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Research Paper

Covariate Detection in Population Pharmacokinetics Using Partially Linear Mixed Effects Models

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Purpose. To introduce partially linear mixed effects models (PLMEMs), to illustrate their use, and to compare the power and Type I error rate in detecting a covariate effect with nonlinear mixed effects modeling using NONMEM.
Methods. Sparse concentration-time data from males and females (1:1) were simulated under a 1-compartment oral model where clearance was sex-dependent. All possible combinations of number of subjects (50, 75, 100, 150, 250), samples per subject (2, 4, 6), and clearance multipliers (1 to 1.25) were generated. Data were analyzed with and without sex as a covariate using PLMEM (maximum likelihood estimation) and NONMEM (first-order conditional estimation). Four covariate screening methods were examined: NONMEM using the likelihood ratio test (LRT), PLMEM using the LRT, PLMEM using Wald's test, and analysis of variance (ANOVA) of the empirical Bayes estimates (EBEs) for CL treating sex as a categorical variable. The percent of simulations rejecting the null hypothesis of no covariate effect at the 0.05 level was determined. 300 simulations were done to calculate power curves and 1000 simulations were done (with no covariate effect) to calculate Type I error rate. Actual implementation of PLMEMs is illustrated using previously published teicoplanin data.
Results. Type I error rates were similar between PLMEM and NONMEM using the LRT, but were inflated (as high as 36%) based on PLMEM using Wald's test. Type I error rate tended to increase as the number of observations per subject increased for the LRT methods. Power curves were similar between the PLMEM and NONMEM LRT methods and were slightly more than the power curve using ANOVA on the EBEs of CL. 80% power was achieved with 4 samples per subject and 50 subjects total when the effect size was approximately 1.07, 1.07, 1.08, and 1.05 for LRT using PLMEMs, LRT using NONMEM, ANOVA on the EBEs, and Wald's test using PLMEMs, respectively.
Conclusions. PLMEM and NONMEM covariate screening using the LRT had similar Type I error rates and power under the data generating model. PLMEMs offers a viable alternative to NONMEM-based covariate screening.

KEY WORDS: linear mixed effects model; NONMEM; regression splines; semiparametric mixed effects models; splines.

INTRODUCTION

One goal of population pharmacokinetics is to identify covariates that may influence drug concentrations and, hence, exposure (1). In a typical population model, the effect of the covariate is mediated through its influence on the primary pharmacokinetic parameters in the model (e.g., the effect of weight on clearance). Through its effect on a pharmacokinetic parameter, the covariate then influences drug concentrations. However, the ability to detect a covariate affecting drug concentrations is dependent on the choice of structural model. Although it has never been shown directly, it seems likely that the effect of a covariate on exposure may be diluted through the use of a structurally defective model. Hence, a more natural goal would be to identify the relationship between covariates and drug concentrations directly without heavy reliance on an appropriate structural model.

Gibiansky *et al.* (2) first tackled this problem using cilostazol. They proposed that the concentration-time data be partitioned into three groups based on the 25th, 50th, and 75th percentiles using a nonparametric cubic spline fit to the concentration-time profile. Observations are then categorized into which group they then fall into. For example, if the cutoff for the 25th percentile at some time was 10 ng/ml and an observation was 5 ng/ml, then this observations is categorized into group 1. Then using either logistic regression or classification regression tree models, the effect of covariates on which group an observation is classified into can be determined. This method would be useful when only a single observation is available per subject but becomes problematic when more than one observation is present, for then it is entirely possible that one observation falls into one group but another observation fall into another group. One then runs into the situation where subjects are classified into multiple groups, and how to determine which group a subject falls into "overall" is questionable. What makes the method of Gibiansky *et al.* so interesting is that they removed the effect of

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time from the analysis and focused solely on how the covariates affect which group an observation was categorized into.

