Optimal Sampling Strategies to Assess Inulin Clearance in Children by the Inulin Single-Injection Method

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Background: Glomerular filtration rate in patients can be determined by estimating the plasma clearance of inulin with the single-injection method. In this method, a single bolus injection of inulin is administered and several blood samples are collected. For practical and convenient application of this method in children, it is important that a minimal number of samples are drawn. The aim of this study was to develop and validate sampling strategies with fewer samples for reliable prediction of inulin clearance in pediatric patients by the inulin single-bolus-injection method.

Methods: Complete inulin plasma concentration–time curves of 154 patients were divided into an index (n = 115) and a validation set (n = 54). A population pharmacokinetic model was developed for the index set. Optimal sampling times were selected based on D-optimality theory. For the validation set, Bayesian estimates of clearance were generated using the derived population parameters and concentrations at two to four sampling times. Bayesian estimates of clearance were compared with the individual reference values of clearance.

Results: The strategies with samples taken at 10/30/90/240 min, 10/30/240 min, 10/90/240 min, 30/90/240 min, and 90/240 min allowed accurate prediction of inulin clearance (bias <3% and not significantly different from 0; imprecision <15%).

Conclusions: Strategies involving two to four samples, including a sample at 240 min after administration of inulin, in the inulin single-injection method allow accurate prediction of inulin clearance in pediatric patients. Even one blood sample at 240 min showed acceptable performance. The proposed strategies are practical and convenient to children, and reduce repetitive blood sampling without compromising accuracy.

Measurement of renal function, i.e., glomerular filtration rate (GFR),3 is essential for evaluating suspected renal diseases and for studying changes in renal function in patients with renal failure. The ideal marker is freely filtered by the glomerulus; not reabsorbed, secreted, or metabolized by the kidney; physiologically inert; and does not alter renal function. Inulin, an exogenous marker, is such a marker, and its renal clearance during continuous intravenous infusion is regarded as the gold standard for measuring GFR in children (1). This method, however, is complex, time-consuming, invasive, and requires urine collection. For these reasons, renal inulin clearance is not routinely used in the clinical setting. An alternative method, which can be performed without collection of urine, is the determination of plasma clearance of inulin. Plasma clearance of inulin can be measured by use of either a continuous intravenous infusion or a single bolus injection. The former method is more accurate, but is time-consuming because a steady-state situation has to be achieved (1). With the latter method, a bolus injection of inulin is administered, and 10 to 12 serial blood samples are collected for the construction of a plasma concentration–time decay curve. Adequate results have been reported with this method in adults (2, 3), but for practical and convenient application in children, it is...
important that the number of blood samples is minimized.

The aim of this study was to design and validate an optimal sampling strategy for the inulin single-injection method that requires fewer samples while allowing reliable estimation of the inulin clearance in pediatric patients with renal disorders.

**Materials and Methods**

**Patients**

We developed and validated the optimal sampling strategies using data collected during routine determination of renal function in 154 pediatric patients treated at the University Hospitals of Rotterdam and Nijmegen between June 1994 and June 1997. Twenty-six children were seen in the follow-up of a hemolytic uremic syndrome (HUS). The other children were known to have impaired renal function attributable to a variety of renal and acquired diseases. Patient demographics and pathophysiologic characteristics are summarized in Table 1.

**Study Design**

All patients had a single cannula in an antecubital vein. They received a single intravenous dose (5000 mg/1.73 m² of body surface area with a maximum dose of 5000 mg) of inulin [polyfructosan-S (InuTest®); Laevosan Gesellschaft] within 1 min. Polyfructosan-S is an inulin-like polysaccharide. Serial blood samples (1 mL) were collected at 0, 10, 20, 30, 45, 65, 90, 120, 150, 180, 210, and 240 min after injection (4) to accurately construct a concentration–time curve (3). Inulin concentrations were measured in serum (0.1 mL) by an enzymatic method (5). The CV for this method was 0.6% at 481 mg/L and 0.4% at 995 mg/L (4). The assay was linear in the range 0–1720 mg/L.

