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Comparison of Some Practical Sampling Strategies for Population Pharmacokinetic Studies

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Using population analysis, sparsely sampled Phase 3 clinical data can be utilized to determine the pharmacokinetic characteristics of the target population. Data arising from such studies are likely to be constrained to certain sampling windows, i.e., the visiting hours at the study clinic. When the sampling window is narrow compared to the half-life of the drug, the advantage of taking more than one sample is not obvious. Study designs with one or two samples per visit have been compared with respect to (i) precision and bias of the population parameter estimates, (ii) the ability to identify the underlying pharmacokinetic model, and (iii) the estimation of individual parameter values. The first point was assessed using simulated data while the latter two were studied using a real data set. Results show: (i) Parameter estimates are more biased and imprecise when only one sample is taken compared to when two samples are obtained, this is true irrespective of the time span between the two samples. (ii) Ability to identify a more complex model is increased if two samples are taken. Specifically, the variability between occasions can be quantified. (iii) Two-sample designs are generally better with respect to prediction of individual parameter values. Even minor changes to commonly employed study designs, in this case the addition of one sample at each study occasion, can improve quality and quantity of the information obtained.

KEY WORDS: study design; sampling design; population analysis; NONMEM.

INTRODUCTION

Population analysis using nonlinear mixed-effects models is a commonly used tool to analyze sparse pharmacokinetic data. This type of data may arise from a number of situations; routinely collected clinical data, toxicokinetic studies, and Phase 2 and Phase 3 clinical studies (1). In recent years

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some work has been done using simulations to evaluate and compare designs of sparse data experiments (2,3,4). These studies evaluated the impact of altering the number of subjects and the number and timing of blood samples on the precision and bias of the estimated parameter values. The conclusions can be summarized broadly as follows: Increasing the number of individuals in the study improves the estimation of the parameters. This is true even if a large fraction of the subjects have only one sample each (2). Increasing the number of samples per individual also leads to increased predictive performance but not to the same extent as designs with more (sparsely sampled) individuals (4). The question of the timing and the number of the samples is a little more complex. It is well known that the sampling times can have a large impact on the precision and bias of parameter estimates in nonlinear regression type of data analysis (5). Unfortunately, to find optimal times for sampling, it is necessary to make quite precise assumptions regarding the model, the values of the parameters to be estimated, and, especially, the weighting scheme to be used in the analysis. If these assumptions can be made it is theoretically possible to devise an optimal design with respect to the precision of the estimated parameters (6). The effect of sampling times on the precision of the parameter estimates from an intravenous monoexponential model was explored by Al-Banna *et al.* (3). The results show that as early and as late as possible were the best time points if only two samples were taken (the model used was a one-compartment intravenous bolus model with constant coefficient of variation residual model). If a third sample was added to the best two-sample design, it mainly improved the estimation of the population random effects and it did not matter when the third sample was taken. Another sampling strategy is to randomize the observations. Hashimoto and Sheiner (4) showed that compared to fixed sampling times, randomization of the sampling times in all the individuals resulted in a high robustness to model misspecification.

It is not always possible to make observations either totally at random or at certain optimal time points. In a Phase 3 outpatient clinical study, for example, it is more likely that the sampling times will vary within certain sampling windows, circumscribed by the visiting hours at the clinic. These sampling windows do not necessarily coincide with the optimal sampling times and do not allow a total randomization. When, in addition, the sampling windows are narrow compared to the half-life of the drug, the main design question becomes one of number of samples. The present study evaluates the consequences of taking two samples instead of one during a visit to a clinic in a Phase 3 study. The influence of taking two samples on parameter precision and bias, as well as the ability to characterize the structural and statistical model and the capability to predict individual parameter values were investigated, using both simulated and real data.

METHODS

Simulations

Study Design

The basic study design of the simulated data sets was aimed at mimicking an outpatient Phase 3 clinical trial (including 100 patients) in which two visits to the study clinic were scheduled for pharmacokinetic observations, i.e., blood samples. Apart from blood sampling these visits are supposed to include other types of examinations and tests. The dosing interval is 12 hr with doses taken at 8 am and 8 pm. Visits can take place either between 10 and 12 am or between 2 and 4 pm (Fig. 1).

Data were generated according to six different sampling designs (Table I). Either the patients had samples taken at both a morning and an afternoon visit (the normal case) or they had only morning samples or afternoon samples (mm/aa). In the latter case, half of the patients had only morning samples and the other half only afternoon samples (Data sets 2 and 5 in Table I). The first sample was taken when the patient arrived at the clinic, which was at a time randomly chosen from 2, 2.5, 3, 3.5, or 4 hr postdose in the case of a morning visit or 6, 6.5, 7, 7.5, or 8 hr postdose in the case of an afternoon visit (which corresponds to 0, 0.5, 1, or 2 hr from the start of the visiting period, see Table I). The second sample, in the case of a two-sample design, could be at a time 0 (a replicate sample), 1 or 2 hr after the first sample. No sample was allowed to be taken outside the sampling window.

