Optimal design of a two-sample test for assessing $[^{125}\text{I}]$iothalamate plasma clearance in peritoneal dialysis

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Abstract

**Background.** Plasma clearance of a tracer in peritoneal dialysis (PD) can be used to assess treatment adequacy without labour-intensive fluid collections. Accuracy and precision of plasma clearance estimates by the bolus injection technique depend on the estimation accuracy of the area under the concentration curve and the measurement precision of plasma concentrations. The first source of error is due to oversimplified, e.g. monoexponential, descriptions of plasma disappearance curves. The second source of error arises from the propagation of measurement errors to the parameter estimates.

**Methods.** The theoretical bias of parameter estimates is determined first for a monoexponential approximation of a biexponential disappearance curve and as a function of the first sampling time at which mixing is still incomplete. The precision of plasma clearance estimates, expressed as coefficient of variation, is then described as a function of the experimental variables and of the standard deviation of measurement error. This allows the determination of the optimal two-sample test that yields most precise estimates of plasma clearance.

**Results.** The optimal two-sample schedules for assessing plasma clearance of $[^{125}\text{I}]$iothalamate in PD patients vary between subjects according to individual clearances and distribution volumes. Our results suggest collecting the first sample 120 min, and the second 2–4 days, after the bolus injection.

**Conclusions.** The proposed two-sample test is suitable to be used in clinical routine for assessment of adequacy of PD treatment but requires a priori estimation of individual tracer kinetics and of laboratory measurement errors. A fixed design with the first sample taken after 120 min and the second sample collected 3 days after the bolus injection should yield the best performance.

**Key words:** bolus injection method; dialysis efficiency; $[^{125}\text{I}]$iothalamate; optimal experiment design; peritoneal dialysis; plasma clearance

Introduction

Many studies have been carried out in the past decades for selecting the best method for assessing in clinical routine glomerular filtration rate in man [1]. The total plasma clearance approach is widely used because of its simplicity and accuracy, and is based on single intravenous bolus-injections of biologically inert substances such as inulin, $[^{51}\text{Cr}]$EDTA and $[^{125}\text{I}]$iothalamate [1–4]. The application of total plasma clearance of an injected tracer to measure peritoneal dialysis (PD) efficiency does not require fluid collections of urine and dialysate and reduces errors due to measurement, mixing, and sampling [5,6].

The plasma disappearance curves of the test substances after bolus injection are usually well described by a biexponential function [2,7–9], whose parameters can be determined only from multiple concentration measurements. Since the minimum number of blood samples that must be collected is equal to the number of estimated parameters, which is four in the case of a biexponential model, several simplified methods have been proposed for reducing the complexity of test protocols [3,10–12]. These simplified methods are based on a monoexponential description of the tail of the plasma disappearance curve, which is characterized by distribution volume and clearance only. Therefore only two measurements are needed for determining uniquely the two parameters of a monoexponential function. Simpler tests based on a single blood sample have also been proposed but they require independent determination of the total distribution volume of the test substance in each individual, or are based on a mean square error sampling design with use of empirical correction formulae [10–12]. This reduces the accuracy and precision of the clearance estimates.

The monoexponential approximation can be sufficiently accurate for determining the area under the concentration curve of a multiexponential plasma dis-
appearance curve if the distribution of the test substance in the body compartments after bolus injection occurs at a much higher rate than elimination. This condition is satisfied at low plasma clearance rates such as in advanced renal insufficiency and in PD. In these situations the design of the sampling schedule is important for the precision and accuracy of clearance measurements [13]. Another important factor that affects precision is concentration measurement noise, which usually depends on the dose of the injected substance. When using radioactive tracers in patients with low plasma clearance rates, due to the long mean residence time of the test substance in the body, the administered activity must be kept low to minimize the radiation dose. In this case, measurement noise can become a major source of errors and it must be explicitly taken into account in the design of the sampling schedule.

