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CLINICAL RESEARCH

Measured GFR by Utilizing Population Pharmacokinetic Methods to Determine Iohexol Clearance

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Introduction: There is an increasing demand for accurately measured glomerular filtration rate (GFR). Iohexol serum clearance has become a new gold standard, but it is challenging when GFR is low and 24-hour sampling is required for accurate results. The primary aim of this study was to develop an iohexol pharmacokinetic population model for accurate determination of individual GFR using limited sampling for up to 5 hours also when renal function is <40 ml/min.

Methods: A nonparametric iohexol population pharmacokinetic model was developed with rich data from 176 patients. In a validation cohort of 43 patients, a model-determined GFR (iohexol clearance) using different limited sampling strategies for up to 5 hours was compared with the strategy currently used in routine care, a log-linear 2-point method. In all, 1526 iohexol concentrations were used, from patients ranging in age from 1 to 82 years and GFR from 14 to 149 ml/min.

Results: The clinical 2-point method showed insufficient agreement compared with reference values; 15% of GFR values had an error of greater than ±10% even when sampling for 24 hours when estimating GFR <40 ml/min per 1.73 m^2 (standard procedure). Restricted sampling the first 5 hours with the population model required 4 samples to determine GFR accurately. This strategy showed excellent agreement with the reference; <3% of GFR values had an error greater than ±10 %.

Conclusion: Using an iohexol population pharmacokinetic model allows for accurate determination of GFR within 5 hours when applying 4 optimally timed samples, even in patients with GFR <40 ml/min.

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Serum creatinine–based algorithms may often be sufficient,[1–3] but there is a clinical need of accurate measures of renal function in many situations: for example, diagnostics, follow-up of kidney disease, and optimal drug dosing. Inulin clearance has been the gold standard measure of glomerular filtration rate (GFR).[4] Inulin clearance, however, is seldom used nowadays, and iohexol is considered by many to be *the new gold standard for GFR*.[5–8]

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Because the kidney is by far the paramount elimination route for iohexol, determination of GFR is based on calculating iohexol serum clearance (CL), that is, the dose given divided by the area under the serum concentration versus time curve from zero to infinity ($AUC_{0\text{-inf}}$). To properly calculate $AUC_{0\text{-inf}}$, multiple serum concentrations are needed, timely taken to include both the initial distribution and the terminal elimination phase. This is difficult to obtain in a clinical setting, and mathematical algorithms, generally including 1 or 2 iohexol concentrations in the elimination phase, are therefore commonly used. These algorithms, however, only indirectly estimate individual differences in the distribution phase.[9,10] In patients with good renal function, sampling at 2 and 5 hours determine GFR adequately. However, in patients with

low renal function, that is, <40 ml/min, the second sample needs to be delayed for up to 8 hours to avoid clinically important GFR deviations, or even up to 24 hours when renal function is even lower.[11–13] The need for such extremely prolonged sampling times with the currently used iohexol method is a serious restriction for its clinical application in patients with chronic kidney disease (CKD) stage 3b or higher.

Nonparametric population modeling of pharmacokinetic data is a powerful method for describing individual pharmacokinetic parameters. It provides the means to store experience of drug pharmacokinetic behavior in a population and then use it as a Bayesian prior for a new patient.[14] The true power of this approach lies in the accurate area-under-the-curve (AUC) predictions even when combined with limited sampling strategies (LSS), for example, as shown for everolimus using 0, 1, and 3 hours.[15] This has proved useful for individualized dosing of drugs such as tacrolimus in transplant recipients.[16] The pharmacokinetic structural model can be parameterized to determine drug CL directly. Applying this methodology on iohexol would potentially provide a means to determine individual GFR values more accurately and with a more flexible sampling scheme than the current clinical method described above, representing a major advantage in clinical practice.

The primary objective of the present investigation is to develop a nonparametric iohexol pharmacokinetic population model for accurate determination of individual GFR. Special emphasis will be placed on establishing optimal sampling times that are clinically applicable and still provide accurate GFR determination, for both adult and pediatric patients, with normal and, importantly, also with reduced renal function.

METHODS

Patients

Data from pediatric and adult patients were used for development and validation of the iohexol population model. Both data from previously published studies (n = 134)[17,18] and prospectively collected data (n = 85) were used. Table 1 presents an overview of the demographic data of the patients. The prospective study was performed at Oslo University Hospital–Rikshospitalet in the period 2014 to 2017. Patients scheduled for an iohexol GFR investigation were asked to provide extra samples whenever clinically feasible among patients giving consent.

The study was performed according to the Declaration of Helsinki and Good Clinical Practice. The Regional Ethics committee of Health Region South-East in Norway approved the study (REK number: 2014/2180). The retrospective data were obtained under ethical approval by the respective relevant ethics committee as described in original papers.[17,18]

Iohexol Administration and Sampling

The investigations were started in the morning. Patients had been instructed to withhold food, caffeine, and medications from 22:00 the evening before. Before administering iohexol, a fasting sample for determination of standard clinical chemical parameters, e.g., plasma creatinine, was obtained.

A 5-ml iohexol solution (Omnipaque 300 mg I/ml, GE Healthcare AS, Oslo, Norway), corresponding to 3235 mg iohexol, was administered through an intravenous cannula in the antecubital vein and flushed with 10 ml saline. Children under 2 years of age received 2 ml iohexol solution. The exact dose of iohexol administered was determined by weighing the syringe before and after administration.