One of the most difficult parts of a population analysis is determining an appropriate structural model, which is dependent on how concentrations change over time. If the effect of time could be neutralized or treated as a nuisance variable, then perhaps the effect of a covariate on drug concentrations could be determined directly. Splines are a good choice for nonparametric modeling the effect of time on drug concentrations because their use effectively removes the time component from a model. The purpose of this paper is to introduce the use of partially linear mixed effects models (PLMEMs) as presented by Ruppert, *et al.* (3) (which they call semiparametric mixed effects models) and Hardle *et al.* (4) in the analysis of population concentration-time data using polynomial basis functions and to examine the power and type I error rate of PLMEMs at detecting important covariates compared to direct covariate screening using NONMEM (5) and *post hoc* examination of the empirical Bayes estimates (EBEs).

Partially Linear Mixed Effects Models Background

Before beginning the development of partially linear models, a basis function must first be defined. Using Heaviside function notation, a linear spline basis function (sometimes called a truncated line function) may be written as

$$u_+ = (x - k)_+ \tag{1}$$

where x is some variable, k is the knot, and for any x, u_+ is equal to (x – k) if (x – k) is positive and equal to 0 otherwise. Hence, if x = 6 and k = 3, the $u_+ = 3$. But if x = 3 and k = 6, then $u_+ = 0$ because (x – k) is negative. A set of such functions is called a linear spline basis and a spline model using these spline bases can be written as

$$Y = \theta_0 + \theta_1 x + \sum_{i=1}^{K} \theta_{i+1}(x - k_i)_+ + \varepsilon \tag{2}$$

where now x is the independent variable, Y is the dependent variable, θ is a set of parameters that are to be estimated, ε is the residual, and K is the number of knots. Ordinary least-squares can be used to estimate θ and obtain the linear spline fit to a data set. A linear spline fit to simulated concentration-time data from a one-compartment model with first-order absorption with samples randomly drawn over a 24-h interval can be found in Fig. 1 using equally spaced knots from 3 h to 21 h every 3 h.

Although easy to compute, this approach has some limitations. First, this algorithm assumes that the observations are independent; it fails to take into account the within-subject correlations in the data and assumes that each data point is a unique observation. Second, the spline model may be unstable because as the number knots increases, the new variables may be collinear (6), although a singular value or QR-decomposition (7) may be done prior to inversion to stabilize the model parameter estimates. Third, the resulting fit may be dependent on the number and choice of knots. This third problem may be minimized by using automated knot selection methods or model selection criteria to find the optimal number and choice of knots, but these methods are computationally intensive and not easily implemented (3).

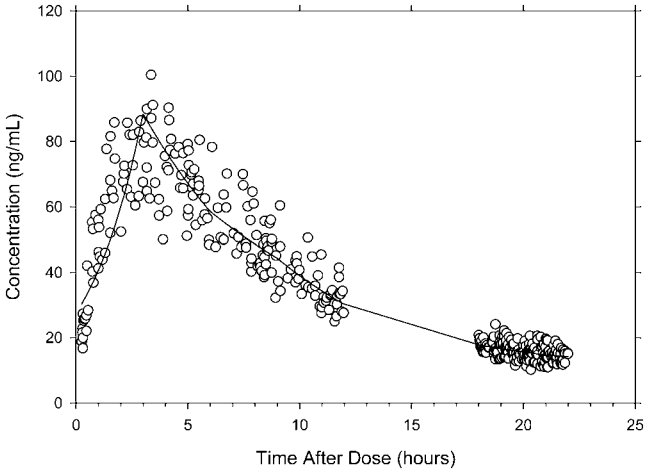

Fig. 1. Linear spline fit to simulated concentration-time data using linear spline basis functions with knots at 3, 6, 9, 12, 15, 18, and 21 h after dosing. Concentration data from 97 subjects were simulated with each subject having from 1 to 4 samples collected at steady-state. Data were fit using PROC REG in SAS using ordinary least squares. Drug concentrations were fit after Ln-transformation and then predicted values were estimated after exponentiation back to the original domain.

