Optimal Sampling Schedule Design for Populations of Patients

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Generation of pharmacodynamic relationships in the clinical arena requires estimation of pharmacokinetic parameter values for individual patients. When the target population is severely ill, the ability to obtain traditional intensive blood sampling schedules is curtailed. Population modeling guided by optimal sampling theory has provided robust estimates of individual patient pharmacokinetic parameter values. Because of the wide range of parameter values seen in this circumstance, it is important to know how the range of parameter values in the population affects the timing of the optimal samples. We describe a new, simple technique to obtain optimal samples for a population of patients. This technique uses the nonparametric distribution associated with a nonparametric adaptive grid population pharmacokinetic analysis. We used the distribution from an analysis of 58 patients receiving levofloxacin for nosocomial pneumonia at a dose of 750 mg. The collection of parameter vectors and their associated probabilities were entered into a D-optimal design evaluation by using ADAPT II. The sampling times, weighted for their probabilities, were displayed in a frequency histogram (an expression of how system information varies with time for the population). Such an explicit expression of the time distribution of information allows rational sampling design that is robust not only for the population mean vector, as in traditional D-optimal design theory, but also for large portions of the total population. For levofloxacin, one reasonable six-sample design would be 1.5, 2, 2.25, 4, 4.75, and 24 h after starting a 90-min infusion. Such sampling designs allow informative population pharmacokinetic analysis with precise and unbiased estimates after the maximal a posteriori probability Bayesian step. This allows the highest probability of delineating a pharmacodynamic relationship.

The goal of anti-infective agent chemotherapy is to give infected patients a dose of drug that has the highest possible probability of achieving the desired therapeutic end point (clinical response, microbiological response, suppression of resistance) while simultaneously having an acceptably low probability of generating a concentration-related adverse event. To achieve this goal, it is necessary, as a first step, to develop exposure-effect as well as exposure-toxicity relationships.

While this has been difficult in the past, the advent of newer mathematical techniques such as population pharmacokinetic modeling, maximal a posteriori probability (MAP) Bayesian estimation and linkage to end points through modeling with logistic regression, and Cox proportional-hazard modeling has allowed the direct delineation of exposure-effect as well as exposure-toxicity relationships.

Most of the data necessary for construction of such relationships (and the most expensive data) have already been collected as part of the Food and Drug Administration-mandated phase II/III clinical trial structure. For a case to be both clinically and microbiologically evaluable, it is necessary that a database that documents that the patient meets the trial entry criteria and does not meet any exclusion criteria be established. In addition, the clinical and microbiological end points at the test-of-cure visit must be documented and a pathogen appropriate to the pathological process needs to be isolated along with demonstration that the pathogen is sensitive to the drug being studied for the infection.

Currently, clinical trials are rarely set up to delineate an exposure-response relationship. The major deficiency in the data set needed to establish an exposure-response (or exposure-toxicity) relationship is the exposure of a particular patient to the drug being studied. Prior studies have demonstrated that, in the clinical arena, even a drug, such as levofloxacin, that exhibits a relatively low degree of pharmacokinetic variability will have a relatively broad spread of clearance in the target population (9). Often, only a single drug dose is studied in clinical trials. Even if different doses are evaluated in a clinical trial, the broad range of clearance observed among patients will then result in a broad range of drug exposures, making delineation of an exposure-response relation by dose or even by the ratio of dose/MIC very difficult, if not impossible.

This means that, to achieve our goals, we need to obtain blood samples to determine a concentration-time profile in the patients being studied. The ill nature of real patients and their need for clinical care preclude attaining robust 10 to 15 sample data sets in most instances. We need a way of minimizing patient invasion while maintaining the ability to generate good point estimates of the patient's pharmacokinetic parameter values.

One method for doing this is to obtain multiple randomly obtained samples from a patient and use population modeling techniques (1, 7) along with MAP Bayesian estimation to obtain point estimates of the patient's parameter values. Our group (3) and Carter et al. (A. A. Carter, M. N. Dudley, C. M. Horton, S. Kaul, L. Dunkle, K. Mayer, K. K. Graham, and S. Geletko, Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. A3, 1995) have shown that random sampling

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may result in biased parameter estimates for an individual patient. Such an outcome for a subset of patients would, because of its nature, bias the pharmacodynamic relationship being developed. On the other hand, we have shown that small data sets of samples that are D-optimally informative generate good parameter estimates (4–6).