Blood samples were obtained at standard time points for clinical assessment of GFR, that is, after 2 and 5 hours (later with estimated glomerular filtration rate [eGFR] <40 ml/min) in all patients. Additional blood samples at other time points were obtained whenever feasible, without any strict sampling scheme. Exact dosing and sampling times were carefully noted. Blood samples were obtained using Vacutainer serum separation tubes (BD Diagnostics, Trondheim, Norway), which were left at room temperature between 30 and 60 minutes before 10 minutes of centrifugation at 2400 *g*. Serum samples were stored at −70 °C.

Table 1. Demographic data (mean ± SD) in the development and validation cohorts, divided into age bins

	Development cohort (n = 176)				Validation cohort (n=43)				Total (n = 219)
Characteristics	0–2 yr	2–21 yr	21–60 yr	>60 yr	0–2 yr	2–21 yr	21–60 yr	>60 yr	
Patients (n)	2	38	63	73	0	16	18	9	219
Age (yr)	1.5	12 ± 5	48 ± 9	69 ± 6	NA	15 ± 4	44 ± 14	66 ± 6	46 ± 23
Male sex (%)	0	47	81	89	NA	62	78	78	75
Weight (kg)	11.4	45.0 ± 24.8	78.9 ± 18.1	87.9 ± 15.4	NA	61.3 ± 20.3	79.7 ± 14.3	77.6 ± 14.1	74.1 ± 24.4
Height (cm)	80	143 ± 28	170 ± 8	170 ± 8	NA	155 ± 17	179 ± 13	176 ± 10	165 ± 20
BSA (m^2)	0.51	1.31 ± 0.47	1.90 ± 0.23	1.99 ± 0.18	NA	1.61 ± 0.34	1.98 ± 0.25	1.94 ± 0.22	1.80 ± 0.39
P-creatinine (μmol/l)	22	98 ± 95	276 ± 168	249 ± 94	NA	155 ± 129	127 ± 30	167 ± 81	208 ± 138

BSA, body surface area: NA, not applicable.

Bioanalytical Methods

Iohexol serum concentrations were analyzed with high-performance liquid chromatography (HPLC-UV) at the respective hospital laboratories, showing a coefficient of variation of <6%. The validated lower level of detection and quantification is 20 mg/L, and the linear range is validated between 20 and 1100 mg/L.

Plasma creatinine concentrations were measured by an enzymatic calorimetric method (reagents from Roche Diagnostics, Rotkreutz, Switzerland) isotope dilution mass spectrometry traceable at the respective laboratories. The coefficient of variation was ≤4%.

Population Pharmacokinetic Modeling

Patients with available iohexol concentrations both in the distribution (the first 1.5 hours) and elimination phase (n = 87) were randomly divided in a ratio of 1:1 into 2 cohorts, 1 cohort for developing the population model and 1 cohort for validation. Data from patients with concentrations available only in the elimination phase (2 hours and later, n = 132) were included in the development cohort.

Model Development

Information about dose and subsequent iohexol concentrations in the development cohort (Table 1, Figure 1) was applied in the nonparametric adaptive grid approach implemented in Pmetrics for R[19] for model development. Among the 44 of 176 patients with samples from the first 2 hours after dosing, the number of individuals with data at the different time points was 42 at 10 minutes, 38 at 20 minutes, 35 at 30 minutes, 9 at 45 minutes, 40 at 60 minutes, and 30 at 90 minutes. The most appropriate pharmacokinetic structural model was assessed by testing 1-, 2-, and 3-compartment models without covariates. The models were parameterized in terms of clearance (CL) and volume of distribution (V_d) and model selection was based on comparison of the relative root mean squared predictive error (RMSE, %), calculated from the relative predictive error (PE, %; [predicted concentration − observed concentration]/observed concentration) of all iohexol concentrations in the development dataset, in addition to linear regression slope and R^2 values of the observed versus predicted plots and individual serum concentration versus time plots. Model selection based on minimization of the Akaike information criterion (AIC) values were treated as less important in the selection of the best model. This was due to the significant influence by the population predictions on the AIC value, and the aim of the current analysis was focused primarily on the individual predictions. Various body size measures, namely, total body weight, height, body mass index (BMI), and fat-free mass[20,21] were centralized to population median values and tested for allometric scaling.[22] Because the model was developed as a clinical tool for assessing GFR, we also tested the effect of plasma creatinine as covariate on CL. Both the gamma (error = SD*γ) and lambda (error = $[SD^{2*}\lambda^2]^{0.5}$) error models were tested, where SD is the standard deviation of the analytical method; SD = 0.1523073 + 0.01747435*obs − 0.000003919581*obs^2, and obs is the observed iohexol concentration.

