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**Effect of Assumptions concerning Assay Error Patterns upon
Pharmacokinetic Parameter Values and Model Behavior**

by

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INTRODUCTION:

In the past, laboratory assay errors have usually been evaluated by determining the standard deviation (SD) of a few sample values and keeping their variation within certain specified acceptable limits. However, no specific or explicit practical clinical use of this information has usually been made. Explicit characterization of the analytic errors associated with each measured serum drug concentration usually has not been done over the entire working range of the assay, and it has not been customary to report, or to make routinely available, the SD of each measured serum drug concentration along with the result itself.

The implementation of population pharmacokinetic modeling and Bayesian fitting of pharmacokinetic models has now changed our needs for knowledge of assay errors. For example, the objective function which is minimized in the Bayesian fitting procedure is

$$\left[\text{SUM} \frac{(\text{Ppop} - \text{Pmod})^2}{\text{SD}^2 \text{ Ppop}} + \text{SUM} \frac{(\text{Cobs} - \text{Cmod})^2}{\text{SD}^2 \text{ Cobs}} \right]$$

where:

Ppop is the collection of population parameters values, (VD, KEL, KA, etc.).

Pmod is the collection of the revised values of each parameter as the model is fitted.

SD² Ppop is the variance (the square of the SD) by which each member of Ppop is known.

Cob is the collection of the patient's observed serum concentrations.

Cmod is the collection of the fitted model estimates of the serum concentrations at the time each was drawn.

Finally, SD² Cobs is the variance (the square of the SD) with which each serum concentration was measured.

This last term shows the importance of knowing explicitly the standard deviation with which each serum concentration has been measured. It shows more than ever how important the laboratory is in evaluating the combined role played by the population parameter values (and each of their SD's) compared with the measured serum concentrations (and each of their SD's) in coming to the correct Bayesian posterior parameter values, weighing and balancing the credibility of the patient's serum concentration data against that of the data of the population pharmacokinetic parameter values. The fitted model based on the correct Bayesian analysis yields the most precise prediction of future serum levels achieved with a subsequent dosage regimen, leading to dosage regimens that

obtain the clinically chosen serum level goals with the greatest possible precision, benefit, and safety (1).

Explicit measurement of the assay error pattern

Many assays, especially immunoassays, EMIT assays, and fluorescence polarization assays, as well as various chromatographic assays, often have a standard deviation that varies in a quite nonlinear manner over the range of the assay. Such an error pattern is said to be heteroschedastic, as opposed to a constant error at all serum levels, which is set to be homoschedastic. Such a heteroschedastic error pattern is well known, and is commonly expressed as a certain coefficient of variation (CV). This implies that the assay standard deviation is a constant percent of the measured concentration, whatever its value. Expressing the error this way, however, gives increasing weight to the lower concentrations, frequently to a quite unrealistic extent. An analysis of the College of American Pathologists (CAP) surveys over several years shows that most assays do not have a constant, but rather a varying CV and SD over their working range (2).

An easy and cost effective way to determine the assay error pattern over its entire working range is simply to measure, in at least quadruplicate, a representative number of serum levels over the entire working range. For example, one can take a blank sample, a low one, a middle one, a high one, and a very high one, and can measure each of them in at least quadruplicate, to obtain the mean and SD of each sample. Thus one has at least five points that cover the entire working range of the assay. One can then fit these points or more, describing the relationship between the serum concentration and the SD with which it is measured, and arrive at a polynomial equation, usually of second order, which usually fits these points quite well for most assays. From this polynomial it is then easy to compute the probable standard deviation with which any single measured serum level has been determined (3).

For example, in the Toxicology and Therapeutic Drug Monitoring Laboratory in the LAC/USC Medical Center, quadruplicate determinations of serum gentamicin concentrations were run on a blank sample and on samples containing 2, 4, 8, and 12 ug/ml (done under the direction of Clifford Walberg, Ph.D., and at the suggestion of Thomas Gilman, Pharm.D.). This work led to the following polynomial equation describing that relationship:

$$\text{SD (ug/ml)} = 0.570628 - 0.106496C + 0.016852C^2$$

where C is the measured serum concentration (3).