In this study a two-sample bolus injection test is analysed with regard to different error sources involved in the estimation of plasma clearance. In particular: the estimation bias introduced by ignoring the so-called fast exponential; the estimation error for the slow exponential related to the first sampling time when the fast exponential is not extinguished yet; and the measurement noise that affects the precision of the clearance estimates. With respect to this latter error source, a particular standard deviation model is assumed, and the optimal time of the second sample that minimizes the estimation variance of the clearance is determined. The theoretical analysis is applied to the results of a pilot study on $^{125}$I}o]othalamate plasma clearance in PD patients.

**Subjects and methods**

**Patients and protocol**

Twenty-one patients of median age 63 years (range 44–82), on PD for a median of 5 months (range 1–80) participated in this study after having given their informed consent.

A dose of 21 kBq/kg (0.57 μCi/kg) of $^{125}$I}o]othalamate (Amersham, Bucks, UK) was injected as a bolus after the filling of the peritoneal cavity at the second exchange of dialysis fluid of the day. Nine blood samples were collected at 20, 40, 60 min, and 2, 6, 12, 18, 24 and 48 h. One additional sample was collected in two patients after 3 days and in one patient also after 5 and 7 days. The administered dose was calculated by precision double weighing and the activity of 0.5 ml plasma samples was measured by a gamma counter (Stratec, Birkenfeld, Germany) for 10 min. Plasma concentrations were expressed as CPM/ml. One hour prior to the test, 1 ml of Lugol solution was administered to the patients to minimize thyroid uptake of the radioactive iodine. Effective radiation dose per kilogram bodyweight was calculated a posteriori for each patient as the product of individual mean residence time of $^{125}$I}o]othalamate and the effective dose equivalent reported for abnormal renal function in [16]. This reference value was corrected for the ratio of actual to reference value was corrected for the ratio of actual to equivalent reported for abnormal renal function in [16].

**Statistical methods**

**Reference model**

It is assumed that the plasma concentration time course, $c(t)$, of $^{125}$I}o]othalamate after an intravenous bolus injection of a given dose $D$ can be accurately described by the biexponential model

$$c(t) = Ae^{-\alpha t} + Be^{-\beta t},$$

where parameters $\{A, \alpha, B, \beta\}$ are non-negative. The time, $t=0$, represents the time instant of the bolus administration (or the centre of the short infusion interval). Let $B e^{-\beta t}$ represent the so-called fast exponential (i.e. $\beta > \alpha$) which typically decays to negligible values within few hours [1,4].

**Measurement noise description**

Noisy plasma concentration measurements are considered as

$$y(t_i) = c(t_i) + \varepsilon(t_i),$$

where $t_i$ represents the blood sampling time and $\varepsilon(t_i)$ the measurement noise. This latter is assumed to be uncorrelated, with zero mean, and known standard deviation, $\sigma(t_i)$, described as

$$\sigma(t_i) = p_1 + p_2 \varepsilon(t_i).$$

Parameters $p_1$ and $p_2$ represent background noise and coefficient of variation at elevated concentration measurements respectively.

The statistical variability for $^{125}$I}o]othalamate measurements with our laboratory procedures was determined through a dilution study with six concentration levels in a range of 1:1000. For each dilution level and with unlabelled water, radioactivity measurements were repeated five times and average counts, $\bar{y}$ (CPM/ml), and standard deviations, $\sigma_y$ (CPM/ml), were calculated. The parameters $p_1$ and $p_2$ of the noise model (3) were estimated by non-linear least-squares with log-transformation of the data.

**Parameter estimation from multiple samples**

For each subject, individual parameter estimates of the biexponential model (1) were obtained by non-linear weighted least-squares fit of the data with weights chosen equal to the inverse of the measurement variance calculated from Eq. (3) [14]. Given the estimates of parameters $\{A, \alpha, B, \beta\}$, estimates of the plasma clearance rate and the total distribution volume were obtained by standard formulae reported in the following.