Model Validation

The final model from the development cohort was used as Bayesian prior and all iohexol dose and concentration data from the validation cohort were included in a single run, without cycling. The RMSE was applied as the main validation metric, in addition to individual imprecision and bias. Prediction-corrected visual predictive checks (pcVPC) were produced according to the method by Bergstrand *et al.*,[23] but were given limited

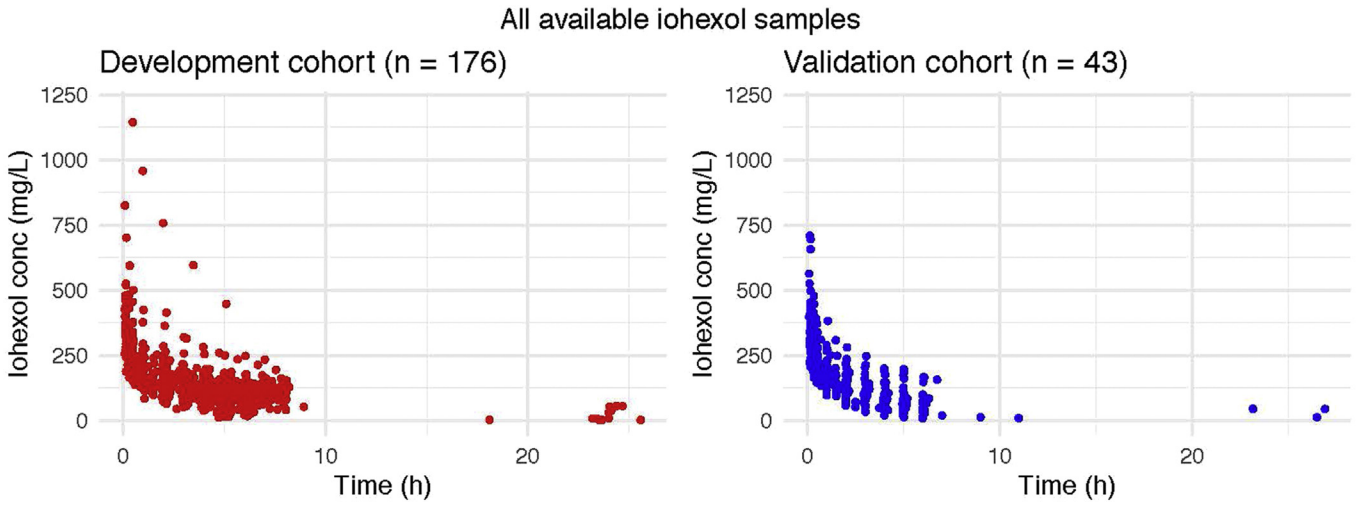

Figure 1. Iohexol serum concentrations (conc) used for development (176 patients, 1131 samples) and validation (43 patients, 395 samples) of the iohexol population model.

consideration during model validation because the objective was to develop a model for accurate individual predictions given both dose and measured concentrations for each individual, not for use in simulations or population predictions.

GFR Determinations

The validation cohort was used to evaluate individual GFR values obtained by the iohexol population model (GFR_{Model}) using different LSS and compared with the standard clinical 2-point log-linear approach (GFR_{Clin2}) for the estimation of the reference GFR (GFR_{ref}). Throughout the present paper, absolute GFR values are reported as ml/min and not as ml/min normalized to 1.73 m^2.

Reference GFR

The individual GFR_{ref} used to assess these methods is usually calculated as follows: dose of iohexol divided by $AUC_{0\text{-inf}}$, where $AUC_{0\text{-inf}}$ is approximated using the trapezoidal rule. Contrary to this method, we used the current validated population model to calculate iohexol clearance for each individual, using the final model as Bayesian prior and including all measured iohexol concentrations in the validation cohort, median 9 concentrations (range 5−11) per patient, for individual predictions allowing the model to cycle until it converged.[19] The GFR for a given individual i (GFR_i) is equal to that patient's iohexol clearance (CL_i) and calculated as follows: $GFR_i = CL_i = CLs_i * (WT_i/85)^{0.75}$ (ml/min), where CLs_i (ml/min) is the individual parameter estimate of clearance using the population model as Bayesian prior on the actual dose and measured iohexol concentrations for patient i and WT_i is the total body weight (in kilograms [kg]) of patient i. The number 85 is the anticipated median body weight (in kilograms) of the population used for centralizing the WT scaling in the model.

Clinical 2-Point GFR

The standard 2-point algorithm used for measured GFR determination (GFR_{Clin2}) was calculated according to the 1-pool clearance method previously described.[24] In short, $CL_1 = dose\ iohexol/(c_1/b_1)$, where c_1 is the y-intercept and b_1 is the slope of the log-linear regression line for the 2 measured iohexol concentrations. Furthermore, $GFR_{Clin2} = CL_1/(1-f \times CL_1)$, where $f = 0.0032 \times BSA^{-1.3}$. The first sample was obtained 2 hours after dosing, whereas the second sample was dependent on anticipated renal function; eGFR > 40 ml/min per 1.73 m^2, 5 hours; eGFR <40 ml/min per 1.73 m^2, 8 hours; and eGFR < 30 ml/min per 1.73 m^2, 24 hours.

Model GFR (GFR_{Model}) by LSS

The final iohexol population model was applied on the validation cohort using different LSS combinations. The LSS tested included 1, 2, 3, and 4 samples restricted to either the first 3 hours or the first 5 hours after dosing (e.g., $GFR_{Model,5h,4s}$). The individual GFR was assessed as outlined above for GFR_{ref}, including only the samples specified by the LSS tested, for example, including only iohexol concentrations at 30 minutes, 2 hours, and 5 hours in a run testing an LSS of 3 samples within the first 5 hours. Patients with missing data at a specific time tested were excluded from that measure.

