them are commonly used. Two of the equations (displayed in figure 1) are Cockcroft-Gault and “Modification of Diet in Renal Disease”(MDRD) formulae[19, 20]. The third type is CKD-EPI, a set of new equations developed in recent years.

New equations have been developed in recent years. These equations are have been implemented into clinical practice as a general test for assessing GFR in adults. They have been developed for use with standardized serum creatinine assays, as has implementation of new equations developed for use with standardized cystatin c as “confirmatory tests” for decreased eGFR have been proposed in clinical practice. The most accurate GFR estimating equations in large diverse population in recent times are The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. The 2009 CKD-EPI creatinine equation was developed to improve on the 2006 MDRD Study equation due to its limitations. The variables included in both equations are the same but they differ in form and coefficients[21]. A comparison of the equations used to estimate GFR prove that the CKD-EPI equations are more accurate than the MDRD Study equation. The CKD-EPI is less biased in most subgroups defined by demographic and clinical characteristics and level of GFR[22, 23], meaning there is less of a systemic difference between eGFR and GFR.
2009 CKD-EPI CREATININE

eGFR = 141 * min(SCr/ κ,1)α * max(SCr/ κ,1)α * 0.993age*[1.018 if female] *[1.159 if African American]

=> If female κ =0.7, α =-0.329
=> If male κ = 0.9, α = -0.411

<table>
<thead>
<tr>
<th>Cockroft Gault:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine clearance(ml/min) = (\frac{140 – \text{age (years)} \times \text{bodyweight (kg)}}{0.815 \times \text{serum creatinine (µmol/L)}} * 0.85 \text{ (if female)})</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MDRD:</th>
</tr>
</thead>
<tbody>
<tr>
<td>(GFR = \frac{\text{ml}}{\text{min} \times 1.73m^2}) = 186 * Serum creatinin^{-1.203} * (0.742 if female) * (1.21 if african american)</td>
</tr>
</tbody>
</table>

Figure 1: Prediction equations based on plasma creatinine for estimating GFR
CKD-EPI, Cockroft Gault and MDRD are prediction equations for determining GFR using creatinine as a marker

1.1.5 mGFR

Exogenous markers

Exogenous markers are in certain cases required, due to the limited accuracy of the endogenous markers. Planning cancer chemotherapy with nephrotoxic agents, staging chronic kidney disease or preparations for renal replacement therapy are typical cases in which the utilization of a more definite measurement of GFR is necessary[24]. If the nonrenal clearance of a substance is negligible, the substance’s plasma clearance is directly correlated with renal clearance of said substance. A single-injection clearance technique can then be used to calculate GFR by monitoring the substance’s rate of disappearance from the plasma after the injection[25]. A continuous intravenous infusion is also a possibility in combination with urine collection and concentration measurements in both plasma and urine for calculation of clearance[26].

The gold standard of mGFR is inulin clearance. Inulin possesses all the attributes required to be a marker of GFR measurement and is known as the ideal marker for determining GFR yet technical difficulties inherent in the measurement of inulin concentration in urine and in plasma creates limitation in its utility in clinical practice. Alternatives to inulin as an exogenous marker are radioactive agents such as \(^{51}\text{Cr-EDTA}, \(^{99m}\text{Tc-DTPA}, \text{and }^{125}\text{I or}
Iothalamte. However, there are problems with each of these agents and none of them are regarded as adequate replacements for the standard inulin clearance. Another alternative that has proved to be a satisfactory marker of GFR both in adults and children is Iohexol.

1.1.6 Iohexol

![Iohexol molecular structure](image)

**Iohexol Properties**

Iohexol is a nonionic contrast medium[28]. It has a molecular weight of 821 Dalton and is used intravenously for radiologic procedures. Iohexol is neither secreted, metabolized nor reabsorbed by the kidney. It diffuses into the extracellular space and it binds less than 2% to plasma proteins. Iohexol is eliminated exclusively via filtration in the kidneys. There are two isomers of iohexol and they both, are handled similarly by the body. Radiolabeled iohexol is a possibility of measurement but less attractive than measurements of serum concentrations with HPLC-UV[24].

**Single-injection Approach**

Iohexol-determined GFR is based on a single injection of Omnipaque®, the brand name of iohexol, and the analyses of blood samples from the patient. According to protocol utilized at OUS-Rikshospitalet, Omnipaque®[29] is injected using a venflon or a threeway-split butterfly syringe. Due to accuracy of the procedure, the whole volume has to be injected and the exact injection volume recorded. For Omnipaque 300mgI/ml, the recommended dosage is 2 mL for children below the age of two and 5 mL for all patients above the age of two. The exact injection volume is achieved by weighing the syringe before and after the injection. The patient’s weight and height are also required for algorithm after the sample analyses[29].
Plasma clearance and sample analyses

Duration of plasma disappearance and number of blood sample collections are important decisions made when using iohexol as a marker[30]. The algorithm used for measurement can calculate the patients GFR using either one time-specific blood sample or two time-specific blood samples. They are respectively referred to as One-point or Two-point measurements. Two-point measurements give more accurate calculations of GFR and are highly recommended when children are involved[29]. Schwartz et al [24] discovered that two sample measurement of iohexol disappearance was just as adequate as a four sample and a nine sample measurement. When using two-point measurement, the first sample cannot be taken earlier than 120 min after injection and the last sample should not be obtained earlier than 300 min after injection. These are sampling times that describe the elimination of iohexol. Timing of the second and last sample depends on the individuals renal function[24]. The recommended time periods for blood sampling in relation to individuals estimated GFR according to the protocol at OUS-Rikshospitalet are displayed in the table 3.