Generation of the Data

A one-compartment, first-order absorption, steady-state pharmacokinetic model was used to generate the concentrations in the simulated data sets

$$C_{ijk} = \frac{F_{ij} ka_i Dose}{V_i\left(ka_i - CL_i/V_i\right)}\left(\frac{\exp\left(-\frac{CL_i}{V_i}\cdot t_{ijk}\right)}{1-\exp\left(-\frac{CL_i}{V_i}\cdot \tau\right)} - \frac{e^{-ka_i\cdot t_{ijk}}}{1-e^{-ka_i\cdot\tau}}\right) \quad (1)$$

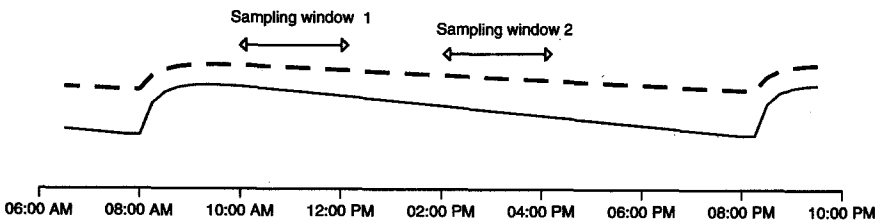

Fig. 1. The timing of the sampling windows. The curves are the log concentration–time curves for the short half-life (solid line) and the long half-life (broken line).

Table I. Sampling Designs Evaluated in the Simulations

Data set no.		Sampling times[a]	
		First visit	Second visit
1	One sample	2, 2.5, 3, 3.5, or 4	6, 6.5, 7, 7.5, or 8
2	One sample (mm/aa)[b]	2, 2.5, 3, 3.5, or 4	2, 2.5, 3, 3.5, or 4
		or	
		6, 6.5, 7, 7.5, or 8	6, 6.5, 7, 7.5, or 8
3	Two samples, $\Delta T=0$	2, 2.5, 3, 3.5, or 4	6, 6.5, 7, 7.5, or 8
		and	*and*
		2, 2.5, 3, 3.5, or 4	6, 6.5, 7, 7.5, or 8
4	Two samples, $\Delta T=1$	2, 2.5, or 3	6, 6.5, or 7
		and	*and*
		3, 3.5, or 4	7, 7.5, or 8
5	Two samples, $\Delta T=1$ (mm/aa)[b]	2, 2.5, or 3	2, 2.5, or 3
		and	*and*
		3, 3.5, or 4	3, 3.5, or 4
		or	
		6, 6.5 or 7	6, 6.5, or 7
		and	*and*
		7, 7.5, or 8	7, 7.5, or 8
6	Two samples, $\Delta T=2$	2	6
		and	*and*
		4	8

[a]Hours postdose.
[b]Half of the patients have only morning samples on both visits and the other half only afternoon samples after both visits.

F_{ij}, V_i, CL_i, and ka_i are the ith individual's parameter values where the subscripts i, j, and k denote individual, occasion, and sample respectively. Individual parameter estimates for CL, V, and ka were obtained according to

$$CL_i = CL \cdot e^{\eta_{iCL}} \tag{2a}$$

$$V_i = V \cdot e^{\eta_{iV}} \tag{2b}$$

$$ka_i = ka \cdot e^{\eta_{ika}} \tag{2c}$$

where CL, V, ka, and F are the typical values of the parameters in the population and CL_i, V_i, and ka_i are the ith individual's parameter value.

The assumption of stationarity is often made in studies performed on two or more occasions. In many cases though, this assumption may be invalid (7,8,9). To evaluate the impact of nonstationarity the following statistical models were used to simulate values for F.

$$F_{ij} = F \cdot e^{\kappa_{ijF}} \tag{3}$$

F_{ij} are the ith individual's value of F at study occasion j. The two random variables η_P (where P is one of CL, V, or ka) and κ_F denote interindividual (IIV) and interoccasion (IOV) variability, respectively. η_P and κ_F are normally distributed with mean zero and variances ω_P^2 and π_F^2 respectively. It was assumed that CL, V, and ka had only IIV and that F had only IOV. In addition to the models for IIV and IOV, a positive correlation between CL and V was also included in the generation of the data. This may be viewed as if there is some covariate that influences both clearance and volume, for example protein binding or body size. "Observed" concentrations ($C_{\text{obs},ijk}$) were generated from the true concentrations by addition of random errors according to

$$C_{\text{obs},ijk} = C_{ijk} e^{\varepsilon_{ijk}} \tag{4}$$

ε_{ijk} is normally distributed with mean zero and variance σ^2. Since the benefit of two narrowly spaced samples is likely to be dependent on the difference in concentration between the sampling times, two different half-lives were used in the simulations, 6 or 12 hr.

Thirty data sets with 100 individuals in each were generated and analyzed for each design and parameter set. The parameter values of both the pharmacokinetic and statistical parameters used in the simulations are displayed in Table II.

Analysis of the Generated Data

The models used to analyze the simulated data differed depending on whether the model used to generate the data included IOV in F or not. The model used to analyze the simulated data without IOV was identical to the model used to generate the data except that ka was not estimated but rather fixed to its true value and that ω_{ka} was fixed to zero. The reason for this

Table II. Summary of Parameter Values Used in the Simulations and How Parameters Were Treated in the Analysis

	Simulation	Analysis
CL (L/hr)	11.55/5.8[a]	Estimated (CL/V)
V (L)	100	Estimated (V/F)
ka (hr^{-1})	2.08	Fixed to 2.08
ω_{CL}	0.3	Estimated
ω_V	0.3	Estimated
ω_{ka}	0.3	Not included
π'_F	0/0.1[b]	0/approximated by IOV in CL/F and V/F ($\kappa_{CL} = \kappa_V$)[b]
$\text{cov}(\eta_{CL}, \eta_V)$	0.045	Estimated
ε	0.15	Estimated

[a] Half-life = 6 hr/half-life = 12 hr.
[b] Data generated without IOV/data generated with IOV.

was that the simulated data sets contained no, or very little, information about the absorption process and hence problems with the identification of *ka* could be anticipated. Wade *et al.* (10) have shown that if the data to be analyzed do not contain information about absorption, then there will be no adverse effect (such as bias appearing in other parameters) if *ka* is fixed to a value similar to the true value.