Penalized spline regression with truncated polynomial basis functions were developed to overcome some of the numerical problems encountered with fitting linear spline functions. The general model for a p-degree spline model is then written as

$$Y = \theta_0 + \theta_1 x + \ldots \theta_p x^p + \sum_{i=1}^{K} \theta_{i+p}(x - k_i)_+^p \tag{3}$$

If D is a (K + p + 1) diagonal matrix whose first (p + 1) diagonal elements are equal to 0 and whose remaining K diagonal elements are equal to 1, that is,

$$D = \text{diag}(0_{p+1}, 1_K) \tag{4}$$

the fitting criteria is then to minimize the penalized objective function

$$(Y - x\theta)^T (Y - x\theta) + \theta^T D \theta \tag{5}$$

The solution to which is

$$\hat{\theta} = (x^T x + \lambda^{2p} D)^{-1} x^T Y \tag{6}$$

where λ is called the smoothing parameter. Hence, the solution is something similar to ridge regression where the term $\lambda^{2p}D$ attempts to reduce the variability of the estimated regression coefficients. The penalty term, the second term inside the parentheses in Eq. (6), attempts to shrink all regression coefficients associated with the spline basis functions to zero and is sometimes called a roughness or curvature penalty. At large values of λ, the penalty term becomes large and the smooth increases to the ordinary least squares fit to the data. As λ approaches zero and the penalty term becomes zero, the smooth becomes an unconstrained spline fit. Luckily, as long as the knots cover the range of x data well, their number and positioning has little effect. Hence, smoothing is largely controlled by the value of λ. Figure 2 presents the same data in Fig. 1 using penalized splines with λ varying from

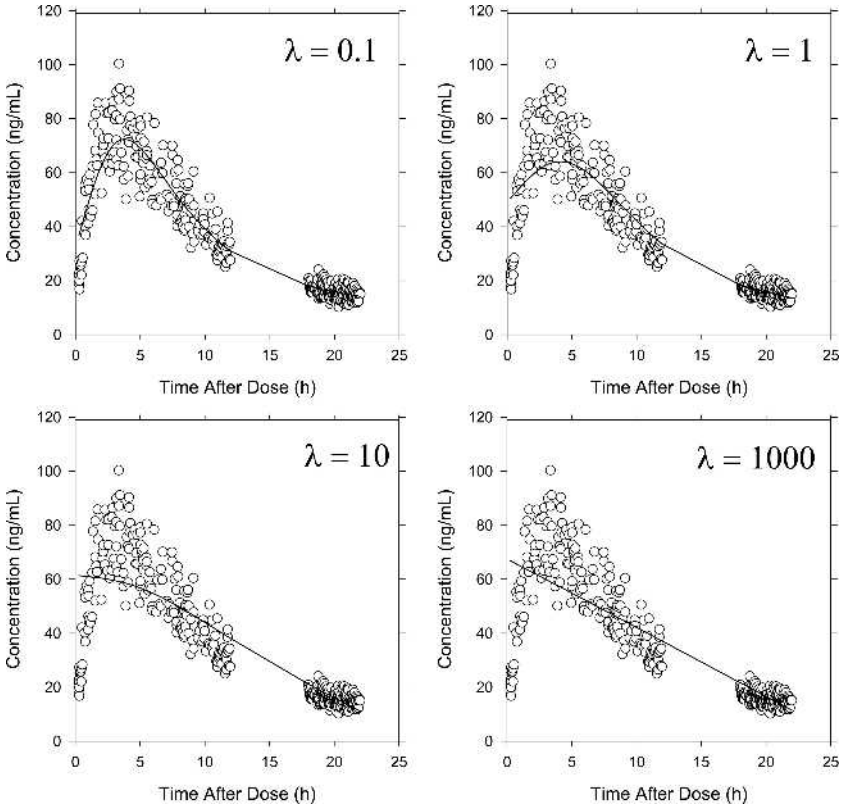

Fig. 2. Linear spline fit to the simulated data example using penalized spline regression with varying values of λ and knots at 3, 6, 9, 12, 15, 18, and 21 h after dosing.. Data were fit using PROC IML in SAS. Drug concentrations were fit after Ln-transformation and then predicted values were estimated after exponentiation back to the original domain. As $\lambda \to \infty$, the penalty term dominates, forcing the curvature toward zero. As $\lambda \to 0$, the penalty term approaches zero with the result being a linear spline fit to the data.