Table 3: Protocol for sampling times at OUS-Rikshospitalet

<table>
<thead>
<tr>
<th></th>
<th>GFR &gt;40</th>
<th>GFR 10-40</th>
<th>GFR &gt;10</th>
</tr>
</thead>
<tbody>
<tr>
<td>One-point</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>measurement</td>
<td>3 hours</td>
<td>8 hours</td>
<td>24 hours</td>
</tr>
<tr>
<td></td>
<td>after injection</td>
<td>after injection</td>
<td>after injection</td>
</tr>
<tr>
<td>Two-point</td>
<td>2 and 5 hours</td>
<td>2 and 8 hours</td>
<td>2 and 24 hours</td>
</tr>
<tr>
<td>measurement</td>
<td>after injection</td>
<td>after injection</td>
<td>after injection</td>
</tr>
</tbody>
</table>

The time of blood sampling does not have to be exact. A leeway of 15 minutes is accepted, given that the exact time of injection and blood sampling is recorded. The blood is collected and sent for centrifugation. A minimum of 0.6 mL blood (0.3 mL serum) is required. The samples are then analyzed using HPLC-UV (liquid chromatography)[29].

Clinical algorithm

The iohexol concentrations achieved from the HPLC analyses are used in an algorithm to calculate a patient’s true GFR. The GFR is based on calculation from the dose of the iohexol divided by area under the plasma disappearance curve (AUC$_0^{-\infty}$), displayed in equation (1), fitted by a double exponential equation.

$$\text{Clearance} = \frac{\text{Dose}}{\text{AUC}_{0^{-\infty}}}$$
The total AUC\textsubscript{0-inf} of iohexol can be divided into two mono exponential decay curves by the residual method. The first curve describes distribution of iohexol and second curve describes elimination. To approximately determine both curves, at least 4 blood samples between 5 minutes and 5 hours after injection are needed. These time periods describe both distribution and elimination of iohexol relatively accurate. Concentrations achieved from the two blood samples are used to determine the AUC by determining the elimination curve. The distribution curve then has to be estimated to be able to determine the whole AUC[24]. Using this technique, the GFR for patients with expanded body spaces are overestimated. This includes patients with oedema or ascites and this technique may be inadequate. Most patients within this groups are highly dependent on accurate GFR measurement due to accuracy in chemotherapeutic regimes[31].

The clinical algorithm combines the patient’s anthropometry (height, weight, sex) with amount and concentration of iohexol given and time of blood sampling to determine the elimination phase. The distribution phase is then estimated based on previous population values and initial estimates of GFR. The result is the patient’s GFR in ml/min/1.73m\textsuperscript{2}. That value is then adjusted based on the patient’s body surface area. The result is divided by 1.73m\textsuperscript{2}, resulting in the patients GFR[24].

1.2 Population modeling

1.2.1 Population Pharmacokinetics

Population pharmacokinetics is the study of the sources and correlates of variety in drug concentrations among individuals who are the target patient population receiving clinically relevant doses of a drug of interest[32]. The aim is to identify and quantify sources of the variability. Associations between patient characteristics and differences in pharmacokinetics can then be used to customize individual prediction of pharmacokinetic parameters[1]. One of the major differences between population pharmacokinetics and the traditional form of non-compartmental analyses of pharmacokinetic study is the samples needed for analyses. Traditionally, healthy volunteers were required and multiple samples were taken at fixed intervals. In contrast, patients involved in population pharmacokinetic studies are patients taking different doses of the drug investigated and blood samples are taking at different times. The use of population pharmacokinetics has vastly increased in the field of drug
development, especially in situations with suspicion of highly variable pharmacokinetics of a drug between subgroups of the population[1].

1.2.2 Population Pharmacokinetics models
Pharmacokinetic modeling is a mathematical method for predicting how the body will handle a drug[1]. It is a concept that involves an estimation of an unknown population distribution based on data from a collection of non-linear models[33]. The primary goal is finding population pharmacokinetic parameters and sources of variability in a population. Observed concentrations relations to administered doses by identifying predictive covariates in a target population are another goal when using population models. The need for many observations per subject, also referred to as “rich” data, is not as highly required when using a population model as it is in analysis of single-subject data. Structured sampling time schedules are not a requirement either. Few observations per subject, also referred to as “sparse” data, or a combination of “sparse” and “rich” data can be used when working with a population pharmacokinetics model[34].

Three important components of population modeling are structural, stochastic and covariate models. The structural models describe the time course of a measured response. It is normally represented as algebraic or differential equations. The stochastic model describes variability or random effects in the observed data. Other factors may influence individual time course of a response. These factors can be the patient’s demographics or a disease and they are described with covariate models[34, 35].

Estimating a typical value, such as drug clearance or oral bioavailability, is normally the point of interest in population pharmacokinetics. Each patient lends information to the population model but then borrows information back from the population model to obtain an estimate of their own pharmacokinetic parameters. This means the individuality of the information supplied by each patient to the population is used to estimate the most likely value of the parameter for each patient. This typical parameter value is usually the most frequent occurring value (the mode) and approaches the population mean value as the number of patients increase. Pharmacometrics can be used to improve our understanding of mechanisms, inform the initial selection of doses to test or personalize dosage for subpopulations of patients and evaluate the appropriateness of the study designs[35].
1.2.3 Pmetrics

Pharmacometrics is the incorporation of pharmacokinetics and pharmacodynamics models and simulations[36]. Pmetrics is a pharmacometric library package for R, a statistical and programming software. The Pmetrics package is for simulation and parameter estimation in linear and non-linear pharmacokinetic/pharmacodynamics systems.

Installation of three components is required when taking use of the Pmetrics package. The R software, Pmetrics package for R and gfortran, a fortran complier. There are three main software programs that Pmetrics control: IT2B, NPAG and a semi-parametric Monte Carlo simulation software program. The software program used in this study is NPAG[36]

**NPAG: Non-parametric adaptive grid**

This software creates non-parametric population models. The models consist of discrete support points, each with a set of estimates, and an associated probability of that set, for all parameters in the model. Each subject in the study population represents, at most, one support point[36].