**Monoexponential approximation**

Plasma clearance, $CL$, is given by the injected dose, $D$, divided by the total area under the concentration curve (AUC) [15]:

$$AUC = \int_0^\infty c(t)dt = \frac{A}{\alpha} + \frac{B}{\beta}$$

A monoexponential approximation of $c(t)$ with the slow exponential, i.e. $c(t) \approx Ae^{-\alpha t}$, is justified by the fact that the
contribution to the total AUC of the fast exponential, \( \int_0^\infty \beta e^{-\beta t} \, dt = \frac{\beta}{\beta} \), can be negligible (see also Figure 1A). The analysis of the errors that arise from this approximation is considered in Table 1 and is based on the ratio \( e^x \) of the two areas.

From Table 1 row three it follows that plasma clearance calculated from the slow exponential alone is related to the true clearance by

\[
\frac{Dx}{A} = CL(1 + e_A),
\]

that is \( Dx/A \) overestimates the true plasma clearance proportionally to the area ratio \( e_A \). In principle, this estimation bias can be compensated, as discussed later.

The formula for computing the total distribution volume, \( V_{tot} \), is also reported in Table 1. The error analysis of row four shows that \( V_{tot} \) is overestimated proportionally to twice the area ratio \( e_A \) if this latter is small such that its squared value is negligible, in fact

\[
\frac{D}{A} = V_{tot}(1 + e_A)^2 = V_{tot}(1 + 2e_A + e_A^2).
\]

**Optimal experiment design for estimating \( A \) and \( \alpha \)**

In order to be able to estimate accurately the two parameters of a monoeXponential function the simplest test that can be performed comprises two measurements. In the following, accuracy and precision of parameter estimates of \( A \) and \( \alpha \) with respect to the design variables of a two-sample test are considered. A qualitative representation of the rationale of error analysis and experiment design is shown in Figure 1.

**Accuracy and the first sampling time**

Under the assumption of a monoeXponential model, the equations for estimating the two parameters \( A \) and \( \alpha \) as well as \( CL \) and \( V_{tot} \) from two measurements taken at times \( t_1 \) and \( t_2 \) are reported in Table 2 column two, where the symbol \( \hat{\alpha}, (\hat{\cdot}), \) denotes estimates from experimental data. Estimation bias arises from the fact that measurements are actually given by

\[
y(t_1) = Ae^{-\alpha t_1} + Be^{-\beta t_1} + e(t_1) = Ae^{-\alpha t_1}(1 + e_1) + e(t_1)
\]

\[
y(t_2) = Ae^{-\alpha t_2} + e(t_2)
\]

where the fast exponential is viewed as a disturbing factor that affects, if \( t_2 \) is much greater than \( t_1 \), only the first sample according to the ratio

\[
e_1 = Be^{-\beta t_2}/Ae^{-\alpha t_2}.
\]

Unlike the error term \( e_A \) in Table 1, \( e_1 \) can be reduced at will by increasing the first sampling time \( t_1 \). Column three of Table 2 was derived by substituting \( e_1 \) in column two and by assuming zero measurement error in Eqs (7) and (8). Column four reports the linearized equations of column three with respect to \( e_1 \), which are useful to understand the effects of small, positive deviations of \( e_1 \) from zero. In fact from row three it can be observed that a suitable choice of \( t_2 \) can, in principle, compensate the bias of the clearance estimate reported in Eq. (5). In particular, if \( t_2 \) is greater than \( 1/\alpha \), the clearance estimate \( Dx/A \) underestimates \( Dx/A \), whereas if \( t_2 = 1/\alpha \) the error term due to \( e_1 \) is cancelled out. This last observation has been used as design criterion in [17]. In this study it is assumed that \( t_2 \geq 1/\alpha \), and the definition of the optimal first sampling time, \( t_1(t_2) \), is given as the one that cancels out the estimation bias of Eq. (5) for a given \( t_2 \) (e.g. this condition is satisfied by the two sampling times of Figure 1A). Similarly from Table 2 row four it follows that \( D/A \) underestimates the distribution volume \( D/A \) and that the effect of \( e_1 \) is proportionally stronger than for the
clearance estimate. It can therefore be expected that a compensation of the bias in the clearance estimate is accompanied by an overcompensation, i.e. underestimation, of the distribution volume.