In a similar manner, the error pattern of the Abbott TDx assay of serum lidocaine levels in our Laboratory of Applied Pharmacokinetics was also nonlinear, and the resulting polynomial was:

$$\text{SD} = 0.042779 + 0.0031294C + 0.0060417C^2.$$

The practical use of such polynomials is facilitated by the ability of the USC*PACK clinical pharmacokinetic computer programs (4) to enter and store the coefficients of their polynomial in a file, along with the population pharmacokinetic parameter values for that drug. In this way, each center and laboratory has the opportunity to develop its own polynomial for its own assays, for each drug whose serum concentrations it monitors, and to enter and store it so that each serum concentration can be correctly evaluated by its own

properly estimated standard deviation, and thus can have proper credibility assigned to it as the reciprocal of its variance, in keeping with its correct Fisher information, a well-known index of credibility (5), which is specifically used in the Bayesian objective function shown earlier.

In this way, just as the credibility of each population pharmacokinetic parameter value is determined by the reciprocal of its variance (the square of the SD), so also can we now similarly evaluate the credibility of each serum concentration according to its Fisher information by similarly weighting each measured serum concentration by the reciprocal of the variance with which it is known. Thus the credibility of the population parameter values gets correctly balanced against the credibility of the patient's serum levels.

If the estimate of the standard deviation of the serum levels is too small, the serum levels will receive too much importance, and the Bayesian posterior parameter values will be too dependent on serum level data. On the other hand, if the standard deviation of the serum levels is estimated to be too large, then the credibility of the serum levels will be evaluated as being too little, and the Bayesian posterior parameter values will hang back too much toward the population priors.

In the same way, when one makes population pharmacokinetic models, the parameter values found will depend greatly upon the credibility assigned to each of the measured serum levels. Each serum level must be correctly evaluated according to its credibility, or erroneous parameter values will be found, even though they may give "good" empirical fits to the data.

All this is nothing new. It merely restates the well-known fact that different weighting schemes result in different fits and in different parameter estimates. Garbage in, garbage out.

METHODS

In this analysis, a carefully simulated data set generously provided by Dr. Marilyn Martinez, consisting of 24 simulated animals, the "original formulation set" was used. A single dose of 2257.2 milligrams of a hypothetical drug was given at time zero to 24 simulated animals. A total of 14 simulated serum levels was obtained at 0.5, 1, 1.5, 2, 3, 4, 6, 10, 16, 24, 36, 48, 60, and 72 hours following drug administration into an absorptive compartment.

The NPEM population modeling program using the nonparametric EM algorithm developed by Schumitzky was used to analyze this data (6-8). This particular study was done using a new prototype version of the NPEM program which permits analysis of doses given into an absorptive compartment, and estimates of up to 7 parameter values to be made. The parameters of interest in the present study were the absorptive rate constant (K_A , hr^{-1}), the apparent volume of distribution (V_D , liters), and the elimination rate constant (K_{EL}). The anticipated range of these parameters was from 0.0 to 0.1 hr^{-1} for K_E , 0.000001 to 2000 liters for V_D , and 0.0 to 3.0 hr^{-1} for K_A .

Twenty four such carefully simulated data sets were examined. The serum levels were stated to be obtained without any assay noise at all. However, what was done here was to make a population model based on these 24 data sets using three different assumptions about assay error patterns.

1. Homoschedastic or Constant Assay Error.

In this analysis the assay error was stated by Dr. Martinez to be noise free. Since the NPEM program will not accept a standard deviation of zero in association with a measured serum level, the standard deviation of all levels was assumed to be 0.000001 ug/ml. Because of this, the error pattern polynomial was

$$SD = 0.000001 + 0.0C + 0.0C^2.$$

2. A Heteroschedastic Error Pattern Assuming a Constant Coefficient of Variation.

In this analysis the original error referred to above was supplemented by a coefficient of variation which represented 10% of whatever serum level was measured. This assay error pattern was