0.1 to 1000. Notice that as λ increased, the smooth became less and less wiggly and curved.

One problem with a linear spline basis is that for data that show curvature, such as concentration-time data after oral administration, the spline may appear discontinuous and may not fit windows where the derivative is changing sign. For example, in Fig. 1 and Fig. 2, near the time of maximal concentration (~3 h), the spline makes an abrupt change from positive slope to negative slope. A better model would have the change from positive to negative slope be a gradual one. One way to escape from such piecewise linearity is to use higher order penalized regression splines, either quadratic or cubic, most often the latter. Figure 3 presents a penalized regression fit to the data in Fig. 1 using quadratic and cubic spline basis functions at the optimal value of λ (i.e., with $\lambda = 2$). With higher order polynomials, the curvature in the concentration-time data was better estimated and smoother fits were obtained compared to a linear spline basis function.

Two points should be noted. First, spline models reported within SAS (SAS Institute, Inc., Cary, NC) or S-Plus (Insightful Corp., Seattle, WA, USA) do not use the method just presented. These packages use cubic splines that minimize the objective function

$$\sum_{i=1}^{n} w_i(Y_i - \hat{Y}_i) + \lambda \int (f''(x))^2 \, dx \qquad (7)$$

where w_i are the weighting factors, and the second term again controls the smoothness. Second, while truncated basis functions are useful to describe splines, their routine use is problematic because as the number of knots increases so does the collinearity between the basis functions with the result being a numerically unstable smooth (ill-conditioned). For example, using the linear basis functions with knots equally spaced from 3 to 21 h as in Fig. 3, the 3 h and 6 h knot have a correlation of 0.9930, 6 and 9 h have a correlation of 0.9906, and so forth. For this reason, alternative basis functions, ones that are more stable numerically, are sometimes used.

The use of splines in population modeling is not new. Park *et al.* (8) reported on the use of splines to analyze oral concentration-time data and obtain estimates of area under the curve, maximal concentration, and time to maximal concentration. However, in their approach, concentrations were modeled as a function of a "template" spline common to all individuals and a "distortion" spline representing individual differences from the template. To estimate the template spline, the coefficients of the basis functions were treated as fixed effects. In contrast, the basis functions are treated as random effects in the PLMEM approach. Further, in order to obtain a reasonable spline fit to their data, Park *et al.* force certain constraints upon the spline, such as a negative tail-slope. No such constraints, like monotonicity or non-negativity, are made with the PLMEM approach. Hence, al-