**Population Pharmacokinetic Analysis**

The patients were randomly divided into an index data set (n = 100) and a validation data set (n = 54). Population pharmacokinetic models were developed independently for both data sets with use of the nonlinear mixed-effect modeling program NONMEM (double precision; Ver. V, level 1.1; GloboMax LLC). External validation of the index set population model was performed by comparison of its pharmacokinetic parameters with those of the validation data set. The validation procedure and the 2:1 ratio of the pharmacokinetic parameters with those of the validation set population model was performed by comparison of its level (1.1; GloboMax LLC). External validation of the index modeling program NONMEM (double precision; Ver. V, level 1.1; GloboMax LLC).

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**Optimal Sampling Strategy**

The residual intraindividual variability was modeled with a combined additive-proportional error model:

\[ \text{Cobs}_{ij} = \text{Cpred}_{ij} \times (1 + \epsilon_1 + \epsilon_2) \]

where Cobs$_{ij}$ is the $i^{th}$ observed concentration in the $j^{th}$ patient, Cpred$_{ij}$ is the predicted concentration, and $\epsilon_1$ and $\epsilon_2$ are independent random variables with a mean of 0 and variances of $\sigma_1^2$ and $\sigma_2^2$. The factors $\epsilon_1$ and $\epsilon_2$ account for the difference between the observed and predicted concentrations.

The population model was built step by step. At each step, a specific assumption was tested (e.g., one-compartment vs two-compartment model or correlation between clearance and body surface area). The main criterion of decision was the likelihood ratio test (7). For hierarchical models, the difference between their respective objective function values is approximately $\chi^2$-distributed, and as a result, formal testing can be performed. The level of significance was set at $P < 0.01$, corresponding to a difference of the objective function values of 6.6 points. The objective function value, which equals minus twice the logarithm of the likelihood of data, is a goodness-of-fit criterion provided by NONMEM and has no units. Secondary criteria were aspects of the various residual plots (goodness-of-fit plots) and the values of random-effects variances.

Possible correlations between the demographic or pathophysiologic indices and the pharmacokinetic parameters were explored by a three-step approach (8, 9). In step 1, an initial NONMEM analysis provided the population pharmacokinetic parameters without taking into account the demographic factors. In step 2, the Bayesian parameter estimates of the individual patients were plotted against the demographic factors of interest. From the scatter plots, demographic factors were selected that correlated with the pharmacokinetic parameters. In step 3, the NONMEM analysis was resumed, and the demographic factors selected in step 2 were entered into the NONMEM regression model in a stepwise manner to test whether a significant correlation was present between the covariate and the pharmacokinetic parameter. Relationships between pharmacokinetic parameters and the following covariates were tested: age (years), body surface area (BSA; m²), body weight (WT; kg), height (HT; cm), body mass index (BMI; kg/m²), HUS/other disorders...
(HUS/OD), center (CENTR, Rotterdam/Nijmegen) and sex (M/F).

The covariates were introduced in the population pharmacokinetic model by use of linear relationships. For example, the relationship between CL and BSA was modeled using:

\[ CL = \theta_1 + \theta_2 \times (BSA - 1.1) \]

where \( \theta_1 \) is the typical clearance of a patient with a body surface area of 1.1 m², and \( \theta_2 \) is the increment of CL per m² of BSA. Dichotomous variables were modeled as follows:

\[ CL = \theta_1 \times \theta_2^{HUS} \]

where HUS is 0 or 1 (absence/presence of HUS), \( \theta_1 \) is the typical clearance of patients with other disorders, and \( \theta_2 \) is the fractional increase of CL in cases with HUS.

The final population model was considered adequate when several criteria were met: (a) adequate fit of each individual concentration–time curve; (b) linear pattern of observed vs predicted inulin concentrations; (c) absence of trend in the weighted residuals-vs-time plot; and (d) an approximately gaussian distribution of the weighted residuals. The graphic plots were created in Xpose 2.0, an S-Plus-based model building aid (Mathsoft Inc.) (10, 11).