Traditional D-optimal design theory has the property of replication. That is, there is an explicit assumption of one true parameter vector. This results in the number of optimal samples being exactly identical to the number of system parameters. If one were willing to collect more than this number of samples, the D-optimal design calculation would ask that the extra sample(s) be a replicate of one of the originally identified samples.

Unfortunately, true variability among patients in pharmacokinetic parameter values exists. This variability still exists after accounting for physiological factors, such as differences in glomerular filtration rate across populations. It is important then to derive a method for choosing sampling times that are robust for obtaining parameter estimates from patients whose true values fall away from the parameter mean. Some investigators have published approaches to this problem using software that is not widely available. We describe below a simple method using easily available software that achieves the same end.

MATERIALS AND METHODS

Patients. The patients whose data were analyzed participated in a trial of levofloxacin for the therapy of nosocomial pneumonia (M. Oross, B. Weisinger, S.-C. Chien, V. Reichl, A. Tennenberg, J. Kahn, and members of the Nosocomial Pneumonia Study Team, Abstr. 10th Int. Congr. Infect. Dis., abstr. DO113, 2002). Patients had six plasma samples drawn over 24 h at steady state (predose, circa 5 min after a 1.5-h drug infusion of 750 mg, and at 2.25, 4.5, 5.5, and 7.5 h of the dosing interval).

Population pharmacokinetic analysis. All plasma concentrations were modeled by the nonparametric adaptive grid (NPAG) approach with optimized gamma (8). A two-compartment model with first-order elimination and first-order intercompartmental transfer was employed, as previous levofloxacin data were available to us to guide model choice (M. Willmann, M. Khashab, A. T. Chow, S.-C. Chien, and J. Kahn, Abstr. 10th Int. Congr. Infect. Dis., 2002). Input was intravenous, with a zero-order, timed infusion of approximately 1.5 h at a rate of approximately 500 mg/h. The infusion times for the studied interval were directly timed. The real infusion time and actual sample acquisition times were employed in the analysis.

Weighting was accomplished by fitting polynomials of up to the third order to the between-day variability data of the high-performance liquid chromatography (HPLC) assay employed. The explicit assumption that overall observation variance was proportional to assay variance was made. The proportionality constant gamma was optimized with each cycle of the analysis. The weighting was as the inverse of the estimated observation variance to provide an approximation to the homoscedastic assumption.

The drug concentrations in plasma were assayed by a sensitive and specific HPLC assay (12). The sensitivity of the assay was 0.0864 mg/liter. The assay was linear over the range of 0.0864 to 10.202 mg/liter. Concentrations exceeding this value were diluted into the linear range. The between-day coefficient of variation of the assay was 9.7% at 0.0864 mg/liter and 4.7% at 10.202 mg/liter.

D-optimal design. The optimal sampling times were estimated by using the design module of the ADAPT II package of programs of D'Argenio and Schumitzky (D. Z. D'Argenio and A. Schumitzky, user's manual for ADAPT II, Biomedical Simulations Resource, University of Southern California.). D-optimality was the design criterion employed (the determinant of the inverse Fisher information matrix was the scalar that was optimized).

Obtaining estimates of sampling times for more than one true parameter vector. Part of the output of the population pharmacokinetic analysis is a so-called MM (multiple-model) file. This file contains the vectors and the associated probabilities for the nonparametric parameter distributions. This allowed us to estimate optimal sampling times for each of these vectors. These times were then plotted for each vector on a frequency histogram where the total number of

TABLE 1. Pharmacokinetic parameter values and their distribution for levofloxacin as determined from 58 patients treated for nosocomial pneumonia^a

Value	Vol (liters)	K_{cp} (h^{-1})	K_{pc} (h^{-1})	CL (liters/h)
Mean	34.42	7.653	6.071	7.238
Median	23.32	2.662	0.924	6.242
SD	33.51	9.591	12.03	4.360

^a Vol, volume of distribution of the central compartment; K_{cp} and K_{pc} , first-order intercompartmental transfer rate constants connecting the central and peripheral compartments; CL, total plasma clearance.