The model used to analyze the data generated with IOV in F differed in one additional respect from the true model. For two samples per occasion data sets Eqs. (5a) and (5b) were used to model the IOV due to IOV in F and the IIV in CL and V, respectively.

$$(CL/F)_{ij} = CL/F \cdot e^{(\eta_{iCL} - \kappa_{ijF})} \tag{5a}$$

$$(V/F)_{ij} = V/F \cdot e^{(\eta_{iV} - \kappa_{ijF})} \tag{5b}$$

The IOV term (κ_{ij}) was omitted from the model when analyzing the single sample per occasion data sets since it is not possible to estimate IOV when having only one sample per occasion. The way the parameters were treated during the analysis is summarized in Table II.

Data were analyzed using both the first-order approximation (FO) and the first-order conditional method (FOCE), as implemented in NONMEM version IV (11). During the analysis, if a run resulted in an unsuccessful termination, all parameter estimates from that particular data set were excluded from the subsequent analysis.

The evaluable parameter estimates were used to calculate mean absolute error (precision), Eq. (6), and mean error (bias), Eq. (7) (12).

$$Precision = \frac{\sum_{n=1}^{N} \left| \frac{\tilde{P}_n - P_T}{P_T} \right|}{N} \tag{6}$$

$$Bias = \frac{\sum_{n=1}^{N} \frac{\tilde{P}_n - P_T}{P_T}}{N} \tag{7}$$

where P_T is the true parameter value, $\tilde{P}_n$ is the estimated population parameter value from the nth simulated data set and N is the number of successfully terminated runs.

Simulations with a Fixed Number of Samples

The cost of bioassay or sample handling may limit the possibility to change from a one-sample design to a two-sample design without other modifications to the study design. If the number of samples per patient is to be increased, the number of sampled patients may have to be decreased.

In order to evaluate a strategy with a fixed number of samples, two additional designs were tested. The first design includes 50 patients with the same sampling scheme as Data set 6 (two samples per visit) in Table II. The second design includes 75 patients of which 25 had the same sampling design as Data set 6 (two samples per visit) and 50 with the same sampling design as Data set 1 in Table II (one sample per visit). The total number of samples in these two designs is 200, the same number of samples as in Data set 1 in Table II.

The data sets with 50 and 75 patients were generated and analyzed in the same way as Data sets 1–6, except that only the short half-life, IOV and FO was used.

Real Data

A real data set was used to investigate the ability of the different sampling designs to identify the correct pharmacokinetic and statistical models and to predict individual parameter values. The data set used herein has been described previously (13,14) as Drug C and B, respectively.

The real data set consists of observations from oral drug therapy, administered twice daily in 64 individuals. After at least 8 weeks of continuous therapy the pharmacokinetics were studied during a 12-hr dosing interval. After a further 4 weeks 35 of the patients were studied again. The sampling scheme on both study occasions was relatively extensive: 10 samples per individual and study occasion (1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 hr postdose).

For the present work the full data set was truncated in order to mimic the sampling designs described Table I. By different combinations of 6 sampling times (2, 3, 4, 6, and 8 hr postdose), 2 single-sample designs (similar to Designs 1 and 2 in Table I) and 3 two-sample designs (similar to Designs 4, 5, and 6 in Table I) were created. This was accomplished by randomly assigning a sampling time for the first sample on each study occasion (the only sample in the case of a one-sample design). The second sampling time was then given by addition of the predefined time span between the samples (i.e., 1 or 2 hr). All of the reduced data sets (including all 64 patients) together with the full data set were used for the purpose of model identification and in the prediction of individual parameter values. The real data sets were analyzed using the FO method.

Model Identification

The model (without covariates—see below) that best described the full data set is denoted the full model. The ability of the various reduced data sets, i.e., different sampling designs, to characterize the full model was

assessed by comparing the final model characterized by each reduced data set to the full model.

Previous analysis of the real data set found that a one-compartment first-order absorption model with both IIV and IOV in all basic parameters (CL/F, V/F, and ka) and a constant CV residual error model best described the full data set [the model is described by Eqs. (1)–(4)]. CL/F and V/F were also found to be correlated, not only between individuals but also within individuals between occasions (14). In addition, a comprehensive covariate model was built (13). In the present work the covariate model was not considered necessary to illustrate the aims of the study and was therefore not included.