DEVELOPMENT OF OPTIMAL SAMPLING STRATEGIES
The population pharmacokinetic model based on the index data set was used for development of the optimal sampling strategies, whereas the validation data set (n = 54) was used for validation of these strategies. We used the population pharmacokinetic parameters derived from the index set to select optimal sampling times by application of the D-optimality theory (12) as implemented in the software package Adapt II (release 4) (13). The D-optimality theory minimizes the total overall variance of parameter estimates based on the Fisher information index.

The optimal sampling strategies were designed with one to four samples and a total sampling time of 90–240 min.

VALIDATION OF SAMPLING STRATEGIES
Plasma concentration–time data for the validation set were used to evaluate the optimal sampling strategies. Individual Bayesian estimates of clearance were calculated using the final population estimates of the index data set and the plasma concentrations at the optimal sampling times. The basis of Bayesian estimation is that, for estimation of the individual pharmacokinetic parameters, information from the population pharmacokinetic parameters is combined with information derived from the actual individual concentrations of the samples (14). A weighted combination of individual and population information where the weighting depends on how much information each individual supplies is applied. If a large number of accurate blood samples are available, the Bayesian estimation will largely be determined by the concentration of those samples alone. If, in contrast, only one blood sample is available, the Bayesian estimation will largely be determined by the information obtained from the population pharmacokinetic parameters. Bayesian analysis was performed using the POSTHOC option in NONMEM with MAXEVAL = 0. The individual reference value for clearance (reference inulin clearance) was obtained by fitting of the individual curves based on all blood samples using extended least-squares estimation in NONMEM (15).

The predictive performance of the Bayesian estimates using the various sampling strategies was evaluated by calculating the mean relative prediction error (MPE%) and its 95% confidence interval (CI) as a measure of bias and the root mean squared relative prediction error (RMSE%) and its 95% CI as a measure of imprecision (16). MPE%, RMSE%, and the SE for MPE% were defined as follows:

\[
\text{MPE\%} = \frac{\sum_{i=1}^{n} pE_i}{n} \times 100\%
\]

\[
\text{SE\%} = \sqrt{\frac{\sum_{i=1}^{n} (pE_i - \text{MPE})^2}{n \times (n - 1)}} \times 100\%
\]

\[
\text{RMSE\%} = \sqrt{\frac{\sum_{i=1}^{n} (pE_i)^2}{n}} \times 100\%
\]

in which \( n \) is the number of clearance pairs (i.e., reference and predicted values), and \( pE \) is the relative prediction error (\( \ln \text{CL pred} - \ln \text{CL ref} \)). The relative prediction error was calculated with the natural logarithm of clearance to avoid bias in favor of high clearance values. The 95% CI for RMSE% was obtained by calculating the 95% CI of the mean squared relative prediction error and extracting the root.

**Results**

POPULATION PHARMACOKINETIC ANALYSIS
The full data set consisted of 1675 samples collected from 154 pediatric patients. Fig. 1 shows all individual inulin plasma concentration–time profiles. Patient characteristics of the index and validation set are summarized in Table 1. Patients in the validation set were slightly younger and had a somewhat lower GFR.

Several compartmental models were evaluated for description of the inulin concentration–time profiles. The analysis was started with a one-compartment model with interindividual variability estimated for both clearance and the volume of distribution. In this model, a clear trend was visible in the plot of weighted residuals (WRES)
vs time. This trend disappeared on introduction of a peripheral compartment; the objective function decreased by 1388.0 points to a value of 10299.8 points ($P < 0.001$). Fitting of a three-compartment model did not converge satisfactorily. With a two-compartment model, interindividual variability could be estimated for all pharmacokinetic parameters. Estimates are given in Table 2.