intervals was 96 for a 24-h steady-state dosing interval (i.e., each interval represented 15 min of the day). This was chosen empirically, and other, finer evaluations (1- or 5-min intervals, etc) are certainly possible. Our choice was based on the reality that in the clinic, while the recording of sample acquisition time can be precise to seconds, the actual ability to obtain the sample is limited to a span of time. As an optimal sampling time was plotted on the frequency histogram, it was multiplied by its probability. When all parameter vectors are evaluated and plotted, we have, in essence, a distribution over the population of the system information. Optimal sampling times can then be chosen based on the peaks of the information content within the dosing interval. To put the distribution in perspective, we also evaluated the population mean parameter vector and plotted the traditional D-optimal sample times for the one true parameter vector, as in classical D-optimal design theory.

RESULTS

There were 58 patients studied. The total number of samples analyzed was 327, giving an average (standard deviation) of 5.64 (0.69) samples per subject. The range of the number of samples analyzed was three to six. The vast majority of patients had either five or six samples available for analysis.

The population pharmacokinetic analysis generated a mean parameter vector and covariance matrix. The means, medians, and standard deviations of the parameter values are displayed in Table 1, and the full covariance matrix is displayed in Table 2.

The fit of the model to the data was good. The observed-predicted plot had a best-fit regression line specified by the following equation: observed value = $0.997 \times$ predicted value + 0.271 ($r^2 = 0.948$; $P \ll 0.001$).

The mean weighted error (measure of bias) was -0.109 mg/liter. The bias-adjusted mean weighted squared error (measure of precision) was 1.126 (mg/liter)². The plot of predicted versus observed concentrations is displayed in Fig. 1. No bias was evident visually.

The MM file contained 42 parameter vectors and their associated probabilities (the MM file contains the parameter vectors and their associated probabilities for the support points present at convergence of the population pharmacokinetic analysis). NPAG analysis examines all members of the popu-

TABLE 2. Covariance matrix^a

Parameter	Vol	K_{cp}	K_{pc}	CL
Vol	1,122.84			
K_{cp}	-28.1030	91.9937		
K_{pc}	34.1635	51.1259	144.654	
CL	39.5756	-1.80660	-7.00593	19.0084

^a Vol, K_{cp} , K_{pc} , and CL are as defined for Table 1.

FIG. 1. Plot of observed versus predicted values for levofloxacin after the MAP Bayesian step from data derived from a clinical study of nosocomial pneumonia.

lation in multidimensional (number of parameters plus associated probability) space. The iteration process improves the resolution with which the parameter vectors are identified to a maximal resolution of 0.02%. At convergence (resolution of 0.02%), 42 parameter vectors were resolvable.

There was an attempt to estimate D-optimal sampling times for each vector. In 20 (weighted probability, 0.42) of the instances, the attempt resulted in a nearly singular Hessian matrix, indicating an inability to calculate the scalar value to be optimized. In the rest of the instances, the four optimal sampling times were each plotted in a frequency histogram with the sum of the attendant probabilities normalized to 1.0.

The sampling times were constrained to be from 5 min after the end of a 1.5-h infusion at a rate of 500 mg/h to hour 24.0 of a steady-state dosing interval. For the first sampling time, there was no variability in the population, with all patients having a first optimal sampling time of 5 min after the end of infusion. Likewise, the fourth sampling time was always at 24 h in a steady-state dosing interval.

The distributions of sampling times for the second and third samples are displayed in Fig. 2A and B. Because there were some overlapping times, the overall sampling information is displayed in Fig. 2C.

From Fig. 2C, one reasonable sampling schedule limited to six samples would be 1.5, 2, 2.25, 4, 4.75, and 24 h after the start of a 90-min infusion. Others are possible. One would, in general, choose sampling times near the peaks of the information content and add additional sampling times until the sampling burden exceeds that which is either clinically reasonable or more than can be afforded.