Preliminary runs showed that all the truncated data sets successfully identified CL/F and V/F but had problems with estimating ka. This was expected since the data sets generally contained very little information about the absorption process. Accordingly, ka was fixed to the estimate from the full model when analyzing the truncated data sets (10). The validity of this simplification was tested by repeating the same analysis but with 50% lower and 50% higher values for the fixed ka. The problem of model identification was therefore reduced to a problem of identifying the different parts of the models for the IOV and IIV in CL/F and V/F, that is ω_{CL}, ω_V, κ_{CL}, κ_V, $\text{cov}(\eta_{CL}, \eta_V)$, $\text{cov}(\kappa_{CL}, \kappa_V)$. Models with different combinations and numbers of these parameters were tested, the complexity ranging from individual estimation of all of the above parameters to a single interindividual variability parameter of $\eta_{CL} = \eta_V$ (Table III). Discrimination between

Table III. Precision and Bias (%) for Parameter Estimates Obtained when Number of Samples Are Kept Constant While Number of Individuals Is Altered

	100 patients 200 samples[a]		75 patients 200 samples[b]		50 patients 200 samples[c]		100 patients 400 samples[d]	
	Precision	Bias	Precision	Bias	Precision	Bias	Precision	Bias
CL/F	5	−5	6	−5	5	−4	6	−6
V/F	7	−4	9	−6	8	−3	5	−4
ω_{CL}	9	4	10	0	11	−1	9	5
$\text{cov}(\eta_{CL}, \eta_V)$	46	14	47	2	58	−7	52	7
ω_V	51	−1	27	4	25	−10	25	−5
κ'_F			34	2	43	−5	32	2
σ	31	19	13	1	8	2	6	5

[a] The same as Data set 1 in Table I (1 + 1 sample).
[b] Fifty patients had the same type of sampling schedule as Data set 1 in Table I (1 + 1 single sample) and 25 patients had the same type of sampling schedule as Data set 6 in Table I (2 + 2 samples).
[c] All 50 patients had the same type of sampling schedule as Data set 6 in Table I (2 + 2 samples).
[d] The same as Data set 6 in Table I (2 + 2 samples). The numerical results from the 2 + 2 samples in 100 patients (Data set 6 in Table I) are included as reference.

different models was via comparison of the objective function values calculated by NONMEM. The difference between the objective function values for two hierarchical models is approximately chi-square distributed and may consequently be used for model selection purposes (15). In this study $p < 0.05$ was used as the significance level.

Prediction of Individual Parameter Values

The ability to predict individual parameters was evaluated by comparing the best individual parameter estimates (the empirical Bayes estimates from the full data set analyzed with the full model) to empirical Bayes estimates obtained from the best model for each of the truncated data sets.

RESULTS

Simulations

When the FOCE method was used, up to three data sets had to be excluded due to unsuccessful termination from a single set of simulation conditions. When the FO method was used, all data sets terminated successfully.

The results from the analysis of the data generated without and with IOV are shown in Figs. 2 and 3, respectively. The symbols represent absolute values of bias (broken lines) and precision (solid lines) for the parameters obtained with the long half-life and FO (east), the short half-life and FO (west), the long half-life and FOCE (north), and the short half-life and FOCE (south). The correlation between the size of the reference stars and the magnitude of the bias/precision is given by the value to the right of each row.

When IOV is absent, the benefits of adding a second sample on each occasion are most pronounced for the precision in ω_V and σ. When IOV is present, the single-sample designs are associated with greater bias and imprecise estimates for all parameters, except CL/F and ω_{CL}, when compared to the two-sample designs. Prolonging the time span between the samples in the two-sample designs had virtually no effect on estimation of any of the parameters. When considering the effect of different lengths of the half-life, it can be seen that the precision is higher for V/F, ω_V, and $\text{cov}(\eta_{CL}, \eta_V)$ when the half-life is short, irrespective of the presence of IOV. When FOCE, rather than FO, is used in the analysis the precision is higher and bias lower for CL/F, ω_{CL} and π_F. In the single-sample designs where the patients have only morning or afternoon samples, the precision is lower in parameters relating to V [i.e., V/F, ω_V, $\text{cov}(\eta_{CL}, \eta_V)$].