Individual empirical Bayesian estimates of the pharmacokinetic parameters were obtained from the basic model with no covariates included. Visual inspection of the plots of the covariates vs individual estimates of parameters indicated correlations between BSA, WT, HT, and CL, $V_1$, $Q$, and $V_2$. For the categorical covariates, the following correlations were observed: CL and HUS and CENTR, $V_1$ and CENTR, and $V_2$ and CENTR. BSA, WT, and HT were highly correlated ($R^2 > 0.9$). Because inulin clearance is commonly normalized for an average BSA of 1.73 m² (units of GFR are $\text{mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$), BSA was selected as the measure for body size in the population model. Stepwise introduction of relationships between all pharmacokinetic parameters and BSA in the population model reduced the objective function significantly by 158.9 ($CL; P < 0.001$), 130.6 ($V_1; P < 0.001$), 77.5 ($Q; P < 0.001$), and 201.1 points ($V_2; P < 0.001$) to a value of 9379.3 points (the objective function value of the basic model was 9947.4 points). Similar introduction of WT in the basic model produced an objective function of 9364.3 points. Despite the 15-point difference in favor of the

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics of the full, index, and validation sets.(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients</strong></td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Weight, kg</td>
</tr>
<tr>
<td>Height, cm</td>
</tr>
<tr>
<td>BSA, m²</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
</tr>
<tr>
<td>Disorders</td>
</tr>
<tr>
<td>HUS</td>
</tr>
<tr>
<td>Other disorders</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Center</td>
</tr>
<tr>
<td>Nijmegen</td>
</tr>
<tr>
<td>Rotterdam</td>
</tr>
<tr>
<td>Inulin dose, mg</td>
</tr>
<tr>
<td>GFR,(^b) $\text{mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$</td>
</tr>
</tbody>
</table>

\(^a\)Data are presented as median (range).

\(^b\)GFR was calculated from serum creatinine and height according to Counahan et al. (29).
weight-pharmacokinetic parameter relationships, we decided to continue with BSA. When we introduced the relationship between CL and BSA, the additive error converged to 0 and was omitted in the subsequent analyses. This indicated that the (residual) difference between observed and predicted concentrations could be described adequately with only a proportional error. Implementation of a relationship between HUS and CL produced the final population model (Table 2). Implementation of CENTR in the population model did not produce significant reductions of the objective function.

The regression equations for the pharmacokinetic parameters for the final model are shown in Table 3. The equations between BSA and the pharmacokinetic parameters were rewritten to investigate whether the (intercompartmental) clearance (CL and Q) and volumes of distribution ($V_1$ and $V_2$) could be expressed in units of mL·min$^{-1}$·m$^{-2}$ BSA and L/m$^2$ BSA, respectively. For example, the relationship between CL and BSA was rewritten as follows: CL = intercept + slope × BSA.

### Table 2. Estimates of the population pharmacokinetic parameters.

<table>
<thead>
<tr>
<th></th>
<th>Index data set</th>
<th>Validation data set, final model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basic model with two-compartment</td>
<td>Final model</td>
</tr>
<tr>
<td>CL, mL/min</td>
<td>30.6 (3.0)</td>
<td>25.3 (1.9)</td>
</tr>
<tr>
<td>$\theta_{\text{BSA}}$, mL·min$^{-1}$·m$^{-2}$</td>
<td>32.9 (3.9)</td>
<td>2.46 (0.30)</td>
</tr>
<tr>
<td>$\theta_{\text{HUS}}$</td>
<td></td>
<td>1.70 (0.38)</td>
</tr>
<tr>
<td>$V_1$, L</td>
<td>1.79 (0.17)</td>
<td>2.07 (0.15)</td>
</tr>
<tr>
<td>$\theta_{\text{BSA}}$, L/m$^2$</td>
<td>90.0 (5.5)</td>
<td>105 (4.0)</td>
</tr>
<tr>
<td>Q, mL/min</td>
<td></td>
<td>104 (10)</td>
</tr>
<tr>
<td>$\theta_{\text{BSA}}$, mL·min$^{-1}$·m$^{-2}$</td>
<td>3.15 (0.32)</td>
<td>3.58 (0.17)</td>
</tr>
<tr>
<td>$V_2$, L</td>
<td></td>
<td>3.90 (0.33)</td>
</tr>
</tbody>
</table>