The values of the model parameters in the population, such as clearance, are random effects. NPAG has no need to make any assumptions about the underlying distribution of random effects. The fixed effect is the error model. This consists of a polynomial that describes assay variance. It also consists of a multiplier of assay variance or an addend to assay variance, respectively referred to as gamma and lambda. They are each estimated as a single value in the population[36]

**Akaike Information Criterion (AIC)**

AIC is an estimate of a measure of fit of a model. The model and the model’s parameters define it, which then gives the minimum of AIC. AIC estimates describe how structured a model is compared to other models[37].
1.3 Aim of the study

The aim of the study is to create a population pharmacokinetic model for iohexol that can be used to determine glomerular filtration rate.

The specific goals for the model are:

- Defining how few sampling times that accurately will determine GFR
- Defining optimal sampling times for measuring GFR in renal transplant recipients.
2 Material and methods

2.1 The Patients

13 renal transplant recipients from OUS-Rikshospitalet were included in this study. An in-depth investigation, including the measured GFR is performed in all renal transplant recipients 8 weeks and 1 year after transplantation. The current method for measuring GFR in these patients is a two-point iohexol method where blood sampling is performed at two and five hours after iohexol injection if eGFR is >40mL/min, 2 and 8 hours if eGFR is between 40 and 15 mL/min and 2 and 24 hours if eGFR is <15mL/min. For this study, patients meeting at the Laboratory for renal physiology for a measured GFR investigation were asked to donate up to ten additional blood samples following iohexol administration. In patients with an eGFR >40ml/min sampling for at least 5 hours were requested and for those with eGFR > an additional 3 samples were warranted between 5 and 24 hours.

2.2 Iohexol analyses

A high performance liquid chromatography (HPLC) system is used to determine iohexol. An isocratic mobile phase with a pH value of 2.5 is prepared using:
- 50mL acetonitrile (C$_2$H$_3$N)
- 950mL deionized water
- 1mL ortho-phosphoric acid 88%

The specimen is mixed with 800uL of perchloric acid 5% and vortex mixed for 30 seconds. It is then centrifuged at 10900 rpm for 6 minutes. The supernatant is placed into an autosampler vial and a 50uL of the specimen volume is injected onto the column[38].

2.2.1 Calibration

Calibration of the method is performed using the same iohexol used for clearance estimation. The calibrators consist of omnipaque solution and pure serum/plasma. An omnipaque solution with a concentration of 1.8mg/mL is made and mixed with the serum/plasma[39]. The calibrator concentrations are presented in table 4.
Table 4: Calibrator concentrations[39]

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Concentration</th>
<th>Total volume</th>
<th>Omnopaque solution</th>
<th>Serum/plasma volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator_10</td>
<td>10ug/mL</td>
<td>10mL</td>
<td>56uL</td>
<td>9.944mL</td>
</tr>
<tr>
<td>Calibrator_50</td>
<td>50ug/mL</td>
<td>10mL</td>
<td>278uL</td>
<td>9.722mL</td>
</tr>
<tr>
<td>Calibrator_100</td>
<td>100ug/mL</td>
<td>10mL</td>
<td>556uL</td>
<td>9.444mL</td>
</tr>
</tbody>
</table>

The calibrators are mixed and distributed to Nunc-cylinders. 500uL are collected to each cylinder and frozen down at -20 degrees[39].

2.2.2 Analyses

The calibrator chromatogram determines iohexol retention time. Iohexol consists of 2 chromatogram peaks and the second peak is used to determine GFR. 3 calibrators are analyzed for each series of serum/plasma analyzed. The results of the iohexol analyses are documented (mg iodine/mL) in an excel sheet, together with patient’s demographics, dosage and blood sampling times[39].

2.3 Iohexol population model development

The software used for pharmacokinetics analyses in this study is Pmetrics version 1.3.1. The software requires two forms of data input for the analyses, an input file and a model file.

Input file

The input file is an excel spreadsheet format with data needed to describe the population. The order, capitalization and names of the header of the first 12 columns are fixed. The first 12 columns have to be “Id, evid, time, dur, dose, addl, ii, input, out, outeq, c0, c1, c2 and c3”. Column descriptions are displayed in table 5

Table 5: Description of fixed columns in the input file.

<table>
<thead>
<tr>
<th>Columns</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID</td>
<td>A numeric character that identifies each individual</td>
</tr>
<tr>
<td>Evid</td>
<td>The event ID field. 0= observation, 1= Input(e.g dose) 4= reset</td>
</tr>
<tr>
<td>Time</td>
<td>Elapsed time in decimal hours since the first event.</td>
</tr>
<tr>
<td>Dur</td>
<td>Duration of an infusion, in hours. (0 for oral doses)</td>
</tr>
<tr>
<td>Dose</td>
<td>The dose amount.</td>
</tr>
<tr>
<td>Addl</td>
<td>Specifies the amount of additional doses.</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>ii</td>
<td>Specifies the interdose interval</td>
</tr>
<tr>
<td>input</td>
<td>This defines which input (i.e drug) the dose corresponds to.</td>
</tr>
<tr>
<td>Out</td>
<td>This is the observation or output value (e.g drug concentration)</td>
</tr>
<tr>
<td>Outeq</td>
<td>The output equation that corresponds to the OUT value</td>
</tr>
<tr>
<td>C0,c1,c2,c3</td>
<td>These are coefficients for the assay error polynomial for that observation.</td>
</tr>
</tbody>
</table>

The next columns are the patient’s demographics and covariates used in the model file. There are no requirements to how the names and order of these columns have to be. Iohexol dosage is documented in the first row for each patient under the “dose” column. The value under the “time” column is “0” and the value under the “evid” and “input” columns is “1” indicating that the row represents dose input of drug nr.1 (Pmetrics can handle up to seven drugs simultaneously). The next rows after, for each patient, consist of the sampling times and the corresponding plasma iohexol concentration. The value under “evid” is “0” for observation and the value under “outeq” is “1” (for drug nr.1), indicating that the row represents an observation and not a dose input. The concentrations are documented under the “out” column. Covariate values are updated if relevant in each new row[36].