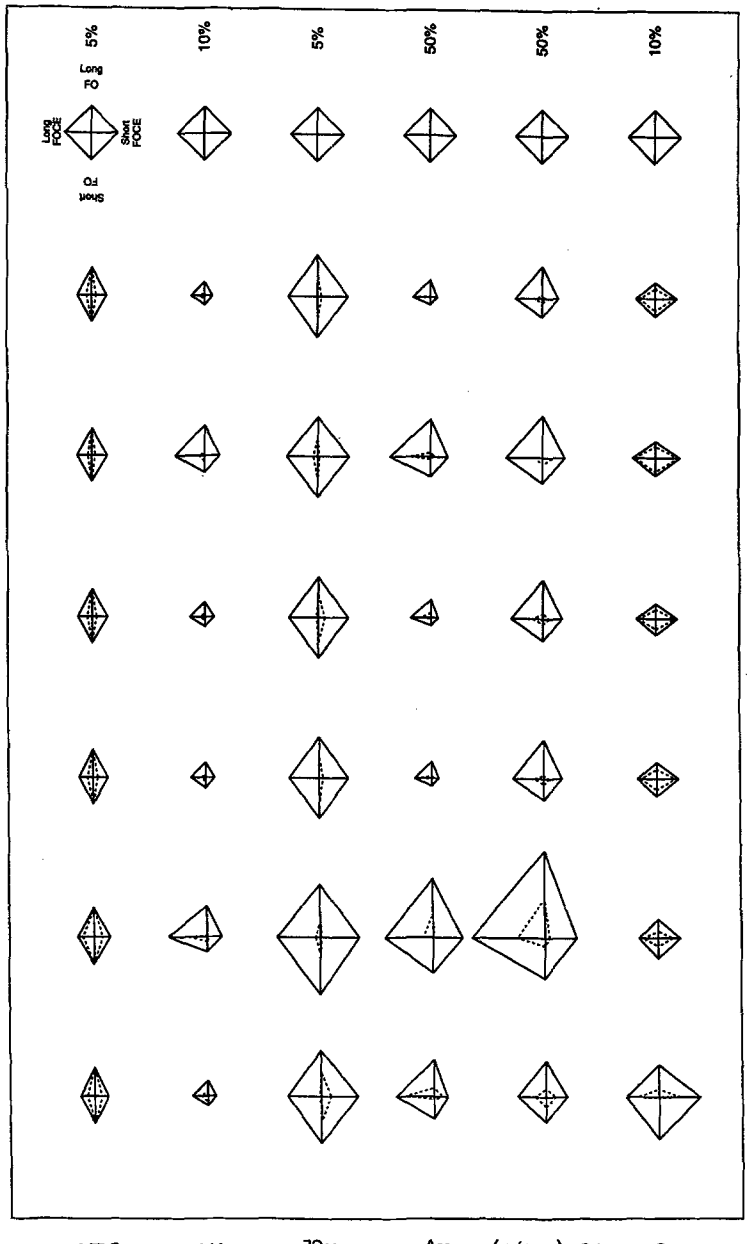

Fig. 2. Parameter precision and bias from the simulations without IOV. The symbols represent absolute values of bias (broken lines) and precision (solid lines) for the parameters obtained with the long half-life and FO (east), the short half-life and FO (west), the long half-life and FOCE (north), and the short half-life and FOCE (south). The size of the reference stars is given by the value to the right of each row. The length of each axis is proportional to the value to the right of the respective row.

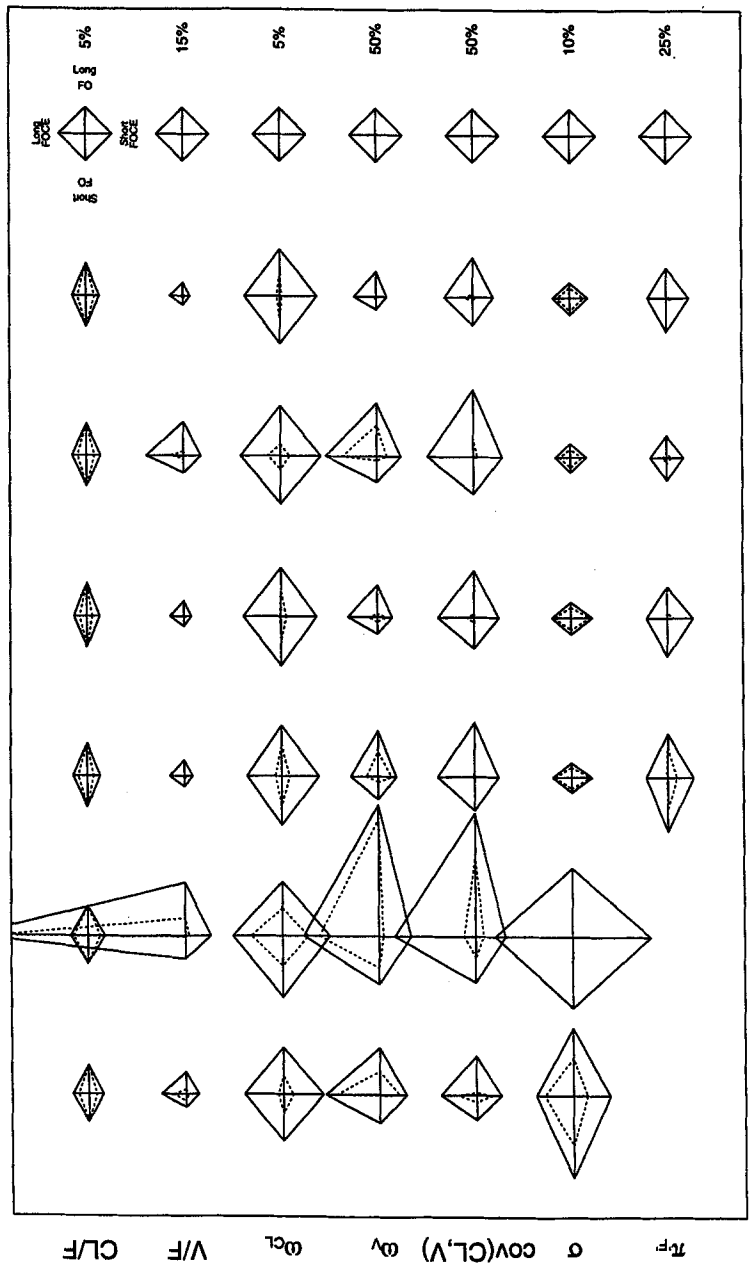

Fig. 3. Parameter precision and bias from the simulations including IOV. The symbols represent absolute values of bias (broken lines) and precision (solid lines) for the parameters obtained with: the long half-life and FO (east), the short half-life and FO (west), the long half-life and FOCE (north), and the short-half life and FOCE (south) when IOV was included in the simulations. The size of the reference stars is given by the value to the right of each row. The length of each axis is proportional to the value to the right of the respective row. π_F is used to denote the IOV estimated in CL/F and V/F.