**Interindividual variability,** a %

- CL: 150, 56, 64
- $V_1$: 73, 56, 50
- Q: 42, 21, 18
- $V_2$: 47, 29, 27

**Residual variability**

- Additional error, b mg/L: 20.9 (3.8)
- Proportional error, c %: 5.69 (1.33), 7.46 (0.64), 7.94 (0.84)

Fitting of this model to the data produced a goodness of fit comparable to that of the final model. Subsequently, the intercept was fixed at 0, thereby expressing clearance as CL/m$^2$ of BSA and reducing the number of estimated parameters (only the slope is estimated). For all parameters, population models were obtained that were significantly worse ($P < 0.001$) than the models with intercepts included.

Inclusion of the covariate relationships reduced the unexplained interindividual variability in CL, $V_1$, Q, and $V_2$. For example, variability in clearance was decreased from 150% to 56%; introduction of the covariates BSA and HUS explained 94% of the variability in CL. The goodness of fit of the final model can be illustrated by plots of population and individual predicted concentration values vs observed inulin concentrations in the index set (Fig. 2).

The population-predicted inulin concentrations were predicted from the population model without taking into account interpatient and residual variability (i.e., $\eta = 0$ and $\varepsilon = 0$). Individual-predicted inulin concentrations were predicted by Bayesian estimation; for each patient, individual $\eta$ values were estimated based on individual concentrations. In the plot of population-predicted vs observed concentrations, a slight bias was present at high concentrations, especially at sampling time 10 min; the population model underpredicted the observations. Underprediction of observations could not be associated with weight, height, BSA, or BMI of the patient, but a preanalytical error was plausible. The bias was absent in the plot of individual-predicted vs observed concentra-

### Table 3. Regression equations describing the relationship between the pharmacokinetic parameters and the covariates for the final model (index data set), with HUS = 1 (HUS) or 0 (other diseases).

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Index Data Set</th>
<th>Validation Data Set, Final Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL, mL/min</td>
<td>[25.3 + 32.9 × (BSA – 1.1)] × 2.46$^{\text{HUS}}$</td>
<td>20.9 (3.8)</td>
</tr>
<tr>
<td>$V_1$, L</td>
<td>2.07 + 1.70 × (BSA – 1.1)</td>
<td>5.69 (1.33)</td>
</tr>
<tr>
<td>Q, mL/min</td>
<td>105 + 104 × (BSA – 1.1)</td>
<td>7.46 (0.64)</td>
</tr>
<tr>
<td>$V_2$, L</td>
<td>3.58 + 3.90 × (BSA – 1.1)</td>
<td>7.94 (0.84)</td>
</tr>
</tbody>
</table>

- a CV in population.
- b Expressed as absolute inulin concentration.
- c Expressed as percentage of inulin concentration.
tions, indicating an adequate fit of each individual concentration–time curve. For each individual in the index set, pharmacokinetic parameters were estimated by Bayesian analysis. The medians (ranges) of the individual estimates of $CL$, $V_1$, $Q$, $V_2$, distribution half-life, and elimination half-life were 28 (2–123) mL/min, 1.93 (0.36–6.15) L, 94 (32–195) mL/min, 2.59 (0.84–8.71) L, 7.9 (3.6–12.3) min, and 143 (30–918) min, respectively ($n = 100$).

The developed population model was validated with use of the validation data set. No bias was observed when the predicted inulin concentrations based on the index population model were plotted against the observed concentrations (plot not shown). Independent development of a population model based on the validation data set produced pharmacokinetic parameter estimates comparable to those of the index data set (Table 2). Separate deletion of the five covariate–pharmacokinetic relationships from the model ($CL$ vs BSA and HUS, $V_1$, $Q$, and $V_2$ vs BSA) increased the objective function significantly ($P <0.001$). No other significant relationships were detected.