There are certain requirements to some of the columns in the input file. The time periods are documented in the input file as relative times (hours) after injection of iohexol. The doses in the input file are documented in milligrams. The concentration of omnipaque used for GFR measurement is 300mg iodine /mL. This is equivalent to 647mg iohexol/mL. The iohexol concentrations achieved from the HPLC analyses are converted (iodine:iohexol ratio =2.16) and documented under the “out” column in mg/L iohexol. The documentation of the patient’s sex in the input file has to be numeric for recognition in the R software. The number “1” is for males and “0” for females. The values in the weight and height columns are documented in kilograms and meters.

The values can be either documented in the excel file and imported to the R software for modification or they can be both documented and modified in excel before import to the R software[36].
Model file

The model file is a text file with up to 11 blocks, each marked by “#” followed by header tags. The model file is the data input containing the primary variables (with boundaries defined) and covariate equations for the model. The blocks used in this study are #PRImary variables, #COVariates, #SECondary variables, #DIFferential equations, #OUTput and #ERRor. The primary variables are the parameters that are estimated by Pmetrics. The secondary variables are defined by equations that are combinations of primary variables and covariates. In the model file, clearance (CL), volume of distribution (V), peripheral volume of distribution (Vp) and intercompartmental clearance (Q) were set up as primary variables. Different secondary variables and covariates were applied in the model file[36].

Different types of compartment models had to be tested to find the best structural model for the distribution and elimination of iohexol. Two and three compartment models were tested and compared. Two-compartment models were coded algebraically while three-compartment models were coded with differential equations, as algebraic equations cannot handle more than two compartments. Extracts of the model files are displayed in figure (3) and (4).

Two-compartment model:

<table>
<thead>
<tr>
<th>#PRI</th>
<th>#SEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL0</td>
<td>VTC= VT/72</td>
</tr>
<tr>
<td>V0</td>
<td>CL = CL0*VTC**0.75</td>
</tr>
<tr>
<td>Vp0</td>
<td>V = V0*VTC</td>
</tr>
<tr>
<td>Q0</td>
<td>Vp = Vp0</td>
</tr>
<tr>
<td></td>
<td>Q = Q0</td>
</tr>
<tr>
<td></td>
<td>Ke = CL/V</td>
</tr>
<tr>
<td></td>
<td>KCP = Q/V</td>
</tr>
<tr>
<td></td>
<td>KPC = Q/Vp</td>
</tr>
</tbody>
</table>

Three-compartment model

<table>
<thead>
<tr>
<th>#PRI</th>
<th>#SEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1S, 1,50</td>
<td>WTC=VT/72</td>
</tr>
<tr>
<td>K10, 1, 50</td>
<td>V1 = V1S*WTC</td>
</tr>
<tr>
<td>K12, 1, 30</td>
<td>V2 = V2S</td>
</tr>
<tr>
<td>K21, 1, 45</td>
<td>V3 = V3S</td>
</tr>
<tr>
<td>K23, 1, 60</td>
<td></td>
</tr>
</tbody>
</table>

XP(1)= RATEIV(1) + K21*X(2)-K12*X(1)- K10*X(1)
XP(2)= K12*X(1)-K21*X(2)+ K32*X(3)- K23*X(2)
XP(3)=K23*X(2)-K32*X(3)

Figure 3: Model file extract

Figure 4: Model file extract
The compartment model describes the distribution and elimination of a substance. Iohexol is injected into the central compartment, displayed as compartment 1. The substance is distributed to peripheral compartment (k12), displayed as compartment 2, and returns to the central compartment (k21). The substance is eliminated from the compartment (k10). A third compartment is added to the model to describe a substance’s prolonged time in a specific organ.

Population pharmacokinetic estimations

The Pmetrics function used to run NPAG algorithm was NPrun. Each NPAG run was run to convergence. A summary of the run is automatically launched in the default browser[36].

2.4 Covariates

Covariates are subject specific data, such as body weight, plasma creatinine etc., and they are listed in the input file in separate columns and the values updated according to changes in relation to the respective input event. They are included in the model file for use in the secondary variable equations. Both covariate and covariate-free models were tested and compared[36].

Covariate-free model

Two- and three-compartment models without covariates were tested. The covariate-free model describes the model without the adjustment of factors that may influence the primary parameters. Having a model without covariates makes it easier to identify the magnitude of
influence a specific factor has on perfecting the model’s ability to predict the observed values.

**Covariates**

Different covariates were tested in this study. The covariates tested were based on pharmacokinetic parameters with a theoretical potential to influence the pharmacokinetics of iohexol. Allometric scales were performed. Clearance parameters were multiplied with the centralized body size measures (Weight, height and BMI) to the power 0.75 and volumes to power of 1. Median covariate values for the population were used for centralization.

The covariates “age” and “plasma creatinine” were also tested. They were first multiplied with the primary parameters and later combined with the body size measures. The idea was to investigate if these covariates influenced the model on their own or influenced the model by enhancing the influence of body size measures.

All covariates models tested in this study were evaluated by comparing r-squared values, bias and imprecision. Changes in AIC was minimal and therefore eliminated as a unit of comparison. Further investigation was inhibited for covariate models that showed no improvement (covariate-free models as reference).