DISCUSSION

We have studied the pharmacokinetics of levofloxacin in seriously ill patients being treated for nosocomially acquired pneumonia. We have used this database to delineate a simple approach for determining the most informative times to obtain blood samples so that minimum-variance pharmacokinetic parameter estimates can be obtained for patients. It should be stated at the outset that such calculations are patient popula-

FIG. 2. Information content distribution for determining sampling times. (A and B) Optimal levofloxacin sampling times for the second (A) and third (B) samples. (C) Overall sampling information. □, locations of the second and third sampling times derived from traditional D-optimal sampling using the population mean parameter vector.

tion specific. One should not use an optimal sampling strategy derived from a population of patients with nosocomial pneumonia (or healthy volunteers) to study a patient population suffering from meningitis or skin and skin structure infections unless no other information is available.

As an example, the population pharmacokinetic analysis identified estimates for the mean and median total clearance of 7.4 and 6.4 liters/h, respectively. Corresponding values from a previous analysis of 500 mg of intravenous levofloxacin at steady state drawn from a population of patients with community-acquired pneumonia were 9.3 and 9.0 liters/h, respectively (9). Clearly, the results from one population should not be extrapolated to another.

In general, the population analysis was precise and unbiased. The plot of predicted versus observed values had a slope that was not significantly different from 1.0 (0.997) and a small positive intercept. The overall amount of variance explained by the regression was excellent at 94.8% ($r^2 = 0.948$). The residual plot did not demonstrate problems with bias by concentration, and the calculated measures of bias and precision were quite acceptable.

The values for the population analysis were also quite consistent with expectations. While we have pointed out above that the clearance was lower than that seen in a population of community-acquired pneumonia patients, this meets with our expectations, as this population was older and sicker than the patients we studied previously.

The problem of delineation of an optimal sampling schedule for a population of patients is different in kind from that of delineation of an optimal sampling design for a single patient. While one could make the latter problem into one that is fully stochastic, the idea that there is but one true parameter vector for a specific patient at a specific time is plausible. This is not the case when one thinks about designing a sampling strategy for a population of patients. Clearly, the existence of true variability among patients in a population violates the underlying assumption of there being one true parameter vector that is at the heart of traditional D-optimal design theory. Other laboratories have made significant inroads into this problem. D'Argenio has examined different design criteria applicable to populations of patients and performed an extensive evaluation (2). At least one of these criteria, the ELD criterion (expectation over the logarithm of the D-optimal design criterion for the population), does not have the property of replication. Tod and Rocchisani have also made inroads into this area (10, 11). All the work in this area requires that one possess specialized software that is not widely available.

In this paper we set forth a simple method for obtaining insight into how the true variability among patients distributes system information in the dosing interval. This allows construction of rational sampling schedules. The method is flexible, in that it allows delineation of any number of sample times, limited only by the decreasing ability to obtain large number of samples in an ill patient population and, perhaps, by the cost of analyzing a large number of samples. Further, the approach requires only software that is widely available. The NPAG program of Leary et al. (8) is necessary to analyze the database and to generate the nonparametric distribution of parameter vectors for the population and their associated probabilities. Each of these can be analyzed in a traditional D-optimal design fashion, and the sampling times can be weighted for the probability of that vector occurring in the population. The resulting times can be displayed in a frequency histogram. The optimal

sampling calculation provides a specific sampling time. However, if the sampling time is offset from the true optimal time, the resulting datum is not worthless. The information content declines but is not zero. That is why the optimal sampling times are displayed in a frequency histogram format.

As the number of sampling times increases, we may see that we are gaining better and better information for a larger proportion of the population. Unless a very large number of samples were obtained (obviating the whole point of the analysis), the sampling schedule cannot be robust for patients who are in the outlier part of the distribution. Nonetheless, we believe this approach is a reasonable way to design the pharmacokinetic study of ill-patient populations where sample numbers are limited. This also has obvious application to other populations such as children and the elderly, where blood sample numbers must be limited.

In summary, we have described a simple method that allows delineation of optimal sampling schedules of populations of patients. In so doing, we hope to be able to obtain precise and unbiased estimates of drug exposure in individual patients when these optimal samples are subjected to a population pharmacokinetic analysis and then individual estimates obtained with MAP Bayesian estimation techniques. Such precise and unbiased estimates can then be normalized to MIC (e.g., peak concentration/MIC ratio, area under the concentration-time curve/MIC ratio, or time above the MIC) and used to delineate relationships between exposure and response. Delineation of such relationships should allow optimal therapy to be provided for our most seriously ill patients.

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