**DEVELOPMENT OF OPTIMAL SAMPLING STRATEGIES**

For a typical patient with a BSA of 1.1 m$^2$, we found the following four optimal sampling times in the observation period from 10 to 240 min: 10, 25, 79, and 240 min. Optimal sampling times were only marginally influenced by BSA and presence/absence of HUS. Samples close to the optimal sampling times were selected from the profiles in the validation set. Differences between theoretical and actual sampling times were within 15 min. The tested sampling strategies are summarized in Table 4.

**VALIDATION OF SAMPLING STRATEGIES**

Individual reference values for the clearance and other pharmacokinetic parameters were calculated with use of the full plasma concentration–time profiles. The bias and imprecision of predicted clearance for the different sampling strategies are given in Table 4. None of the sampling strategies was significantly biased. Imprecision was 10.7–68.9%. Sampling strategies 1 (10/30/90/240 min), 4 (10/30/240 min), 5 (10/90/240 min), 6 (30/90/240 min), and 9 (90/240 min) had good predictive performance with a bias $<3\%$ and an imprecision not exceeding 15%.

As an example, Fig. 3 shows Bland–Altman plots of the Bayesian predicted clearance and the reference clearance for sampling strategies 1 (10/30/90/240 min) and 2 (10/30/65/120 min). Typically, in sampling strategy 2, we observed a bias at low inulin clearance values: the predicted clearance was larger than the reference clearance. This can be explained by the fact that the elimination half-life is underestimated. We further investigated this phenomenon by stratifying predictive performance for an individual $CL <40$ mL $\cdot$ min$^{-1} \cdot 1.73$ m$^2$ vs individual $CL >40$ mL $\cdot$ min$^{-1} \cdot 1.73$ m$^2$. For $CL <40$ mL $\cdot$ min$^{-1} \cdot 1.73$ m$^2$, we observed no bias, and the imprecision was $<15\%$ for sampling strategies 1 and 4–11. For $CL >40$ mL $\cdot$ min$^{-1} \cdot 1.73$ m$^2$, the clearance was slightly (but not significantly) overestimated with bias ranging from 2.8% to 4.8% for sampling strategies 1 and 4–10. The imprecision was $<15\%$. Strategy 1, for example, produced a bias of 2.8% (95% CI, –0.5% to 6.1%) and an imprecision of 9.8% (95% CI, 0–13.9%).

Shown in Fig. 4 are Bland–Altman plots of the inulin clearance for sampling strategies 9 (90/240 min) and 10 (240 min). The mean differences of the predicted and the reference inulin clearances were $–0.8$ and $–1.2$ for strategies 9 and 10, respectively. For both sampling strategies,
we obtained a difference \(>10 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}\) for only one patient. The difference between the predicted inulin clearance and the reference clearance showed a larger variation at higher GFR.

**Discussion**

For assessing renal function and the rate of progression of renal diseases, reliable measurement of GFR is necessary. The reference method (gold standard) to determine GFR in children (in a research setting) is the assessment of renal clearance of inulin during a continuous intravenous infusion. This method, however, is complex, invasive, and time-consuming. Alternatively, plasma clearance of inulin can be assessed by a single injection of inulin, which is less time-consuming. Plasma clearance of inulin can be calculated by dividing the dose by the area under the plasma concentration–time curve. For practical and convenient application in children, it is important that the total number of blood samples is minimized. The present study focused on the development of an optimal sampling strategy for the inulin single-injection method in pediatric patients. We investigated whether inulin clearance could be estimated accurately with a smaller number of blood samples.

A population pharmacokinetic model for inulin was developed based on the plasma concentration–time profiles of 100 pediatric patients in the index data set. During nonlinear mixed-effect modeling, the pharmacokinetic model was fitted to the data of all patients simultaneously. Typical pharmacokinetic parameters and their corresponding interindividual variabilities were estimated.

Inulin data were adequately described by a two-compartment model with first-order elimination from the central compartment. In the literature, both two- and three-compartment models have been used to describe the disposition of inulin (3, 17). In the NONMEM analysis, addition of a second peripheral compartment did not improve the fit of model to the data. This can be explained by the fact that the total sampling time in the present study was limited to 240 min. Odeh et al. (17) described the pharmacokinetics of inulin based on a three-compartment model, but in that study, samples were collected until 480 min after injection.