Simulations with a Fixed Number of Samples

The results from the simulations with a fixed number of samples are displayed in Table II. The results from Data set 6 in Table I (400 samples in 100 patients) are included as reference. The three designs perform similarly with respect to precision and bias in CL/F, V/F, and ω_{CL}. The 100- and 75-patient designs are better with respect to precision in $\text{cov}(\eta_{CL}, \eta_V)$ compared to the 50-patient design while the 100-patient design yields the most biased estimate. The 50- and 75-patient designs are more precise in the estimation of ω_V and σ compared to the 100-subject design. The 100-patient design also produces the most biased estimate of σ while the 50-patient design gives the most biased estimate of ω_V.

Real Data Set

The results of the model identification are displayed in Table IV. None of the reduced data sets were able to identify the full model but the best models for the two-sample designs were closer to the full model than the best models for the corresponding single-sample designs. It can also be seen that the mm/aa designs performed worse than the corresponding balanced morning–afternoon design.

The parameter values from the final models are displayed in Table V. The basic pharmacokinetic parameters (CL/F and V/F) are fairly well estimated, except for the one-sample mm/aa design. The estimated magnitudes of the interindividual variabilities (both ω_V and ω_{CL}) increases when the number of samples decreases. Analyzing the final models with an incorrectly fixed *ka* resulted in values of precision and bias in parameter estimates that differed no more than 10% (not displayed) from the estimates obtained when *ka* was fixed to the value found with the full model.

The individual parameter estimates for the different sampling designs are plotted against the parameter values obtained from the full data set in Fig. 4 for CL/V and V/F, respectively. The dashed line is the line of identity and the solid line is the regression line. Individual predictions are shrunk towards the population mean value (a horizontal solid line would indicate total shrinkage to the population mean value) when the number of observations decreases. In addition, it can be seen that the mm/aa designs suffer to a greater extent from this shrinking effect than do their more balanced counterparts.

DISCUSSION

Traditionally the characterization of pharmacokinetic parameters during drug development has been performed using experimental protocols that

Table IV. The Difference in Objective Function Value Obtained with Different Models Compared to Best Model for Each of the Real Truncated Data Sets[a]

Model no.	Variance parameters estimated	n^d	Two samples $\Delta T=2$	Two samples $\Delta T=1$	Two samples $\Delta T=1$ (mm/aa)	One sample	One sample (mm/aa)
1	ω_{CL}, ω_V, π_{CL}, π_V, cov(η_{CL}, η_V), cov(κ_{CL}, κ_V)	6	−2.7[b]	−0.1	−2.7	−4.6	−0.4
2	ω_{CL}, ω_V, π_{CL}, π_V, cov(κ_{CL}, κ_V)	5	1.5	4.1	−2.5	−4.5	16.8
3	ω_{CL}, ω_V, π_{CL}, π_V, cov(η_{CL}, η_V)	5	−0.1	0.6	−1.4	−3.0	−0.3
4	ω_{CL}, ω_V, π_{CL}, cov(η_{CL}, η_V)	4	1.6	0.6	−0.1	0.0	−0.2
5	ω_{CL}, ω_V, π_V, cov(η_{CL}, η_V)	4	11.6	10.1	−1.4	−2.3	−0.2
6	ω_{CL}, ω_V, $\pi_{CL}=\pi_V$, cov(η_{CL}, η_V)[b]	4	**0.0**	**0.0**	−0.1	0.0	−0.2
7	ω_{CL}, ω_V, π_{CL}, π_V	4	14.8	22.6	5.0	7.3	20.0
8	ω_{CL}, ω_V, π_{CL}	3	22.8	22.6	5.0	7.3	20.0
9	ω_{CL}, ω_V, π_V	3	30.8	35.8	5.0	7.3	20.0
10	ω_{CL}, ω_V, $\pi_{CL}=\pi_V$[b]	3	6.1	9.0	5.0	7.3	20.0
11	ω_{CL}, ω_V, cov(η_{CL}, η_V)	3	11.3	10.1	**0.0**	**0.0**	−0.8
12	ω_{CL}, ω_V	2	31.8	35.8	5.0	7.3	20.0
13	ω_{CL}	1	39.8	83.9	5.0	7.3	22.0
14	ω_V	1	121.9	141.5	19.0	19.2	31.1
15	$\omega_{CL}=\omega_V$[c]	1	—	—	—	—	**0.0**

[a]The value for the best models for the respective designs are in boldface type and have the value of zero.
[b]corr(κ_{CL}, κ_V)=1.
[c]corr(η_{CL}, η_V)=1.
[d]Number of estimated parameters in the model for IIV and IOV.