Statistical Analysis

The agreement between the GFR values obtained with the reference method (GFR_{ref}), the standard clinical method (GFR_{Clin2}), and the novel model-determined GFR (GFR_{Model}) was assessed by the concordance correlation coefficient (CCC), total deviation index (TDI), coverage probability (CP), and proportion within ≤10% relative bias (P10).[25] The CCC varies from 0 to 1, and a CCC >0.90 reflects optimal concordance between measurements. The TDI captures a large proportion of data within a boundary for allowed differences between 2 measurements.[25] The CP varies from 0 to 1, and is a statistic that estimates whether a given TDI is less than a prespecified fixed percentage. An empirical TDI was calculated for a theoretical TDI of 10% and a CP of 90%. We defined that acceptable agreement between the reference and the evaluated GFR methods should be a TDI <10%. P10 values between the methods were compared using the McNemar χ^2 test using the *stats* package in R.[26] Agreement between the reference method and GFR_{Clin2} and GFR_{Model}, respectively, was also visualized by relative and absolute difference plots. The statistical package AGP (Agreement Program) v.1.0 (IGEKO, SP; http://ecihucan.es/lfr/apps/?dir=agreement_installer [last accessed February 15, 2019]) was used.

RESULTS

Study Population

In total, 219 patients, mainly Caucasian, aged from 1 to 82 years, and 1526 iohexol concentrations were included. Demographic data of the development and validation cohort, divided in age bins, are shown in Table 1. The development cohort included 1131 iohexol concentrations, with a median of 7 (range 2−12) per patient, and the validation cohort included 395 concentrations, with a median of 9 (range 5−11) per patient (Figure 1).

Iohexol Population Model

Model Development

The 1-compartment model showed an individual RMSE of 17.3% and was discharged. Both the 2- and 3-compartment models (without covariates) described

Table 2. Pharmacokinetic parameter values[a] (weighted by support point probability) for the final iohexol model (n = 176)

Parameters	Mean	Median	95% CI	Shrinkage (%)
CLs (L/h)	2.60	1.55	2.22–2.97	0.8
Qs (L/h)	9.44	5.99	8.03–11.42	4.0
Vs (L)	10.96	10.47	10.25–11.68	4.1
Vps (L)	9.44	8.02	8.66–10.23	7.3

CI, confidence interval; CLs, clearance; Qs, intercompartment clearance; Vps, peripheral volume of distribution; Vs, central volume of distribution.
[a]Slope values are presented. To obtain individual values, CLs and Qs should be multiplied by (*actual patient weight* [*kg*]/85 [*kg*])$^{0.75}$ and Vs and Vps by (*actual patient weight* [*kg*]/85 [*kg*])$^{1.00}$. Shrinkage is obtained from the approximations integrated in Pmetrics package for R.

the data almost equally well. The individual RMSE for the 2-compartment model was 3.5% and for the 3-compartment model was 4.7%. The AIC values of the 2- and 3-compartment model were 6911 and 6776, respectively. The 2-compartment model was taken further in the development and was allometrically scaled. Total body weight (WT) scaling resulted in a somewhat lower individual RMSE (3.0%) compared to body surface area (BSA) scaling (3.3%). The AIC value was somewhat lower for the WT-scaled model (6858) as compared to the BSA-scaled model (6876). The WT was chosen as scaling factor in the further development of the model. This model converged after 3209 cycles with 151 support points and showed population and individual RMSE values of 52.4% and 3.0%, respectively (Supplementary Figure S1). The model showed an individual bias and imprecision of −0.0328 mg/L and 1.81 mg/L, respectively. The final γ value was 1.947.

Adding creatinine as a covariate on clearance; $CL = CLs \times WTc^{0.75} \times EXP(CLCRE \times CREAT)$, where *CLCRE* is the parameter for individually scaling the effect of plasma creatinine (*CREAT*) on *CL*, improved primarily the population estimates (AIC was reduced to 6805 and the population RMSE to 32%), but the individual predictions deteriorated. Individual RMSE increased severalfold to 20%. Considering the aim of using the model to determine individual iohexol clearance when concentrations are available, we chose the model with the lowest individual RMSE, namely, the model without creatinine. Accordingly, the parameterization of the final model was as follows: $CL = CLs \times WTc^{0.75}$ (*clearance*), $Q = Qs \times WTc^{0.75}$ (*intercompartment clearance*), $V = Vs \times WTc$ (*central* V_d), and $Vp = Vps \times WTc$ (*peripheral* V_d). Table 2 shows the population parameter estimates for the final model, and Figure 2 shows the population and individual predicted versus observed plots. Diagnostic plots of the pcVPC and weighted residual errors are shown in Supplementary Figures S2 and S3. The pcVPC show a slight overprediction of the upper level of the population predictions.