$$SD = 0.00001 + 0.1C + 0.0C^2.$$

3. A Polynomial Assay Error Pattern

A third assay error pattern was used in which the error was assumed to be that of the EMIT assay for gentamicin used at the LAC/USC Medical Center as described earlier. Its polynomial therefore was

$$SD \text{ (ug/ml)} = 0.570628 - 0.106496C + 0.016852C^2$$

RESULTS

1. Using the Homoschedastic Assay Error Pattern

In this analysis the mean values obtained were as follows: $KE = 0.0403 \text{ hr}^{-1}$, $VD = 593.26$ liters, and $KA = 3.278 \text{ hr}^{-1}$. The elimination half time was 17.199 hours. Simulation of the behavior of the drug using these parameter values resulted in a peak serum concentration (C_{max}) of 3.6 ug/ml which was reached (T_{max}) at one hour and 24 minutes after the dose. During elimination, the time required to reach a computed residual serum concentration of 0.1 ug/ml was 91.2 hours after administration of the dose. The computed serum concentration remaining at 120 hours after the dose was 0.03 ug/ml.

2. Using the Constant Coefficient of Variation Assay Error Pattern

This analysis yielded quite different values for VD , and KE . Here the mean KE was 0.0997 hr^{-1} , and the mean V was 726.02 liters. The mean KA was 3.323 hr^{-1} . The elimination half time was much shorter, only 6.952 hours. Simulation with this model yielded a much lower C_{max} of 2.79 ug/ml at a T_{max} of one hour and 6 minutes after the dose. The

time to a computed residual serum concentration of 0.1 ug/ml was much shorter, only 36.87 hours, and the computed serum concentration remaining at 120 hours was essentially 0.00 ug/ml.

3. Using the Polynomial Assay Error Pattern

This analysis just happened to yield parameter values similar to those found with the constant or homoschedastic assay pattern. However, in other unpublished studies of other data sets, that has not been the case at all. In the present study, the mean KE was 0.0383 hr⁻¹, the mean VD was 596.74 liters, and the mean KA was 3.294 hr⁻¹. The resulting C_{max} was 3.59 ug/ml at a T_{max} of 1 hour and 24 minutes. The elimination half time was 18.10 hours, the computed residual serum concentration of 0.1 ug/ml was reached at 96.0 hours, and the computed serum concentration remaining at 120 hours was 0.04 ug/ml. These results are summarized in Table I.

DISCUSSION

It is common knowledge that different methods used to weight data will result in different parameter estimates. In the past, different weighting schemes have been used to obtain “the best fit” to the data. Often some criterion such as the coefficient of the determination (r^2) or the correlation coefficient (r) between the measured concentrations and their model estimates has been used.

The Bayesian objective function shown earlier does not do this. Instead, it specifically gives weight to the credibility of both the population and the serum level data according to their Fisher information, weighting all data points, whether the pharmacokinetic parameter values or the measured serum concentrations, by the reciprocal of their variances.

In the same way, as shown in the present study, different schemes for weighting serum level data result in visibly different population pharmacokinetic parameter values. Models made having these different parameter values result in very different estimates of serum serum concentrations at various times. For example, a residual value such as 0.1 ug/ml might be used to evaluate the time after dosage at which an animal is felt to be safe to slaughter, when residual concentrations may be felt to be acceptable for human consumption. In the present study, this time ranged from 36.87 to 96.0 hours, depending on the assay error assumption, almost a threefold difference.

Because of this, one must be highly skeptical of any scheme that is used to give weight or to assign relative importance to measured serum drug concentrations when using such data for pharmacokinetic modeling or for adaptive control of drug dosage regimens. It is likely that a carefully determined assay error pattern, analyzed at least in quadruplicate for probably at least five serum samples, including a blank, which cover the entire range of the assay, may be a reasonable strategy to use at the present time.

This is especially the case when it comes to measuring low serum concentrations sampled many hours after a dose. For toxicological purposes, it has been quite reasonable to set the lower detectable limit of an assay at something like 2 SD above the blank, to be relatively sure that one is measuring drug and not a blank. Some procedure like this is surely

necessary to be able to say, in the absence of other information or history, that a drug is or is not present.