**Precision and the second sampling time**

Precision of parameter estimates is affected by measurement noise and depends significantly on the experiment design variables (in particular the sampling times). The design problem considered in this paper is the straightforward minimization, with respect to the sampling time \( t_2 \), of the estimation variance of the plasma clearance. The monoexponential model with two noisy measurements is considered according to Eqs. (7) and (8), with \( t_1 \) chosen sufficiently large such that \( e_1 \) is negligible. The measurement noise description given by Eq. (3) becomes

\[
\sigma(t) = p_1 + p_2 A e^{-ut}.
\]

The estimate of plasma clearance, see \( \hat{CL} \) in Table 2, becomes thus a function of the dose \( D \), the parameters \( A \) and \( x \), the sampling times \( t_1 \) and \( t_2 \), and the two error terms \( v(t_1) \) and \( v(t_2) \). Its estimate is indicated, for simplicity, by:

\[
\hat{CL} = CL(D, A, x, t_1, t_2, v(t_1), v(t_2)).
\]

The estimation variance of \( \hat{CL} \), \( \sigma_{\hat{CL}}^2 \), can be calculated by linearizing Eq. (11) with respect to the random variables. By assuming independent errors and no measurement error on the injected dose, the estimation variance of the clearance is:

\[
\sigma_{\hat{CL}}^2 = \left( \frac{\partial CL}{\partial v(t_1)} \right)^2 \sigma^2(v(t_1)) + \left( \frac{\partial CL}{\partial v(t_2)} \right)^2 \sigma^2(v(t_2))
\]

where \( \frac{\partial CL}{\partial x} \) denotes the partial derivative of Eq. (11) with respect to the generic variable \( x \) evaluated at \( v(t_1) = v(t_2) = 0 \). Equation (12) yields the following expression:

\[
\left( \frac{\partial CL}{\partial v(t_1)} \right)^2 = \frac{(p_1 A e^{t_1} + p_2 A e^{-t_1})(t_2 - 1)}{t_2 - t_1} + \frac{(p_1 A e^{t_2} + p_2 A e^{-t_2})(t_1 - 1)}{t_2 - t_1}.
\]

**Results**

**Measurement noise description**

The estimated parameters of the standard deviation model of measurement noise, Eq. (3), were \( p_1 = 25.4 \) (CPM/ml) for background noise, and \( p_2 = 0.0084 \), representing the coefficient of variation at high concentrations.

**Parameter estimates and error analysis**

Statistics of estimates of \(^{125}\)Iothalamate plasma clearance and of total distribution volume obtained in our PD study group are reported in Table 3 together with the variables introduced in Tables 1 and 2 for the error analysis. In particular \( e_1 \), which is defined as the AUC ratio between fast and slow components of a biexponential model (see also Figure 1) and quantifies the fraction by which the monoexponential approximation overestimates the true clearance, was always small with a median value around 1% and a maximum value of 3.1%.