External Validation

The analyses indicate that the final model appropriately describes the pharmacokinetics of iohexol over the age and GFR range of 5 to 77 years and 14 to 149 ml/min. Comparing the data from the *external* patients (n = 43) with the 176 patients used to develop the model, there were only marginal differences in individual predicted

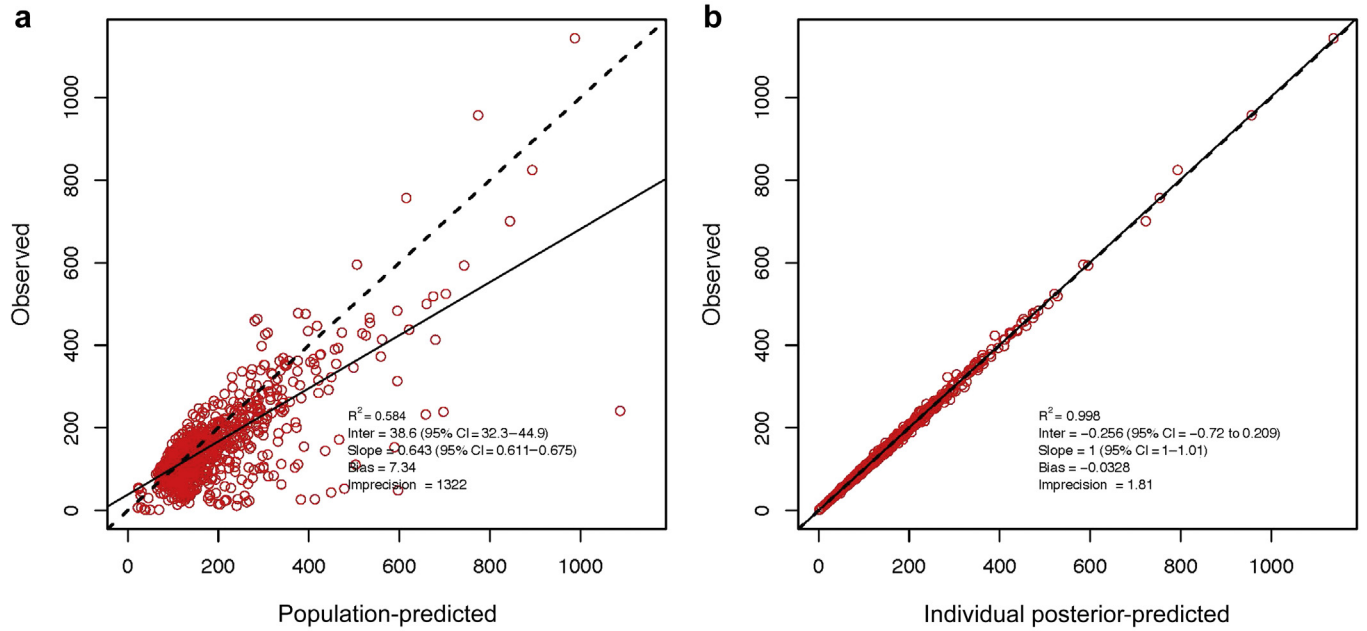

Figure 2. Observed (*y*-axis) versus predicted (*x*-axis) iohexol serum concentrations of the final 2-compartment model (n = 176). (a) Population-predicted. (b) Individually predicted plot. The model was allometrically scaled by total body weight with no further covariate. The model included 151 support points and showed a population and individual root mean squared error of 52% and 3%, respectively. CI, confidence interval; Inter, y-intercept.

Table 3. Absolute and relative predictive error (mean ± SD) and agreement C-statistics measures of the GFR predictions against the reference GFR performed in the validation cohort by the different methods investigated[a]

Variables	Absolute PE (ml/min)	Relative PE (%)	Absolute RMSE (ml/min)	Relative RMSE (%)	CCC	TDI	CP	P10	n
GFR_{Clin2}	–0.3 ± 3.8	–0.2 ± 7.1	2.4 ± 2.9	4.4 ± 5.6	0.994 (0.990)	11.4 (13.7)	84.5 (75.9)	91%	43
$GFR_{Model5h,4s}$	–0.5 ± 1.9	–0.9 ± 4.5	1.4 ± 1.5	2.8 ± 3.6	0.997 (0.995)	7.3 (8.7)	97.2 (92.6)	98%	42
$GFR_{Model3h,4s}$	–0.1 ± 6.9	–1.4 ± 18.0	4.8 ± 4.9[b]	11.0 ± 14.0[b]	0.951 (0.921)	35.5 (43.5)	386. (32.6)	65%[b]	43
$GFR_{Model5h,3s}$	–2.0 ± 5.7	–2.0 ± 5.7	3.0 ± 5.3	5.2 ± 6.8	0.987 (0.981)	14.8 (18.1)	73.2 (63.4)	86%	42

CCC, concordance correlation coefficient; CP, coverage probability; GFR, glomerular filtration rate; PE, predictive error (*predicted concentration – observed concentration*); RMSE, root mean squared error ($\sqrt{PE^2}$).
[a]Reference GFR was obtained by using all individual iohexol concentrations with the final population model as Bayesian prior. Predictions were done with limited sampling strategies according to the following: current clinical 2-point method (GFR_{Clin2}), final population model as Bayesian prior using 4-point, restricted to the first 5 hours ($GFR_{Mod5h,4s}$); using 4-point, restricted to the first 3 hours ($GFR_{Mod3h,4s}$); and using 3-point, restricted to the first 5 hours ($GFR_{Mod5h,3s}$). Values in parentheses are upper limits of 95% confidence intervals for TDI and lower limits of 95% confidence intervals for Accuracy, Precision, CCC, and CP. P10 is the proportion of GFR values obtained by the evaluated methods that are within 10% of GFR_{ref}. TDI allowance, 10; CP allowance, 0.9; α, 0.05.
[b]$P < 0.05$ vs. clinical 2-point method.

bias and imprecision (0.006 mg/l and 3.990 mg/l and −0.033 mg/l and 1.807 mg/l in the validation and development dataset, respectively). In the validation cohort, the RMSE was 6% ± 4%.