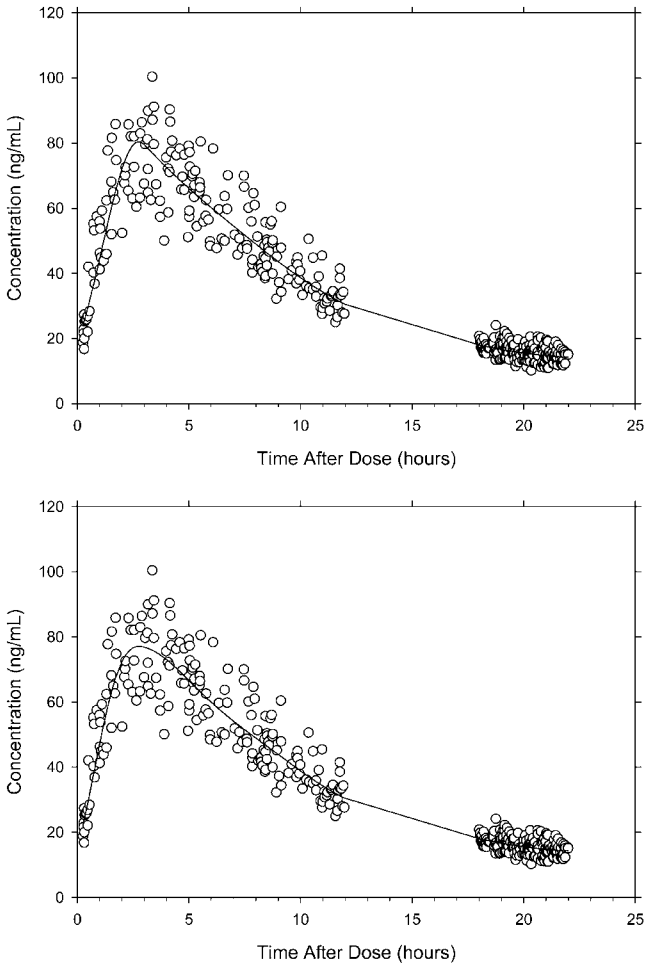

Fig. 3. Penalized regression spline fit to the simulated concentration data using quadratic spline basis functions (top) and cubic spline basis functions (bottom) with knots at 3, 6, 9, 12, 15, 18, and 21 h after dosing and λ fixed at 2. Data were fit using PROC IML in SAS using ordinary least squares. Drug concentrations were fit after Ln-transformation and then predicted values were estimated after exponentiation back to the original domain.

though the approach by Park *et al.* and the approach presented herein are similar, they are different methodologies with different assumptions. Because of their superficial similarities, it was decided that these models would be presented herein under the name partially linear mixed effects models, as called so by Hardle *et al.* (4), instead of under the name semiparametric mixed effects models, which unfortunately is the same name used by Ruppert *et al.* (3).

Penalized Regression Splines Using Mixed Model Methodology

Penalized regression splines using truncated power basis functions can easily be extended to a linear mixed effects model by treating the basis functions as random variables. Within a pharmacokinetic context, time is treated as a fixed effect. In this context, these models will be referred to hereafter as PLMEMs. Let x be the matrix of fixed effects, which includes time and any covariates, and U be the matrix of spline basis functions, which are treated as random effects. For a quadratic spline basis function having K knots at k_1, k_2, . . . k_K, the linear mixed effects model can be written as

$$Y = x\beta + zU + \varepsilon \tag{8}$$

where

$$x = \begin{bmatrix} 1 & t_1 & t_1^2 \\ 1 & t_2 & t_2^2 \\ & \cdots & \\ & \cdots & \\ 1 & t_n & t_n^2 \end{bmatrix}, \quad \text{and}$$

$$z = \begin{bmatrix} (t_1 - k_1)_+^2 & (t_1 - k_2)_+^2 & \cdots & \cdots & (t_1 - k_K)_+^2 \\ (t_2 - k_1)_+^2 & (t_2 - k_2)_+^2 & \cdots & \cdots & (t_2 - k_K)_+^2 \\ & & \cdots & & \\ & & \cdots & & \\ (t_n - k_1)_+^2 & (t_n - k_2)_+^2 & \cdots & \cdots & (t_n - k_K)_+^2 \end{bmatrix} \tag{9}$$