With reference to the error \( e_1 \) of Eq. (9), which is

<table>
<thead>
<tr>
<th>Estimate</th>
<th>Mathematical expression</th>
<th>Equivalent expression</th>
<th>Linearized expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \hat{x} )</td>
<td>( \frac{\ln(y(t_1)) - \ln(y(t_2))}{t_2 - t_1} )</td>
<td>( \frac{\ln(1 + e_1)}{t_2 - t_1} )</td>
<td>( x + \frac{e_1}{t_2 - t_1} )</td>
</tr>
<tr>
<td>( \hat{A} )</td>
<td>( y(t_1) e^{m_1} )</td>
<td>( A(1 + e_1) e^{m_2 - t_1} )</td>
<td>( \hat{A}\left(1 + \frac{t_2 e_1}{t_2 - t_1}\right) )</td>
</tr>
<tr>
<td>( \hat{CL} )</td>
<td>( \frac{D \hat{x}}{\hat{A}} )</td>
<td>( \frac{D \hat{x}}{A} \left[ 1 + \left(1 - \frac{1}{e_1}\right) \frac{t_2}{t_2 - t_1}\right] )</td>
<td>( \frac{D}{t_2 - t_1} )</td>
</tr>
<tr>
<td>( \hat{\nu}_m )</td>
<td>( \frac{D}{\hat{A}} )</td>
<td>( \frac{D}{A} \left(1 + \frac{t_2 e_1}{t_2 - t_1}\right) )</td>
<td>( \frac{D}{t_2 - t_1} )</td>
</tr>
</tbody>
</table>

\[\text{Table 2. Error analysis of estimates based on monoexponential description in presence of an additional error term due to a fast exponential}\]

<table>
<thead>
<tr>
<th>Estimate</th>
<th>Mathematical expression</th>
<th>Equivalent expression</th>
<th>Linearized expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \hat{x} )</td>
<td>( \frac{\ln(y(t_1)) - \ln(y(t_2))}{t_2 - t_1} )</td>
<td>( \frac{\ln(1 + e_1)}{t_2 - t_1} )</td>
<td>( x + \frac{e_1}{t_2 - t_1} )</td>
</tr>
<tr>
<td>( \hat{A} )</td>
<td>( y(t_1) e^{m_1} )</td>
<td>( A(1 + e_1) e^{m_2 - t_1} )</td>
<td>( \hat{A}\left(1 + \frac{t_2 e_1}{t_2 - t_1}\right) )</td>
</tr>
<tr>
<td>( \hat{CL} )</td>
<td>( \frac{D \hat{x}}{\hat{A}} )</td>
<td>( \frac{D \hat{x}}{A} \left[ 1 + \left(1 - \frac{1}{e_1}\right) \frac{t_2}{t_2 - t_1}\right] )</td>
<td>( \frac{D}{t_2 - t_1} )</td>
</tr>
<tr>
<td>( \hat{\nu}_m )</td>
<td>( \frac{D}{\hat{A}} )</td>
<td>( \frac{D}{A} \left(1 + \frac{t_2 e_1}{t_2 - t_1}\right) )</td>
<td>( \frac{D}{t_2 - t_1} )</td>
</tr>
</tbody>
</table>

\[\text{Table 3. Cumulative statistics of results and error analysis}\]

<table>
<thead>
<tr>
<th>Estimate</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>( CL ) (ml/min)</td>
<td>6.09</td>
<td>3.74</td>
<td>10.03</td>
<td>6.61</td>
<td>1.81</td>
</tr>
<tr>
<td>( V_{tot} ) (litre)</td>
<td>20.8</td>
<td>13.3</td>
<td>26.5</td>
<td>20.1</td>
<td>3.2</td>
</tr>
<tr>
<td>( e_1 )</td>
<td>0.91%</td>
<td>0.51%</td>
<td>3.12%</td>
<td>1.08%</td>
<td>0.66%</td>
</tr>
<tr>
<td>( e_2 )</td>
<td>1.87%</td>
<td>0.00%</td>
<td>17.15%</td>
<td>3.45%</td>
<td>4.79%</td>
</tr>
<tr>
<td>( t^b_1 )</td>
<td>2.18\pm3m</td>
<td>1.48\pm22m</td>
<td>4.59\pm14m</td>
<td>2.9153m</td>
<td>17.15m</td>
</tr>
<tr>
<td>1/3 ( t^b_1 )</td>
<td>2.44\pm40m</td>
<td>1.79\pm28m</td>
<td>3.86\pm11m</td>
<td>2.6\pm24m</td>
<td>14.48m</td>
</tr>
</tbody>
</table>