Glomerular Filtration Rate

The mean GFR_{ref} was 59 ± 29 ml/min, ranging from 14 to 149 ml/min, in the validation cohort. Table 3 show different measures of agreement between GFR_{ref} and the different methods tested, and Table 4 presents individual values. In general, the GFR values obtained using the clinical 2-point method demonstrated good agreement with GFR_{ref} over the whole range of age and renal function when including sampling later than 5 hours for patients with low renal function. The optimal sampling times using 4 samples within the first 5 hours in the population model were 10 minutes, 30 minutes, 2 hours, and 5 hours after dosing ($GFR_{Model,5h,4s}$). This approach showed better agreement with GFR_{ref} as compared to the clinical 2-point method (GFR_{Clin2}) for all patients, including patients with low renal function (Table 3). These results demonstrated that 90% of the $GFR_{Model,5h,4s}$ values showed an error ranging from −7.3% to +7.3% of GFR_{ref} and that less than 3% of the $GFR_{Model,5h,4s}$ values had an error greater than ±10% of GFR_{ref}.

Restricting the sampling times to the first 3 hours (10 minutes, 30 minutes, 2 hours, and 3 hours) showed suboptimal agreement with GFR_{ref} (Table 3). The population model also resulted in lower agreement when less than 4 samples per patient were used (3 samples first 5 hours; 10 minutes, 30 minutes, and 5 hours; Table 3).

Figure 3 shows the relative and absolute predictive error of the clinical 2-point method, $GFR_{Mod,5h,4s}$ and $GFR_{Mod,3h,4s}$ versus GFR_{ref}.

DISCUSSION

The main and novel finding in the present study is that using modern pharmacokinetic tools to determine individual iohexol clearance is applicable in a clinical setting. The current 2-point method used is based on log-linear estimations originally developed by Brøchner Mortensen some 50 years ago.[27] With the present model, we achieved accurate measures of GFR with sampling limited to 5 hours even in patients with low renal function (i.e., <40 ml/min). Taken together, the current results show excellent concordance, precision, and accuracy between the reference and GFR values derived from the model approach. To achieve comparable agreement using the current clinical 2-point method, sampling needs to be extended to 8 or even 24 hours in patients with low renal function.[12] Use of the novel population pharmacokinetic method is, first of all, an advantage compared to today's standard for patients with CKD 3b and beyond. To achieve this high level of performance, however, at least 4 samples need to be included, and they should cover both the distribution phase (<1.5 hours) as well as the elimination phase (>2 hours). This underlines the importance of obtaining actual individual information from both the distribution and elimination phase of iohexol by representative and timely iohexol measurements in order to accurately determine GFR. We believe that when a clinician considers an accurate GFR measurement indicated and an i.v. injection of iohexol is administered, 2 extra serum samples is a low price to pay to obtain accurate results on a single day and within normal laboratory opening hours.

An additional advantage of population pharmacokinetic methods, as compared with log-linear algorithms, is more flexibility with regard to sampling times. In comparison, as it is a prerequisite to sample only in the elimination phase (≥2 hours) when using the current clinical 2-point method, a first sample obtained already after 1.5 hours (in the distribution phase) will significantly bias the GFR determination. It should, however, be noted that the sampling time points cannot be completely random using the population model. Samples in both the distribution and elimination phase, as mentioned above, need to be included, and 2 consecutive

Table 4. Individual demographic data and measured GFR in the validation cohort using the clinical 2-point algorithm and 2 LSSs in the final iohexol population model