Table V. Parameter Estimates and Relative Standard Errors Obtained with Final Models for the Truncated Real Data Sets

	Full data	Two samples $\Delta T=2$	Two samples $\Delta T=1$	Two samples $\Delta T=1$ (mm/aa)[a]	One sample	One sample (mm/aa)
Model no.[b]		6	6	11	11	15
ka (hr^{-1})	1.7 (11%)	1.7[c]	1.7[c]	1.7[c]	1.7[c]	1.7[c]
CL/F (L/hr)	19 (5%)	18 (5%)	18 (5%)	18 (5%)	18 (4%)	17 (6%)
V/F (L)	87 (4%)	85 (8%)	81 (7%)	85% (10%)	60 (12%)	60 (12%)
ω_{ka}	62% (105%)	—[d]	—	—	—	—
ω_{CL}	33% (22%)	30% (25%)	33% (21%)	35% (32%)	34% (27%)	37% (21%)
ω_V	35% (24%)	25% (119%)	40% (45%)	40% (123%)	47% (91%)	(same as above)
$\text{cov}(\eta_{CL}, \eta_V)$	0.09 (28%)	0.07 (42%)	0.10 (33%)	0.11 (53%)	0.12 (41%)	—
π_{ka}	100% (33%)	—	—	—	—	—
π_{CL}	19% (20%)	22% (38%)	19% (42%)	—	—	—
π_V	12% (35%)	(same as above)	(same as above)	—	—	—
$\text{cov}(\kappa_{CL}, \kappa_V)$	0.04 (27%)	—	—	—	—	—
σ	13% (14%)	17% (27%)	11% (23%)	23% (74%)	20% (85%)	20% (26%)

[a]mm/aa means that half of the patients have only morning samples and the other half only afternoon samples.
[b]The model number refers to the models listed in Table III.
[c]*ka* was fixed to the estimate from the full model when analyzing the truncated data sets.
[d]Parameter was not included in the model.

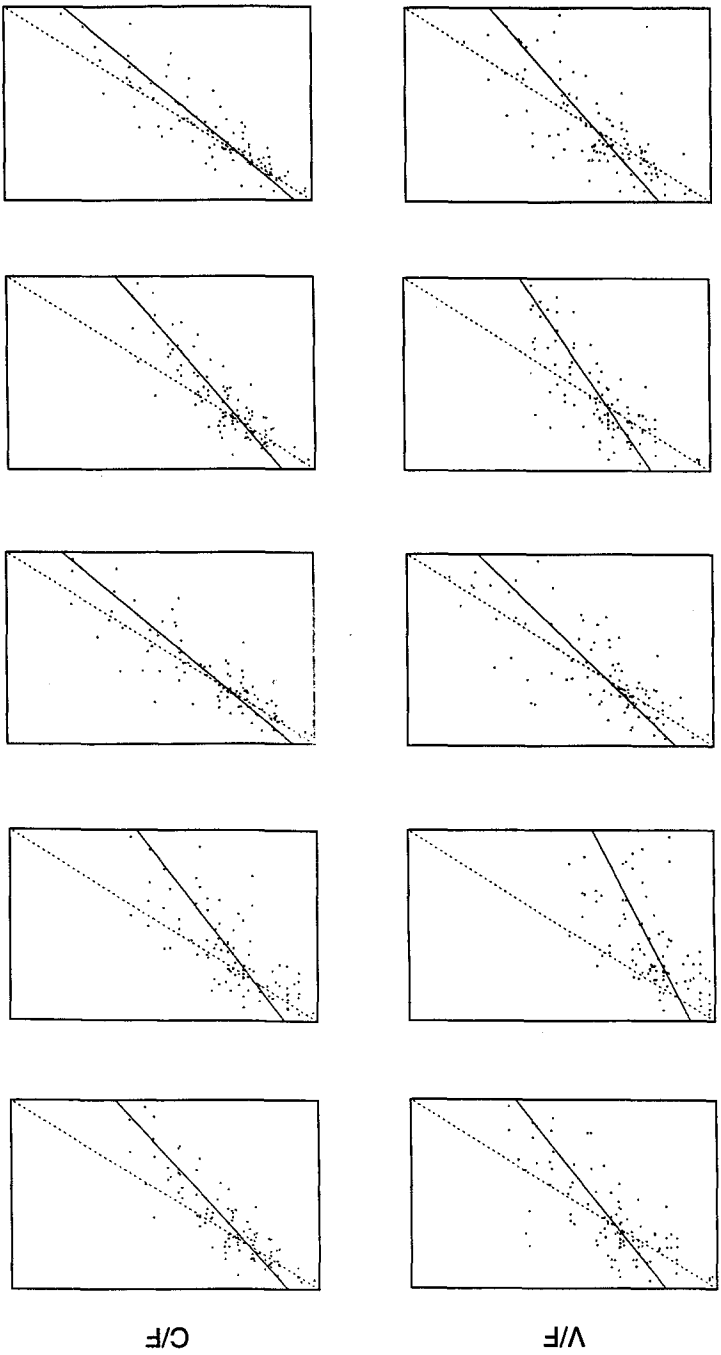

Fig. 4. The individual Bayesian parameter estimates from the truncated data sets plotted against the estimates obtained with the full data set. The broken lines are the lines of identity and the solid lines are the regression lines. The best model for each data set, as given in Table IV, was used to obtain the individual estimates.

employ intensive sampling. This may not be possible in the target population. With the introduction of population pharmacokinetics it is no longer necessary to make frequent observations in each individual participating in the study. It has been shown that as few as one to two samples in the majority of the study population is sufficient to estimate the mean of the pharmacokinetic parameter values and their respective variabilities in the population (2). This opens up the possibility of using fragmentary (i.e., 1–2 samples per individual) clinical trial data to estimate pharmacokinetic parameters. In this study we propose and evaluate one possible way of increasing the amount of information contained in the data collected during a Phase 3 clinical trial without a dramatic change in the sampling design. This is accomplished by taking two samples instead of one on each or some of the times the patients visit the clinic.