Under this framework, U ~ N(0, G) and ε ~ N(0, R). A common between-subject variance model for the random effects (the G-matrix) is a Toeplitz(1) structure (9), which has common diagonal variance components and zero off-diagonal elements for all the random effects

$$G = \begin{bmatrix} \sigma^2 & 0 & 0 & 0 \\ 0 & \sigma^2 & 0 & 0 \\ & \cdots & \cdots & \\ 0 & 0 & 0 & \sigma^2 \end{bmatrix} \tag{10}$$

while the within-subject variance (the R-matrix) is usually, but not always, modeled using a simple covariance model. The empirical best linear unbiased predictors (EBLUP) evaluated at the design points under a mixed model structure is the same as the penalized regression spline solution to the problem where

$$\hat{\lambda} = \left(\frac{R}{G}\right)^{1/2p} \tag{11}$$

Within a general linear mixed effects software package, such as SAS (10) or S-Plus (11), Eq. (8) can be fit using either restricted maximum likelihood (REML) or maximum likelihood (ML) estimation, although REML is the usual choice, as REML takes into account the loss in degrees of freedom in estimating the fixed effects in the model (9). The advantage of this approach is that now the model is easily computable using standard linear mixed effects software, but more importantly, the within-subject correlations can be modeled, as can the effect of covariates. Indeed, failure to account for within-subject correlations can result in an undersmoothed spline (12). Penalized splines formulated within this framework by allowing for the inclusion of covariates into the fit. Hence, entire population models can be developed without having to specify a compartmental structural model. Further these models can be easily used to screen for important covariates to take forward into further model development using NONMEM.

Figure 4 presents a penalized regression spline fit to the data in Fig. 1 using REML estimation with quadratic spline

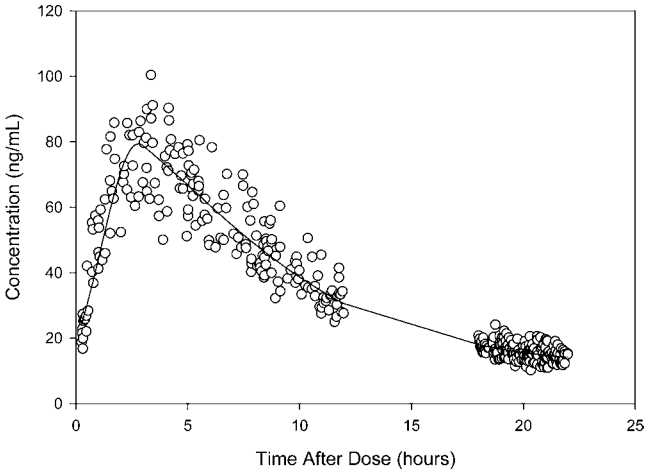

Fig. 4. Linear mixed effects model regression fit to the simulated concentration data using REML estimation with quadratic spline basis functions and knots at 3, 6, 9, 12, 15, 18, and 21 h after dosing. A Toeplitz(1) covariance matrix was used for the random effects, and a spatial power covariance matrix was used to model the within-subject errors. Drug concentrations were fit after Ln-transformation and then predicted values were estimated after exponentiation back to the original domain. The optimal value of λ was estimated at 1.7.

basis functions and knots at 3, 6, 9, 12, 15, 18, and 21 h after dosing. The appendix presents the SAS code used to analyze the data. A Toeplitz(1) covariance matrix was used for the random effects and a spatial power covariance matrix was used to model the within-subject errors. The SAS code used to fit the model is shown in the Appendix. The between-subject (G-matrix) and within-subject variance (R-matrix) were estimated at 0.002798 and 0.02424, respectively. Hence, the optimal value of λ was estimated at 1.7.

Example

Teicoplanin was developed as a glycopeptide antibiotic active against most gram positive bacteria. Steer *et al.* (13) reported on the pharmacokinetics of teicoplanin in children older than 2 months with burns over 10% total body surface area (TBSA) and adults with burns more than 15% TBSA—20 patients in total. The data set in it entirety was later published and reanalyzed by Podczeck *et al.* (14). Because of the wide range of weights, teicoplanin was injected 12 mg/kg intravenously as a bolus dose. Blood samples were drawn at structured times after dosing until concentrations were below the limit of detection of the bioassay. The following covariates were available before starting treatment: sex, age, total burn surface area in percent (TBSA), and creatinine concentration (CD0). Of interest would be, besides weight, are any of these other covariates important predictors of teicoplanin pharmacokinetics?