Patient no.	Age (yr)	Weight (kg)	GFR_{ref} (ml/min)[a]	No. available samples	GFR_{Clin2} (ml/min)[b]	$GFR_{Model5h,4s}$ (ml/min)[c]	$GFR_{Model3h,4s}$ (ml/min)[d]	Sampling times (min)																
								10	20	30	45	90	120	180	240	300	330	360	420	540	660	1380	1620
1	50	44	14	8	14[e]	14	18	X	X	X		X	X	X	X		X					X	
2	73	73	15	9	15[e]		26	X	X	X		X	X	X	X	X							X
3	18	47	16	9	14[e]	15	15	X	X	X		X		X	X	X	X	X					
4	61	64	17	11	16[e]	16	19	X	X	X		X	X	X	X	X	X	X	X				
5	14	73	17	10	17[e]	16	15	X	X	X		X		X	X	X	X	X					
6	12	38	32	10	31[e]	30	32	X	X	X		X		X	X	X	X	X					
7	14	45	33	10	31[e]	34	14	X	X	X		X		X	X	X	X	X					
8	77	66	34	10	37[e]	34	39	X	X	X		X	X	X	X	X	X	X					
9	64	90	37	11	47	45	44	X	X	X	X		X	X	X	X	X	X					X
10	12	49	40	9	39	40	39	X		X	X	X		X	X	X	X	X					
11	54	64	45	9	44	44	50	X	X	X		X	X	X	X	X	X	X					
12	67	79	45	9	45	44	46	X	X	X		X	X	X	X	X	X						
13	57	52	46	9	45	44	44	X	X	X		X	X	X	X	X	X						
14	13	52	49	9	59	50	53	X	X	X		X		X	X	X	X	X					
15	17	94	51	5	50	51	46			X				X	X	X	X						
16	14	48	51	10	51	50	54	X	X	X		X		X	X	X	X	X					
17	5	18	52	10	62	51	49	X	X	X		X		X	X	X	X	X					
18	20	66	52	10	49	52	52	X	X	X		X		X	X	X	X	X					
19	30	92	53	9	52	52	54	X	X	X		X	X	X	X	X	X						
20	55	83	54	9	52	51	46	X	X	X		X	X	X	X	X	X						
21	25	67	54	8	54	54	51	X	X	X	X		X	X	X	X	X						
22	60	83	54	9	55	54	70	X	X	X		X	X	X	X	X	X						
23	17	68	55	10	54	51	65	X	X	X		X		X	X	X	X	X					
24	37	90	57	9	57	58	63	X	X	X		X	X	X	X	X	X						
25	64	64	59	8	57	58	49	X	X			X	X	X	X	X	X						
26	57	79	60	8	57	58	60	X	X	X		X	X	X	X	X	X						
27	60	84	60	9	59	60	62	X	X	X		X	X	X	X	X	X						
28	56	82	61	8	59	60	56	X	X	X		X	X	X	X	X	X						
29	58	83	62	9	62	62	62	X	X			X	X	X	X	X	X	X					
30	61	77	66	9	64	65	61	X	X	X		X	X	X	X	X	X						
31	66	78	69	9	65	68	70	X	X	X		X	X	X	X	X	X						
32	21	63	69	10	67	66	67	X		X		X		X	X	X	X	X					
33	20	85	72	9	71	67	50	X			X	X		X	X	X	X	X					
34	27	95	75	9	75	74	69	X	X	X		X	X	X	X	X	X						
35	13	70	76	9	72	76	77	X	X	X		X		X	X	X	X	X					
36	40	81	79	9	81	78	76	X	X	X		X	X	X	X	X	X						
37	17	75	79	10	79	77	85	X	X	X		X		X	X	X	X	X					
38	21	96	91	9	86	91	95	X	X	X		X	X	X	X	X	X						
39	61	108	94	9	103	92	96	X	X		X	X	X	X	X	X	X						
40	47	83	102	10	102	106	103		X	X	X	X	X	X	X	X	X		X	X	X		
41	32	94	111	9	113	110	118	X	X	X		X	X	X	X	X	X						
42	11	89	136	10	132	136	138	X	X	X		X		X	X	X	X	X					
43	25	83	149	10	139	147	139	X	X	X		X		X	X	X	X	X					
Mean	38	72	59	9.2	59	60	59																
SD	22	19	29	1.0	28	29	29																
Min	5	18	14	5	14	14	14																
Max	77	108	149	11	149	147	139																

GFR, glomerular filtration rate; GFR_{Clin2}, standard clinical 2-point log-linear approach; GFR_{Model}, glomerular filtration values obtained by the iohexol population model; GFR_{ref}, estimation of the reference glomerular filtration rate; LSS, limited sampling strategy; max, maximum; min, minimum.
[a]Obtained using the final population model as Bayesian prior and including all available iohexol concentrations from the validation cohort.
[b]Obtained using the clinical 2-point method and 2- and 5-hour concentrations (later if eGFR $<$ 40 ml/min).
[c]Uses iohexol concentrations measured 10 minutes, 30 minutes, and 2 and 5 hours after dosing with the final model.
[d]Uses iohexol concentrations measured 10 minutes, 30 minutes, and 2 and 3 hours after dosing with the final model.
[e]Last sampling after 5 hours. The missing value in the $GFR_{Model5h,4s}$ is due to the missing 5-hour sample in that patient.

samples should probably be separated by at least 15 minutes. Within these limitations, this new method presented could be a valuable clinical asset, particularly in patients with CKD stage 3b and beyond. This would also include a substantial part of kidney transplant patients.

In the development of the present population model, data from patients ranging from 1 to 82 years