*Evaluated at \( t_1 = 120 \) min; **Determined with \( t_1 \) fixed at 120 min for all subjects; †Determined for each subject with individual optimal sampling time \( t^b_1 \).
defined as the ratio between fast and slow components of a biexponential model at \( t_1 \) (see also Figure 1), Table 3 reports the values calculated at \( t_1 = 120 \) min. Despite its values being small on average, the dispersion was significant with a maximum value of 17%.

The optimal time for the second sample, \( t_2^* \), was determined for each subject separately, assuming a fixed value of \( t_1 = 120 \) min, and resulted on average 30% larger than the mean residence time of 1\(^{35}\)I-iodotamalate in the body given by \( 1/\alpha \). This value has been proposed as the optimal sampling time in [17]. However, it can be shown that the optimal time of the second sample falls between \( 1/\alpha \) and \( 2/\alpha \), depending on the measurement noise and model parameters [Thomas et al., to be published]. In particular, for the constant variance case \((p_2 = 0)\) and with \( t_1 = 0\), the optimal sampling time is \( t_2^* = 1/\alpha \) as proposed in [17], whereas for the case of constant coefficient of variation \((p_1 = 0)\), the optimal sampling time is \( t_2^* = 2/\alpha - t_1 \).

Finally, Table 3 reports the optimum first sampling time, \( t_1^* \), which is defined as the sampling time point that yields, for a given \( t_2^* \), the same AUC with the monoeXponential approximation as with the ‘true’ biexponential model (this particular situation is represented in Figure 1). This optimized first sampling time was on average slightly smaller than the tentative value of 120 min. Simultaneous optimization of \( t_1^* \) and \( t_2^* \) did not provide any significant improvements with respect to the described procedure (results not shown).

Validation of two-sample estimates vs full sampling schedule

The original protocol of the study did not comprise the optimal samples determined a posteriori. Estimates of clearance and total distribution volume were thus calculated from the two available samples closest to 120 min and the individual \( t_2^* \) (in all but one patient the second sample was that taken after 2 days). The comparison between clearance and total distribution volume estimates obtained with the reference multisample–biexponential modelling approach and with the described two-samples procedure is shown in Figure 2. Accuracy and precision for the simplified procedure are high regarding the assessment of clearance, Figure 2A, whereas less accurate estimates are achieved for the total distribution volume, Figure 2B. Summary statistics of sampling times and residuals are reported in Table 4, which confirms the good agreement of the clearance estimates obtained with the simplified method compared to the reference values both in terms of precision (mean and median differences close to zero) and in terms of dispersion (mean and median absolute percent difference around 4% and maximum deviations within 0.7 ml/min). The distribution volume estimates were significantly underestimated with the simplified method (mean difference \( = -0.61 \)). However, the absolute percentage differences were comparable with those of the clearance estimates.

**Table 4.** Validation with suboptimal sampling schedule and statistics of residuals

<table>
<thead>
<tr>
<th>( t_1^* ) (min)</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_2^* ) &amp; 2#0^w &amp; 3#3#2#1^w &amp; 3#3#0^w &amp; 2#1#4^w &amp; 5#5#7^w</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( CL_{120}^{tot} - CL_{120}^{ref} ) &amp; -0.06 &amp; -0.67 &amp; 0.57 &amp; -0.04^b &amp; 0.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( CL_{122}^{ref} - CL_{122}^{tot} ) %e &amp; 4.4 &amp; 0.4 &amp; 12.1 &amp; 4.2 &amp; 2.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_{120}^{tot} - V_{120}^{ref} ) %e &amp; -0.6 &amp; -2.6 &amp; 1.8 &amp; -0.6^d &amp; 1.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_{122}^{tot} - V_{122}^{ref} ) %e &amp; 3.5 &amp; 0.1 &amp; 14.3 &amp; 4.8 &amp; 3.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Effectively used sampling times according to available data; ^aWith null hypothesis: mean = 0, \( P = 0.56 \); ^bAbsolute percent difference evaluated as: 100 \times |\( CL_{120}^{tot} - CL_{120}^{ref} \)|/\( CL_{120}^{ref} \); ^cWith null hypothesis: mean = 0, \( P = 0.022 \); ^dAbsolute difference evaluated as: 100 \times |\( V_{120}^{tot} - V_{120}^{ref} \)|/\( V_{120}^{ref} \).