### 2.5 Calculating GFR

“makeNCA “ is a Pmetrics function used to calculate $AUC_{0-\text{inf}}$ for each individual in the input file, using the trapezoidal approximation. $AUC_{0-\text{inf}}$ was calculated with the observed values for each individual. Iohexol dosages for each patient were then divided by the patient’s corresponding AUC value, resulting in their clearance (L/h). Based on our assumption that clearance of iohexol is directly correlated to renal clearance, the patients GFR value (mL/min) is equal to the iohexol clearance. The value is simply converted from L/h to mL/min. equations are displayed as (2) and (3) The GFR values calculated from the observed values were used as reference.

\[
\text{(2): Clearance(L/h) = Dose/AUC_{0-\text{inf}}}
\]

\[
\text{(3): GFR(ml/min) = CL* (1000/60)}
\]
2.6 Clinical algorithm

An excel file, incorporated with the clinical algorithm was used to calculate GFR. Information required for the clinical algorithm was:

- Sex
- Child or adult
- Iohexol concentrations at 2 and 5 hours after administration (mg iodine/L)
- The specific time of iohexol administration (hours:minutes)
- The time periods for 2 and 5 hours concentrations (hours:minutes)
- Weight and height
- Volume of Ominipaque injected (mL)
- Omnipaque concentration (mg iodine/mL)

After plotting the values into the file, GFR was calculated by the algorithm, resulting in a value with the unit mL/min/1.73m². The value was adjusted with patient’s body surface area value, resulting in the patient’s GFR.

2.7 Two-point and three-point measurements

The MMopt function in Pmetrics was used to find the optimal sampling times for the model. A new input file was created with two test persons and standardized sampling times outlined. The covariates were different for the two test persons in order to get the MMopt function to estimate sampling times over a relevant range of potential patients. The demographics of the two representative test patients in the input file is displayed in table 6.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Weight</th>
<th>Height</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62</td>
<td>M</td>
<td>76.9</td>
<td>1.8</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>F</td>
<td>95.5</td>
<td>1.7</td>
<td>33</td>
</tr>
</tbody>
</table>

The MMopt() function was run to determine both two- and three optimal sampling times within the first 5 hours after iohexol administration. The sampling interval was based on intervals in the input file.
The sampling time closest to those determined from the MMopt function was used for patients which did not have a sample drawn at the exact time periods according to the MMopt results. The same was done for the three-point measurements test. The input files were imported in the R software together with the model file from the final model and simulations were run. New patient input files were created only including the measurements according to the MMopt results, i.e. 2 or 3 samples.

2.8 Comparing GFR-values

GFR values from the two- and three-point measurements were calculated with the same method as the reference. The GFR values calculated from the two- and three measurements were compared to the GFR values calculated from the clinical algorithm, using the observed values as reference.

A residual plot was used to display the comparison. GFR calculated from the clinical algorithm and from the two- and three-point measurements were subtracted from the reference and the difference was displayed in the residual plot.
3 Results

3.1 The Patients

The demographics of the patients included in the study are displayed in table 7.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Post tx [weeks]</th>
<th>Age [years]</th>
<th>Height [cm]</th>
<th>Weight [kg]</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>8</td>
<td>49</td>
<td>187</td>
<td>101</td>
<td>29.0</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>8</td>
<td>62</td>
<td>179</td>
<td>76.9</td>
<td>24.0</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>8</td>
<td>57</td>
<td>180</td>
<td>79.3</td>
<td>24.5</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>8</td>
<td>54</td>
<td>161</td>
<td>63.8</td>
<td>24.6</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>8</td>
<td>60</td>
<td>178</td>
<td>84.0</td>
<td>26.5</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>8</td>
<td>46</td>
<td>183</td>
<td>113</td>
<td>33.6</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>8</td>
<td>63</td>
<td>184</td>
<td>82.2</td>
<td>24.3</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>8</td>
<td>56</td>
<td>178</td>
<td>69.3</td>
<td>21.9</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>8</td>
<td>21</td>
<td>170</td>
<td>95.5</td>
<td>33.0</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>8</td>
<td>25</td>
<td>177</td>
<td>66.9</td>
<td>21.4</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>8</td>
<td>60</td>
<td>187</td>
<td>82.8</td>
<td>23.7</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>8</td>
<td>55</td>
<td>184</td>
<td>82.6</td>
<td>24.4</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>8</td>
<td>37</td>
<td>181</td>
<td>90</td>
<td>27.5</td>
</tr>
</tbody>
</table>

Mean: 49.6 179.2 83.6 26.0

SD: 13.8 7.13 13.82 3.82

*M = male  *F = female  *tx = transplantation  *BMI = body mass index  *SD = standard deviation
3.2 Structural model

The two-compartment model without covariates converged after 264 cycles. The three-compartment model without covariates converged after 1011 cycles.

Figure (6): Observation-prediction plot
The figure displays the observation-prediction plot for the two-compartment model. The plot describes the correlation between the observed and the predicted values, for both population and individual prediction.

Figure (7): Observation-prediction plot
The figure displays the observation-prediction plot for the three-compartment model. The plot describes the correlation between the observed and the predicted values, for both population and individual prediction.
The two-compartment model showed lower bias and imprecision values as seen in table (7)

Table (7): Values from run summaries

<table>
<thead>
<tr>
<th></th>
<th>Two-compartment</th>
<th>Three compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bias</td>
<td>Imprecision</td>
</tr>
<tr>
<td>Population</td>
<td>0.921</td>
<td>10</td>
</tr>
<tr>
<td>Individual</td>
<td>0.124</td>
<td>0.684</td>
</tr>
</tbody>
</table>

The table displays bias and imprecision values for both two-compartment and three compartment models. Lower bias and imprecision values describe a more structural model.

### 3.3 Covariate model

#### 3.3.1 Two compartment model

The three-compartment model showed no improvement with the allometric scaling. The implementation of covariates was then carried on with two-compartment models, which proved to have been positively influenced by the allometric scaling.

Table 8: Run summary from allometric scaling of the three-compartment model

<table>
<thead>
<tr>
<th></th>
<th>K12/WTc^0.25</th>
<th>K21/WTc^0.25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R-squared</td>
<td>Bias</td>
</tr>
<tr>
<td>Population</td>
<td>0.90</td>
<td>1.06</td>
</tr>
<tr>
<td>Individual</td>
<td>0.987</td>
<td>0.411</td>
</tr>
</tbody>
</table>
3.3.2 Allometric scaling

Although the difference between results of the allometric scaling was minimal body weight proved to be the best allometrically scaled model. Summary of the run is displayed in table 8.