The major conclusions to be drawn from the work presented here are:

1. A design with a second sample at each study visit produces parameter estimates that are at least as, and often more, precise and unbiased as a design with only one sample per visit and the same number of subjects. This is true irrespective of the time-span between the two samples.
2. A two-sample design improves the ability to identity a more complex statistical model. Especially, it enables the IOV to be quantified.
3. The capability to predict individual parameter values is greatly improved when a second sample is added.
4. If it is not possible to increase the number of samples it may still be better, with respect to parameter precision and bias, to take two samples per visit in some of the patients, even if the total number of patients has to be reduced to keep the total number of samples constant.
5. Sampling designs where one fraction of the patients have only early samples and the other fraction have only late samples are inferior to designs in which the majority of the patients have both early and late samples. This is true even if the total number of early and late samples are the same in both designs.

The finding that an increased amount of data improves the results of the data analysis is not surprising. More interesting is that the timing of the second sample, within the interval studied, is virtually unimportant. It can be seen in Figs. 2 and 3 that increasing the time span from zero to 2 hr does not lead to a markedly increased performance.

It is logical to assume that a more complex model, i.e., a model closer to the true model, produces better and more meaningful parameter estimates than a less complex model suffering from over simplification. A good example of this is the ability to include IOV in the model. By being able to quantify IOV it is possible to draw conclusions about, for example, the

possibilities of individualizing drug therapy (14). In addition to providing more information, including IOV (if it is present in the data), is also important in order to prevent bias from appearing in other parameters and to avoid erroneous covariate models from being selected (14). Consider the simulations with IOV as an example. When only one sample per occasion was taken the IOV could not be modeled leading to considerably worse predictions of the parameters concerned with volume compared to those estimated when IOV was accounted for.

The importance of being able to predict individual parameter values depends on the objectives of the study in question. If the investigator wishes to explore the possible effects of demographic factors (such as weight, age, and clinical laboratory data) on the pharmacokinetic parameters, as often is the case, predictions of individual parameter values provide a useful tool to accomplish this. It has been shown that the use of empirical Bayesian estimates of the individual parameter values leads to a reduction of the time necessary to build covariate models (13,16). The usefulness of such model-building strategy is however related to the accuracy and precision of the individual parameter estimates. A second sample will decrease the degree of shrinkage towards the population mean value of the parameter in question. In Fig. 4, where the shrinkage effect is investigated using the real data set, the value of the addition of a second sample can be seen.

If it is not possible to influence the timing of the samples, as assumed in the designs used in the present work, the main design factors are the number of samples and number of patients. If the total number of samples collected is the limiting factor in a design, any increase in the number of samples per individual must be accompanied by a similar reduction in the number of studied patients. When designs with a fixed number of samples were compared, the most advantageous design, with respect to parameter estimation, was a design where two samples were collected in only part of the study population (e.g., the data set with 75 patients). Such a design allows all variability components to be estimated and still has a relatively large number of patients (which is beneficial from a covariate model-building point of view). It is worth noting that there is no great loss in parameter precision with the 50-patient design compared to the corresponding 100-patient design.

The inferior performance of the unbalanced designs, i.e., mm/aa designs, is quite clear throughout the results of this study. These designs are, with two exceptions, consistently inferior to the designs in which each subject has both morning and afternoon samples. These exceptions are the estimation of the residual error in the single-sample mm/aa case when no IOV was included in the generation of the data and in the two-sample mm/aa case when the data were simulated with IOV. In both these cases σ was

estimated with better precision and less bias compared to the corresponding single- or two-sample design. On the other hand, the rest of the variance parameters estimates from the mm/aa designs were more imprecise and biased compared to their balanced counterparts. It seems preferable to design studies so that each individual has a balanced sampling design.

That unsuccessful termination appear with the FOCE algorithm and not with the FO algorithm is not unexpected since rounding error problems can occur with the FOCE method even if the FO algorithm terminates successfully (11). The unsuccessfully terminated runs were evenly spread between the different designs and no pattern could be found.

The fact that FOCE performs better than FO for some of the parameters shows that FOCE might improve the precision and bias of the estimates even in situations with sparse data and a relatively linear model (11). Since the data analyzed, both simulated and real, are steady state data, it is not surprising that the estimates pertaining to V are less precise and more biased compared to CL (note the difference in reference value for CL/F and V/F in Figs. 2 and 3). This is due to the fact that steady state data contain more information about CL/F (the main source of information about this parameter are the drug levels) than about V/F, which, with steady state data, is determined mainly by the fall in the concentration–time curve. This also explains why only the parameters relating to V/F are influenced by the length of the half-life. Expressing the length of the sampling windows as percentage of the half-life (approximately 17 and 33% for the long and short half-life, respectively) shows that the samples taken within a sampling window cover a larger part of the elimination phase when the half-life is short, i.e., the fall in concentration is better described and hence the V/F.

As pointed out in the Introduction, the optimality of the sampling times depends on the error model used. The numerical results (i.e., the actual figures) consequently are dependent upon the weighting scheme used, but it is doubtful whether the other, more qualitative results (model-finding ability, for example), are as sensitive. However, an error model with (essentially) constant CV is often appropriate when analyzing pharmacokinetic data (authors experience).

The present work shows that even minor alterations to a practical and "natural" study design lead to improvements in the outcome of the data analysis. In this special case the improvement was due to the addition of a second blood sample at each or some of the study occasions. The benefits included generally improved parameter estimation, facilitated model-finding capability, and better predictions of the individual parameter estimates.

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