However, in pharmacokinetic studies and in therapeutic drug monitoring, much more information is available. One knows that the dose was given at a certain time. Except when compliance is questionable, and poor compliance shows itself as a large VD , one knows that the drug actually was given. Indeed, most clinical laboratories request the time of the last dose to be entered on the lab slip.

Because of this, measuring serum drug concentrations for purposes of therapeutic drug monitoring or pharmacokinetic studies is quite different from doing it for purposes of clinical toxicology. One knows the drug was given from the orders and the nurses' or pharmacist's notes, for example. The question is not whether or not there is drug present. The question is rather how much drug is present. A low serum concentration, even one felt to be below traditional detectable limits, can now be used. Its credibility is exactly that found by using the assay error polynomial to calculate its probable SD , as described above.

CONCLUSION

This easy and cost - effective method of computing the probable SD of each serum sample appears to be a reasonable way to determine explicitly the assay errors for purposes of pharmacokinetic analysis. Skepticism must be maintained throughout. Weighting schemes such as unity weighting, or the assumption of a constant assay error as done in the present study, or other assumptions such as that of a constant assay CV, will result in quite different pharmacokinetic parameter estimates. A carefully determined polynomial describing the assay error pattern stands a good chance of assigning reasonable degrees of credibility and skepticism to each measured serum concentration. Since “garbage in” is “garbage out”, the less one assumes about assay errors the better.

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REFERENCES

1. Jelliffe R, Iglesias J, Hurst A, Foo K, and Rodriguez J. Individualizing Gentamicin Dosage Regimens. A Comparative Review of Selected Models, Data Fitting Methods, and Monitoring Strategies. Clin. Pharmacokinetic. 1991: 21:461-478.
2. Jelliffe R, Schumitzky A, Van Guilder M, Liu M, Hu L, Maire P, Gomis P, Barbaut X, and Tahani B: Individualizing Drug Dosage Regimens: The Roles of Population Pharmacokinetic and Dynamic Models, Bayesian Fitting, and Adaptive Control. Therapeutic Drug Monitoring, in press.
3. Jelliffe R: Explicit Determination of Laboratory Assay Error Patterns - A Useful Aid in Therapeutic Drug Monitoring. American Society of Clinical Pathologists Check Sample Continuing Education Program, Drug Monitoring and Toxicology No. DM 89 - 4 (DM-56), March 1990, pp 1-6.
4. Jelliffe R, D'Argenio D, Schumitzky A, Hu L, and Liu M. The USC PC PACK Programs for Planning, Monitoring, and Adjusting Drug Dosage Regimens. Proceedings of the 23rd Annual Meeting of the Association for the Advancement of Medical Instrumentation, Washington DC, May 14-18, 1988, p 51.
5. DeGroot M. Probability and Statistics, 2nd ed, Addison-Wesley Publishing Company, Reading, MA, 1986, p 423.
6. Schumitzky A. Nonparametric EM Algorithms for Estimating Prior Distributions. App. Math. & Computation. 1991: 45:143-157.
7. Dodge W, Jelliffe R, Richardson J, McCleery R, Hokanson J, and Snodgrass W. Gentamicin Population Pharmacokinetic Models for Low Birth Weight Infants Using a New Nonparametric Method. Clin. Pharmacol. Therap. 1991: 50:25-31.
8. Kisor D, Watling S, Zarowitz B, and Jelliffe R. Population Pharmacokinetics of Gentamicin. Use of the Nonparametric Expectation Maximization (NPEM) Algorithm. Clin. Pharmacokinetic. 1992: 23:62-68.

TABLE I

Results Obtained from Different Assay Error Assumptions

Result	Constant Error	Constant CV	Polynomial Error
KE (hr-1)	0.0403	0.0997	0.0383
VD (L)	593.26	726.02	596.74
KA (hr-1)	3.278	3.323	3.294
T1/2 (hrs)	17.199	6.952	18.098
Cmax (ug/ml)	3.60	2.79	3.59
Tmax (mins)	84	66	84
Time to 0.1 ug/ml (hrs)	91.2	36.9	96.0
Conc at 120 hrs (ug/ml)	0.03	0.00	0.04