The covariates BSA and HUS correlated significantly with inulin clearance and were included in the final model. They reduced the interindividual variability from 150% to 56% and therefore explained 94% of the variability between the patients. BSA was a significant covariate, which is to be expected because GFR varies with body size. For children, GFR is usually expressed per 1.73 m² BSA (i.e., mL \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}) for standardization and comparison between individuals of different sizes. Patients with HUS had, on average, a higher clearance compared with patients with other disorders (factor = 2.46). This may be explained by the fact that the patients with HUS in our data set were totally or partly recovered from HUS and had a (nearly) normal renal function (all patients with HUS in the validation set had an inulin clearance \(>100 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}\)). Kinowski et al. (18) found that BSA is also a significant covariate for clearance and volume of distribution of inulin in adults with diabetes or obesity.

The population pharmacokinetic model described the data adequately (Fig. 2) and showed a low residual variability (proportional error of 7.5%). We validated the population model by performing an identical analysis with the validation data set, which yielded comparable results for mean pharmacokinetic parameters, variance, and relationships with covariates.

Because 11 blood samples were available for each patient, the standard two-stage method may also be used to produce population parameter estimates. Although application of this method usually produces unbiased

### Table 4. Predictive performance of inulin clearance for the sampling strategies in the validation data set.

<table>
<thead>
<tr>
<th>Strategy</th>
<th>No. samples</th>
<th>Sampling times, min</th>
<th>Bias MPE% 95% CI</th>
<th>Imprecision RMSE% 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>10/30/90/240</td>
<td>1.8 (−1.1 to 4.7)</td>
<td>10.7 (6.3–13.8)</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>10/30/65/120</td>
<td>6.7 (−2.7 to 16.1)</td>
<td>34.9 (0–49.4)</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>10/30/90</td>
<td>0.5 (−18.4 to 19.5)</td>
<td>68.9 (50.1–83.6)</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>10/30/240</td>
<td>1.7 (−2.0 to 5.6)</td>
<td>14.1 (4.8–19.3)</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>10/90/240</td>
<td>2.6 (−0.5 to 5.7)</td>
<td>11.7 (6.9–15.0)</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>30/90/240</td>
<td>0.3 (−3.1 to 3.7)</td>
<td>12.5 (8.8–15.2)</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>10/240</td>
<td>1.7 (−2.5 to 6.0)</td>
<td>15.7 (9.4–20.1)</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>30/240</td>
<td>−0.1 (−4.7 to 4.4)</td>
<td>16.4 (9.6–21.2)</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>90/240</td>
<td>1.3 (−2.2 to 4.8)</td>
<td>12.8 (9.1–15.7)</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>240</td>
<td>1.0 (−4.0 to 5.9)</td>
<td>18.2 (12.1–22.7)</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>210</td>
<td>3.9 (−1.2 to 9.0)</td>
<td>19.0 (12.3–23.8)</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>180</td>
<td>3.3 (−5.9 to 12.5)</td>
<td>33.6 (0–49.8)</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>150</td>
<td>3.2 (−6.9 to 13.3)</td>
<td>37.0 (0–52.9)</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>120</td>
<td>6.8 (−4.0 to 17.7)</td>
<td>40.1 (0–57.1)</td>
</tr>
</tbody>
</table>
mean estimates of parameters, random effects (variance and covariance) are likely to be overestimated in all realistic situations (19).