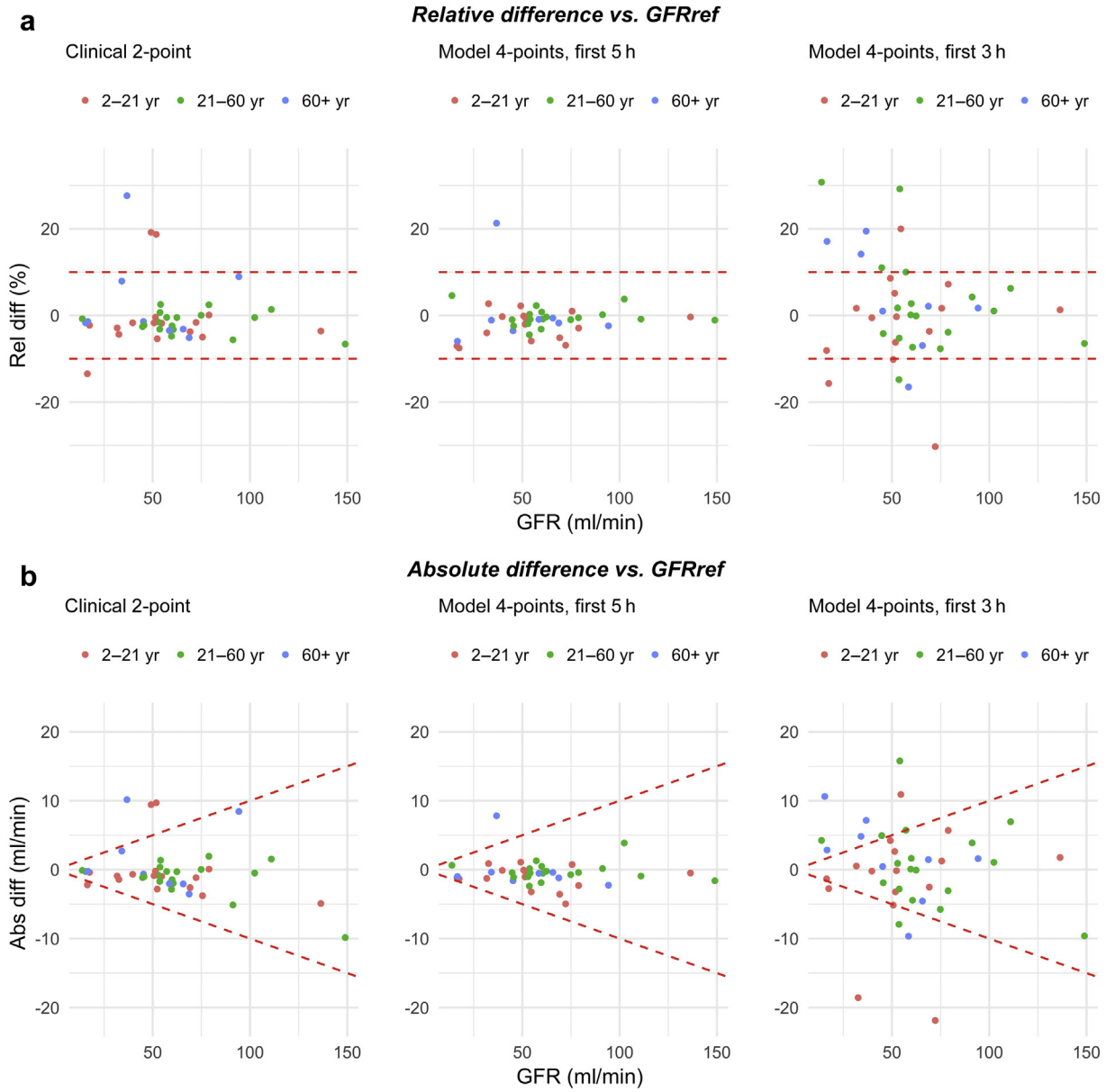

Figure 3. (a) Relative and (b) absolute predictive error (% and ml/min, respectively) on the *y*-axis and reference glomerular filtration rate (GFR) (ml/min) on the *x*-axis for GFR predictions in the validation cohort (n = 43). Reference GFR (GFRref) was obtained by using all individual iohexol concentrations with the final population model as Bayesian prior. Predictions were done with limited sampling strategies according to the current clinical 2-point method (GFR_{Clin2}) and the final population model as Bayesian prior using 4 points, restricted to the first 5 hours ($GFR_{Mod5h,4s}$), and using 4 points, restricted to the first 3 hours ($GFR_{Mod3h,4s}$). Age bins are marked in color. Abs diff, absolute difference; Rel diff, relative difference.

old were included. However, the model is only validated down to 5 years and further validation in younger individuals is needed. The estimated renal function distribution was somewhat different between the development and validation cohort, as indicated by the S-creatinine distribution (Table 1). Potentially this could affect the relevance of the model validation. However, as shown in Figure 3, there are no indications of any skewed agreement by degree of renal dysfunction.

The main strengths of the present analyses is that data from a large cohort of pediatric and adult patients were included and that the model was developed with data from 3 different countries on 2 different continents. A potential drawback with the model approach is that it is not as readily available for all laboratories to use as the well-established and simple clinical 2-point method. To overcome this hurdle, we have recently made the model available on the Web (http://folk.uio.no/anderas/iohexol.html), and relevant population model files can be requested from the corresponding author. Comparing the agreement between methods is a challenge when no true values are obtainable, as in the present study. The reference GFR used in these

analyses is obviously dependent on a good specification of the 2-compartment model. The validations performed, however, do not indicate any major model misspecification.

In conclusion, a pharmacokinetic population model for iohexol was developed for determination of individual measured GFR in pediatric and adult individuals. Using 4 samples drawn during 5 hours after iohexol dosing allows for at least as accurate GFR determination as the clinical 2-point method. In addition, the new method provides good GFR predictions even in individuals with low renal function, even without a final sample after 24 hours. The new method represents a novel approach for GFR measurement that is possible to perform in a single day (5 hours) in all patients, regardless of renal function.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Plot of the probability distribution of the support points of the parameters of the final 2-compartment model (see text for details of the model).

Figure S2. Prediction-corrected visual predictive check (pcVPC) plot of the final 2-compartment model was produced according to a previously described method.[23] Dose, total body weight, and sample times were used for binning in the process of prediction correction and each individual (n = 176) were used as template for 1000 simulations using the binned values by the simulator in Pmetrics. The prediction corrected median (solid red), 5th and 95th percentiles (dashed blue) of the measured iohexol concentrations are shown as lines and the corresponding 95% confidence intervals of the population simulations are shown as shaded areas (see text for model details).

Figure S3. Weighted residual error plots versus individually predicted iohexol concentrations and versus time as well as a frequency distribution of the weighted residual error from the final 2-compartment model (see text for model details).

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