Table 9: Values from run summary for the body weight allometrically scaled two-compartment model.

<table>
<thead>
<tr>
<th>Body weight</th>
<th>R-squared</th>
<th>Bias</th>
<th>Imprecision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>0.930</td>
<td>1.32</td>
<td>9.19</td>
</tr>
<tr>
<td>Individual</td>
<td>0.996</td>
<td>0.02</td>
<td>0.578</td>
</tr>
</tbody>
</table>

Centralized body size measures “height” and “BMI” showed promising results, as well. BMI showed better population prediction values than the body weight model. Values for the summary are displayed in table 10.

Table (10): Values from run summary for height and BMI

<table>
<thead>
<tr>
<th>Height</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-squared</td>
<td>Bias</td>
</tr>
<tr>
<td>Population</td>
<td>0.923</td>
</tr>
<tr>
<td>Individual</td>
<td>0.994</td>
</tr>
</tbody>
</table>

The “Age” covariate models showed no improvement compared to the covariate-free model. The R-squared values were lower, bias and imprecision values were higher and observation-prediction plot showed a lower correlation between observed and predicted values than the covariate-free model. The plasma creatinine model yielded no better compared to the covariate-free model either.
Figure 8: Observation-prediction plot
Run summary with age as a covariate. Population prediction shows low r-squared values and high bias and imprecision.

3.3.3 Assay Variance
The Lambda models proved to be slightly better than the gamma model based on r-squared, bias and imprecision values. The Lambda and gamma models were tested with the covariate-free model and final model.

Table(11): Run summary from Lambda and gamma models

<table>
<thead>
<tr>
<th></th>
<th>Lambda model</th>
<th></th>
<th>Gamma model</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R-squared</td>
<td>Bias</td>
<td>Imprecision</td>
<td>R-squared</td>
</tr>
<tr>
<td>Population</td>
<td>0.922</td>
<td>0.921</td>
<td>10</td>
<td>0.92</td>
</tr>
<tr>
<td>Individual</td>
<td>0.995</td>
<td>0.124</td>
<td>0.684</td>
<td>0.993</td>
</tr>
</tbody>
</table>
3.4 The final model

The final model was a two-compartment model that converged after 322 cycles with an AIC value of 830 and 13 support points. Clearance and intercompartmental clearance were multiplied with centralized body weight to the power of 0.75 while peripheral volume was multiplied with centralized body weight to the power of 1. The observation-prediction plot is displayed in figure 9.

Both input file and model file for the final model can be found in the appendix. The secondary variables in the final model were:
- CL=CL0*WTc**0.75
- V=V0
- Vp=Vp0*WTc
- Q=Q0*WTc**0.75

Predicted plasma concentration versus time plots for all 13 patients included, based on the final model, was designed using the R software. Based on both R-squared values from the observation-prediction plot and visual evaluation of the AUC-plots, this model was then used to find the optimal sampling times for Omnipaque. The plots are displayed in figure (10).
Figure (10): Plasma concentration vs time plot.
The figure display the AUC plots created with prediction values from the final model.
A robust model creates AUC plots with correlation to the observed values.
3.5 Two-point measurement vs Clinical algorithm

Two-point and three-point measurements were tested in this study. The optimal sampling times achieved from the MMopt function are displayed in table (12).

Table (12): Optimal sampling times based on...

<table>
<thead>
<tr>
<th>Two-point measurement</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Samples</strong></td>
<td><strong>Time (hours)</strong></td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Three-point measurements</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Samples</strong></td>
<td><strong>Time (hours)</strong></td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Sample 3</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

The MMopt function in Pmetrics recommends optimal sampling times. These sampling times are the most descriptive and an accurate AUC plot can be created using concentration at these times.

Using the sampling times provided, AUC for each patient was predicted based on two and three samples. The AUC values are displayed in table (13).

Table (13): AUC values

<table>
<thead>
<tr>
<th>ID</th>
<th>2-point measurement</th>
<th>3-point measurement</th>
<th>Trapezoidal method</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>910.7</td>
<td>1173.0</td>
<td>945.8</td>
</tr>
<tr>
<td>38</td>
<td>784.3</td>
<td>784.3</td>
<td>798.6</td>
</tr>
<tr>
<td>42</td>
<td>880.6</td>
<td>876.2</td>
<td>853.1</td>
</tr>
<tr>
<td>44</td>
<td>1455.9</td>
<td>1160.0</td>
<td>1158.5</td>
</tr>
<tr>
<td>66</td>
<td>848.2</td>
<td>884.1</td>
<td>868.1</td>
</tr>
<tr>
<td>70</td>
<td>1983.7</td>
<td>971.2</td>
<td>741.2</td>
</tr>
<tr>
<td>74</td>
<td>1141.8</td>
<td>719.2</td>
<td>687.9</td>
</tr>
<tr>
<td>76</td>
<td>651.0</td>
<td>588.0</td>
<td>607.0</td>
</tr>
<tr>
<td>81</td>
<td>619.0</td>
<td>556.6</td>
<td>561.7</td>
</tr>
<tr>
<td>84</td>
<td>954.5</td>
<td>902.5</td>
<td>972.3</td>
</tr>
<tr>
<td>93</td>
<td>919.7</td>
<td>1896.9</td>
<td>901.6</td>
</tr>
<tr>
<td>94</td>
<td>1212.2</td>
<td>1786.2</td>
<td>982.8</td>
</tr>
<tr>
<td>97</td>
<td>887.2</td>
<td>875.4</td>
<td>893.7</td>
</tr>
</tbody>
</table>

AUC-values calculated for both two-point and three-point measurements using predicted concentration values. The reference was calculated with trapezoidal method using observed concentration values.
GFR was then calculated using the AUC values. The GFR values are displayed in table (14).