Discussion

The two-sample bolus injection test is the simplest test that allows simultaneous estimation of clearance \((CL)\) and distribution volume \((V_{tot})\) of a substance in a subject. This test has been previously indicated as a reliable method to determine plasma clearance in advanced renal insufficiency [13]. In this study the
different sources of errors involved with this approach have been analysed theoretically and the optimal sampling schedule design has been solved by direct minimization of the estimation variance of the clearance estimate.

Given the low level of plasma clearance in PD, the monoeXponential approximation of the biexponential plasma disappearance curve is justified by the fact that the contribution of the fast exponential, which is related to initial mixing of the test substance, to the total area under the curve is marginal. Nevertheless, care must be placed on reducing the first sampling time error, $e_1$, which is defined as the ratio between the value of the fast exponential and that of the slow exponential. The interval between the bolus injection and the first sampling time, $t_1$, should be therefore sufficiently long to allow a good mixing of the test substance in its distribution space. The results of this study suggest that 120 min is a good choice on average, but it may not be sufficient in all cases, as demonstrated by a high value of $e_1$ observed in one patient. Clinical characteristics of patients that indicate a potential slow mixing, such as cardiovascular diseases and prolonged bed resting, should be taken into account by increasing $t_1$. In any case the estimates of plasma clearance with the two-sample test appear to be relatively insensitive to deviations of the sampling schedule from the optimal one as observed with the small deviations of the estimates based on two samples compared to the reference values.

The precision of clearance estimates depend on individual kinetic parameters as well as on the measurement noise level which has been modeled in this study as heteroscedastic noise. Measurement noise becomes a relevant source of errors with low doses of radioactive tracers, which must be kept at minimum for reducing the effective radiation dose administered to patients with low clearance rates, as in PD. It is therefore important to optimize the sampling schedule which is directly related to the mean residence time of the test substance in the body, given as the ratio between the total distribution volume and the clearance. The sampling design approach adopted in this study differs from previous studies, e.g. [17], by the choice of the design criterion which is the direct minimization of the estimation variance of the plasma clearance. This criterion allows also the determination of test design rules for choosing the minimal dose to be injected for achieving a desired measurement precision in plasma clearance estimates [Thomaseth K, to be published].

The individualization of the optimal experiment design for each subject must therefore take into account clinical factors as well as laboratory measurement procedures whose precision varies with time and following periodic maintenance of the equipment. In order to avoid a priori estimation of individual $^{125}$Iiothalamate kinetics and of laboratory measurement errors, a fixed design with the first sample taken after 120 min and the second sample collected 3 days after the bolus injection should yield the best overall performance. These results obtained with $^{125}$Iiothalamate as test substance may be applicable also to other test substances, including non-radioactive compounds, e.g. [8] that exhibit a similar kinetic pattern.

The proposed two-sample test is simple to perform in PD and is suitable for use in clinical routine for the assessment of adequacy of PD treatment. In particular simultaneous measurement of plasma clearance and of total distribution volume allows the evaluation of dialysis dose and of fluid balance. Moreover, plasma clearance measured over 3 full dialysis days gives an average measure that allows a reliable determination of dialysis efficiency.

References


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