We used the D-optimality theory for selecting optimal sampling times. From the optimal sampling times, we developed different sampling strategies with one to four sampling times and tested the validation data set using Bayesian estimation. With Bayesian estimation, the individual pharmacokinetic parameters are estimated using the derived population model and the plasma concentrations at the selected sampling times. Application of Bayesian analysis provides the advantage of estimating a pharmacokinetic parameter from only a few sampling times. Furthermore, there is no need for the sampling times to be exact. This is an advantage over other methods, such as multivariate linear regression techniques, which require that the actual sample is taken at the specified, optimal sampling time (20, 21). The Bayesian estimation method

![Fig. 3. Bland–Altman plots of predicted and reference inulin clearances in the validation data set for sampling strategies 1 (10/30/90/240 min; top) and 2 (10/30/65/120 min; bottom). Solid line, mean difference; dashed lines, 2 SD limits.](image)

![Fig. 4. Bland–Altman plots of predicted and reference inulin clearances in the validation data set for sampling strategies 9 (90/240 min; top) and 10 (240 min; bottom). Solid line, mean difference; dashed lines, 2 SD limits.](image)
has been implemented in several commercially available pharmacokinetic programs (e.g., NONMEM, PKS, USCPACK, P-PHARM, and MWPharm).

For all of the tested sampling strategies, we found that inulin clearance was assessed well by Bayesian estimation when at least a sample at 240 min was included. Limiting the total sampling time window from 0–240 to 0–120 min (strategy 2) decreased the predictive performance, indicating that the sampling time of 240 min after injection is critical for adequate estimation of inulin clearance. The authors of one study in children and adults reported that a total sampling time of 180 min was sufficient to estimate inulin clearance (22), but in that study, the predictive performance of the strategy was not tested and only a minority of patients presented a CL < 40 mL min⁻¹ 1.73 m⁻². In our study, the predictive performance of the sampling strategy with even one sample at 240 min (strategy 10) was acceptable for CL < 40 mL min⁻¹ 1.73 m⁻² [bias, 4.8% (95% CI, −0.1% to 9.6%); imprecision, 14.6% (95% CI, 0–20.6%)].

Sampling strategies 1 (10/30/90/240 min), 4 (10/30/240 min), 5 (10/90/240 min), 6 (30/90/240 min), and 9 (90/240 min) allowed accurate prediction of inulin clearance with a bias not significantly different from 0 and an imprecision not exceeding 15%. The predictive performance of strategy 10 with only one sample taken at 240 min was acceptable as well [bias, 1.0% (95% CI, −4.0% to 5.9%); imprecision, 18.2% (95% CI, 12.1–22.7%)]. These results are in accordance with the results in a previously reported study on the development of a limited sampling model using Bayesian estimation in adults with diabetes or obesity; the authors of that report showed that with one or two samples, inulin clearance was well estimated (18).

Swinkels et al. (4) reduced the total number of required blood samples from 11 to 6 for the determination of inulin clearance in children. Four samples were used to describe the distribution phase and two samples to describe the elimination phase. Because a model built in the program SAS was used for nonlinear fitting, the authors were unable to reduce the required number of blood samples to less than four. This also applies for the results of Florijn et al. (2) (SIPHAR program) and Orlando et al. (3) (GraphPad Prism) in adults. With Bayesian estimation, however, which combines population information and individual information, it is possible to reduce the total number of required blood samples to as few as one (14). In addition, no optimal sampling strategies were developed in the study of Swinkels et al. (4), and the model was not validated. Several other authors have used the slope clearance method instead of Bayesian estimation to simplify the inulin single-injection method for the determination of GFR in adults (23–28). The slope clearance method is based on calculation of the area under the curve by the slope (rate constant) and the extrapolated inulin concentration at 0 min (y-intercept), but in nearly all those studies, no optimal sampling times were determined and the simplified method was not validated.

In conclusion, sampling strategies of two to four sampling times and a total sampling time of 240 min allow good prediction of inulin clearance in children with a nonsignificant bias and good imprecision (<15%). The sampling strategy with two samples (90 and 240 min) seems very practical, but it relies heavily on a flawless preanalytical procedure and assay of both samples with no room for errors. Four sampling times (10/30/90/240 min) provide additional accuracy. Even the sampling strategy with one blood sample at 240 min could be used in certain cases. With the development of optimal sampling strategies, the burden of repetitive blood sampling from children to estimate GFR by the inulin single-injection method can be reduced considerably. As a result, estimation of GFR based on the approach presented here becomes more practical and offers more patient convenience, without compromising accuracy.

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