<table>
<thead>
<tr>
<th>ID</th>
<th>Clinical algorithm</th>
<th>2-point Measurement</th>
<th>3-point Measurement</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>57</td>
<td>58</td>
<td>45</td>
<td>56</td>
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<tr>
<td>38</td>
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</tr>
<tr>
<td>42</td>
<td>57</td>
<td>61</td>
<td>61</td>
<td>63</td>
</tr>
<tr>
<td>44</td>
<td>44</td>
<td>37</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>66</td>
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</tr>
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<td>86</td>
<td>86</td>
<td>96</td>
<td>95</td>
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<tr>
<td>84</td>
<td>54</td>
<td>56</td>
<td>59</td>
<td>55</td>
</tr>
<tr>
<td>93</td>
<td>55</td>
<td>58</td>
<td>28</td>
<td>59</td>
</tr>
<tr>
<td>94</td>
<td>52</td>
<td>44</td>
<td>30</td>
<td>54</td>
</tr>
<tr>
<td>97</td>
<td>57</td>
<td>60</td>
<td>61</td>
<td>59</td>
</tr>
</tbody>
</table>

GFR values calculated from their respective AUC values. The clinical algorithm was used to calculate GFR for each individual in comparison to the model.

Comparison between GFR calculated with the clinical algorithm and the 2-point/3-point measurement was displayed by using a residual plot. The final model was used as a blueprint due to the assumption that more sampling times yield a more accurate AUC calculation. GFR from the 2-point/3-point measurements and clinical algorithm are subtracted from GFR calculated from the final model. Comparison are displayed in figure (11).
Figure 11 shows the absolute difference between the trapezoidal-AUC derived GFR and the clinical 2-point algorithm GFR and the 2- and 3-sample model derived GFR, respectively. Values above 0 represent overprediction while values below 0 represent underprediction. Each number on the x-axis represents a patient.
4 Discussion

In this study, a population pharmacokinetics model was developed to determine glomerular filtration rate in renal transplant recipients by using iohexol as a marker. The current clinical practice is to determine measured GFR by using an algorithm that is based on calculation of the elimination constant in the distribution phase of iohexol plasma concentration versus time curve. One blood concentration two hours after administration is combined with a second measurement after 5 to 24 hours, depending on the estimated renal function of the patient, to determine elimination constant. The distribution phase and volume of distribution on the other only is estimated based on population means and the individual patient’s demographics.

Benz de Bretagne et al[40] designed a bayesian model with iohexol samples obtained during the elimination phase. A one-compartment model was the best model that described the data[40]. The final model that best described the population in this study was a two-compartment model with primary parameters allometrically scaled by centralized body weight. Based on this model, optimal sampling times for measuring GFR in renal transplant recipients were determined to be 30 minutes and 5 hours after iohexol administration for two-point measurement. For a three-point measurement, optimal sampling times were 30 minutes, 1.5 hours and 5 hours after iohexol administration.

4.1 The patients

13 renal transplant recipients were included in this study. All 13 renal transplant recipients were in the early post transplant phase 8 weeks after transplantation, and 2 of the 13 had an estimated GFR < 40 mL/min. This is in accordance with the general renal transplant recipient in Norway. When having an estimated GFR less than 40 mL/min the protocol for mGFR measurement using iohexol indicate that the last sample should be taken 8 hours after administration, which is cumbersome in a policlinic setting. The present iohexol population model indicate that also these patients will be able to get an accurate mGFR determination just using samples up to 5 hours after administration.
4.2 Evaluating the model

Between nine to twelve iohexol samples were obtained per individual in the period 5 minutes to 5 hours after administration of iohexol. The total of the samples were able to describe both the distribution and elimination phase of iohexol well in the population.

Evaluation of the models was based on bias, imprecision and r-square of the observation-prediction plots. Two- and three-compartment models were tested and compared. As expected, the two-compartment model proved to be the better model based on the available values. The two-compartment observation-prediction plot showed better correlation between predicated and observed values for both population and individual predictions. Implementation and investigation of the covariates was carried on with the two-compartment model due to lack of improvement in evaluation values for the three-compartment model.

The primary parameters were multiplied with centralized body size measures to the power of 0.75 for clearance parameters and to the power of 1 for the volume parameters. Minimal difference in r-squared was registered for the values with and without allometrical scaling. The difference was noticed in the bias and imprecision values. Bias and imprecision values were lower for the body size measure models with allometric scaling. The allometrically scaled model showed higher r-squared values for both the population and individual predictions than the model without covariates. The improved r-squared values compared to the non-scaled model indicate that the allometric scaling of the primary parameters improved the models ability to estimate both population and individual predicated concentrations. Population prediction bias was lower for the model without covariates but bias for individual prediction and imprecision was lower for the allometrically scaled model.
4.3 Covariates

Allometrically scaled height and BMI models showed improved bias and imprecision values compared to the model without covariates but allometrically scaled body weight showed a higher improvement in the evaluation values. Clearance, intercompartmental clearance and peripheral volume were allometrically scaled to centralized body weight in the final model.

Both “age” and “plasma creatinine” were investigated as potential covariates in the model. The covariate models tested, were developed by multiplying the primary parameters with the centralized covariates. Age did not show any relevant effect on the model while plasma creatinine showed a distinctive difference on the population observation-prediction plot. R-squared values as low as 0.5 were registered. As iohexol is fully dependent on renal elimination it was expected that plasma creatinine whole have such an effect on the population prediction in the iohexol model. The incorporation of plasma creatinine in the model reduced however its ability to estimate individual predictions. This is probably due to the fact that plasma creatinine is a worse marker of renal function than iohexol itself and including this covariate will introduce a new variability in the model. Since the aim of the development of the population model is to determine mGFR and it will never be an issue to utilize it without actually having iohexol concentration measurements available the model without covariates were chosen to be the most appropriate model for the present objective.