Because the concentration data were based on different doses (i.e., all the absolute doses administered were different), plasma concentrations were first dose-normalized prior to fitting the model. In a linear pharmacokinetic system, dose-normalization should result in all concentrations being superimposable, thus allowing concentrations to be compared across children and adults. The proposed PLMEM to the data was a quadratic spline basis function and knots at 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 288 h post-dose. The dose-normalized concentration data were skewed, which would probably violate the assumption of residual normality. So, the first thing done was to find a suitable transformation to the data that would lead to approximate normality in the residuals. A Box-Cox transformation (15) was applied to the dose-normalized concentrations, and the PLMEM was fit to the transformed concentrations. The Box-Cox parameter was varied from –2 to 2 by 0.5 and the correlation between observed residuals and expected residuals under a normal distribution was calculated (normal probability plot). The value of the Box-Cox parameter maximizing the correlation between observed residuals and expected residuals was 0 (Pearson r: 0.98, $p < 0.0001$), so the logarithmic transformation appeared to be the best transformation for this data.

Figure 5 presents a scatter plot of log-transformed dose-normalized concentrations and model predicted concentrations under a PLMEM with no covariates fit using ML and REML. The REML fit was almost exactly the same as ML as the two curves were not distinguishable. The base model without covariates appeared to do an adequate job at characterizing the data. PLMEMs for each of the covariates (1 model for each covariate) were developed. Using a critical value of 0.01 based on the likelihood ratio test (LRT) for statistical significance and ML estimation, all covariates were identified as being important predictors of teicoplanin pharmacokinetics (sex p value: <0.0001; age p value: <0.0001; TBSA p value: <0.0001; CD0 p value: <0.0001). Based on the solution to the least-squares equations, concentrations were 13% higher in females (90% confidence interval: 1.0–27%), suggesting that the sex effect would not be of clinical relevance. Further, concentrations tended to decline as age, TBSA, or CD0 increased. The results from this analy-

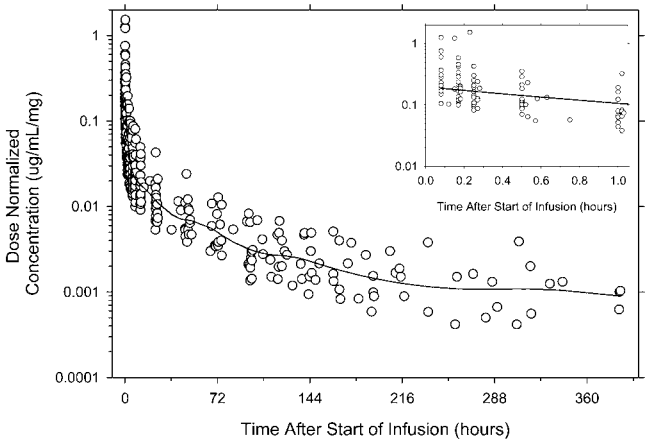

Fig. 5. Mixed model regression fit to the teicoplanin data set in 20 patients with advanced burns using ML and REML estimation with quadratic spline basis functions and knots at 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 288 h after dosing. A Toeplitz(1) covariance matrix was used for the random effects and a simple covariance matrix was used to model the within-subject errors. Dose-normalized concentrations were fit after Ln-transformation and then predicted values were estimated after exponentiation back to the original domain. Solid line is the predicted model fit to the data—both estimation methods lead to indistinguishable predicted values so only a single line is apparent. The optimal value of λ estimated using ML and REML was 6.3 and 8.5, respectively. No covariates were included in the model.

sis were consistent with the conclusions of Steer *et al.* (13) who demonstrated a significant correlation between teicoplanin concentrations at 12 h and age, TBSA, and CD0. However, only age ($p < 0.0001$) was statistically significant using REML estimation, thus highlighting the importance of estimation method on statistical significance with small sample sizes.