4.4 Validation

Measuring the performance of the model’s predictive abilities can test the validity of a population model. This is done by either testing the same dataset (internal validation) or testing on a different dataset of new patients. (external validation).

Internal validation

Internal validation in this study was performed through model evaluation during the development. Visual evaluation of the observation-prediction plot was the method used to internally validate the dataset. Normal methods of internal validation are jack knife validation, likelihood-based method and cross-validation analysis. None of these methods were used in this study due to the limited number of subjects included in the study so far.
External validation
The model was not externally validated due to the lack of an evaluation population. The number of patients included was not sufficient for both building the model and validating the model. Generally, a group of new patients are used to validate the model but due to time constraint and inclusion conditions, 13 patients were included and only used to build the model.

4.5 Comparing GFR values
The aim of the study was defining optimal sampling times for measuring GFR in renal transplant recipients. Two- and three-point sampling were tested and compared with both each other and the algorithm utilized in clinical practice. The algorithm utilized in clinical practice is an algorithm based on the calculations that measure the elimination phase of iohexol disappearance, meaning the distribution phase is only estimated. The model on the other hand estimated the most plausible full-PK curve for each individual with the utilization of two- or three-point measurements. This includes hence and individual estimation of both the distribution and elimination phase of individuals, potentially giving a more complete description of iohexol disappearance in each individual.

$AUC_{0\text{-}inf}$ was calculated with the trapezoidal method using the observed concentrations. This was used as reference. GFR calculated from the clinical algorithm showed a relatively even distribution of GFR values close to the reference values. Although the clinical algorithm showed a more even distribution than the two- and three-point measurements, the two- and three- point measurements achieved results closer to the reference values in the majority of the patients in this particular data set. In some cases however, the values were underpredicted massively. Although both two- and three-point measurements based on the final model show promising results, the massive underprediction is likely the result of lack of patients included, especially patients with an estimated GFR less than 40 ml/min and demographic variety. Underprediction as high as a difference of 45 ml/min was registered, indicating that the model does not fully describe the population. More individuals need to be included in the model before this method can be fully validated with regard’s to use in clinical practice.
4.6 Future perspectives

Based on the results of the two- and three-point measurements, it is clear that the model has the potential for future use in clinical practice. The sampling times utilized in this study seem to accurately determine GFR based on the reference calculated with the trapezoidal method. Due to the inclusion of few patients in the model, certain patients were massively underpredicted. This indicates that future work on building a better model will require the inclusion of more patients. Similarly, more patients are required in order to enable both external and internal validation of the model. This will ensure more insight into the strengths and weaknesses of the model.
5 Conclusion

Using Pmetrics, a population pharmacokinetics model was designed to determine GFR in renal transplant recipients. This was done in order to define optimal sampling times for determining GFR.

The final model that best described the population in this study was a two-compartment model with primary parameters allometrically scaled with centralized body weight. Future work on building a better model will require the inclusion of more patients, especially more data in the later post administration phase in patients with low renal function. More insight into the strengths and weaknesses of the model will require validation of the model, either internally or externally.

Preparations for renal replacement therapy require a more definite measurement of GFR. A good population pharmacokinetic model could prove to be an invaluable tool for accurately determining GFR, particularly in cases concerning patients with severe loss in renal function.
6 References


38. James, T.J., et al., *Validity of simplified protocols to estimate glomerular filtration rate using iohexol clearance*. (0004-5632 (Print)).

Benz-de Bretagne, I., et al., *New sampling strategy using a Bayesian approach to assess iohexol clearance in kidney transplant recipients.* (1536-3694 (Electronic)).
# 7 Appendix

## 7.1 Requisition for measured GFR with iohexol clearance

### ETIKETT

**Målt GFR ved joheksol-clearance**

<table>
<thead>
<tr>
<th>Kliniske opplysninger:</th>
<th>- evt. tilleggsinformasjon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kroppsvekt:</td>
<td>kg</td>
</tr>
<tr>
<td>Høyde:</td>
<td>cm</td>
</tr>
<tr>
<td>Vekt av sprøyte før inj. (med Omnipaque):</td>
<td>gram og etter injeksjon:</td>
</tr>
<tr>
<td>Injiseret volum avlest fra sprøyte:</td>
<td>ml</td>
</tr>
<tr>
<td>Tidspunkt for inj. av Omnipaque 300 mg J/ml, dato:</td>
<td>kl:</td>
</tr>
<tr>
<td>Tidspunkt for første blodprøve, nøyaktig kl:</td>
<td></td>
</tr>
<tr>
<td>Tidspunkt for andre blodprøve, nøyaktig kl:</td>
<td></td>
</tr>
<tr>
<td>Kryss av her hvis prøve 2 vil bli ettersendt:</td>
<td></td>
</tr>
<tr>
<td>Cirka klokkeslett når prøve 2 vil bli tatt kl:</td>
<td></td>
</tr>
</tbody>
</table>

**Avdeling for farmakologi, OUS-Rikshospitalet**

**Kontaktinformasjon:** Laboratoriet telefon 23071014.

For mer informasjon GFR målt ved joheksol, se [www.anx.no/GFR](http://www.anx.no/GFR) som også har kalkulator for utregningene samt denne relevansblanketten for nedlasting. Dette finnes også på OUS internett og internett under Avdeling for farmakologi.
7.3 The model file

```
#PRI
CL0,1,10
V0,0.1,20
Vp0,1,25
Q0,1,50

#COV
AGE
SEX
WT
HGT
BMI
KREATININ

#Sec
WTc=WT/72
BMIC=BMI/25
KREATININC=KREATININ/60
+IF (SEX.GT.0)KREATININC=KREATININ/45
CL=CL0*WTc**0.75
V=V0
Vp=Vp0*WTc
Q=Q0*WTc**0.75
Kec=CL/V
KCP=Q/V
KPC=Q/Vp

#Out
Y(1)=X(1)/V

#ERR
L=1
0.1523073,0.01747435,-0.000003919581,0
```