METHODS

The purpose of this simulation was to examine the power and type I error rate of PLMEMs at detecting important covariates compared to screening directly using NONMEM. Single dose population concentration-time data were simulated from males and females (1:1) under a one-compartment model with first-order absorption after an oral dose of 1 mg. The number of subjects was systematically varied from 50 to 250. The number of observations per subject was fixed and varied from 2, 4, or 6 samples per subject using a pharmacokinetic screen approach. Subjects having 2 samples per subject were randomly sampled from the time intervals 0 to 6 h and 6 to 24 h. Subjects having 4 samples per subject were randomly sampled from the time intervals 0 to 2 h, 2 to 4 h, 4 to 10 h, and 10 to 24 h. Subjects having 6 samples per subject were randomly sampled from the time intervals 0 to 2 h, 2 to 4 h, 4 to 6 h, 6 to 10 h, 10 to 16 h, and 16 to 24 h. Clearance (CL) was log-normal in distribution with a mean value of 15 L/h in males and 20% between-subject variability (BSV). Volume of distribution was log-normal in distribution with a mean value of 100 L and 20% BSV across sexes. The absorption rate constant was log-normal in distribution with a mean value of 1 per hour and a 30% BSV across sexes. One covariate was introduced into the simulation: patient sex on clearance. The effect of sex on clearance was treated as a constant multiplier which was systematically varied from 1.0 (no effect) to 1.25 (25% increase in clearance in females compared to males). Hence, under the simulation three factors were explored: sex effect on clearance, number of samples per subjects, and total number of subjects.

Knots were generated equally spaced every 3 h for 24 h and a PLMEM was fit to the data using REML. Quadratic spline basis functions were used as the random effects. Between-subject variability was modeled as a Toeplitz(1) covariance structure, whereas within-subject variability was modeled using a simple covariance structure. Time was treated as a quadratic polynomial fixed effect. Patient sex was also treated as a fixed categorical effect. Numerator degrees of freedom was estimated using Satterthwaite's approximation (16). The statistical significance of sex in the model was tested two ways. First, using the p value obtained from the Wald test on sex, that is, parameter/(standard error of parameter) (9). Second, a reduced PLMEM without patient sex as a covariate was developed, and then the likelihood ratio test with 1 degree of freedom was used to compare the full (with patient sex as a covariate) and reduced model (without patient sex as a covariate) (17).

Nonlinear mixed effects models were also used to analyze the same concentration-time data as the partially linear model. A one-compartment oral model (ADVAN2 TRANS2) was fit to the data using NONMEM (version 5, GloboMax, Inc., Hanover, MD, USA) using first-order conditional estimation (FOCE). Two models were developed for each data set: one with sex included in the model for clearance (full model) and one without sex in the model (reduced model). The starting values were the known population means and variances used to simulate the data. Because of the sparseness in the data, the model had difficulty estimating the absorption rate constant, and many runs resulted in rounding errors. To avoid this problem, the absorption rate constant was fixed to its mean value, 1.0 per hour, in both the full and reduced model, and then treated as a nonestimable model parameter. It should be pointed out that the full model used to fit the data was the same model used to simulate the data.

The likelihood ratio test was calculated for the full and reduced model and the statistical significance for including sex in the model was tested based on a χ^2 distribution with 1 degree of freedom (17). Statistical significance was declared if the p value from the LRT was less than 0.05. The significance of sex on CL was also tested using a covariate screening approach wherein the empirical Bayes estimates (EBEs) for CL were calculated for each subject. Analysis of variance on the log-transformed EBE was done treating sex as a classification variable. Statistical significance was declared if the p value from the ANOVA was less than 0.05.

For each combination of effect size, samples per subject, and total number of subjects, 300 simulations were done, except for when the effect size was zero in which 1000 simulations were done. Power was determined as the number of simulations that rejected the null hypothesis of no sex effect at $p < 0.05$. Type I error rate was determined as the power when the effect size was fixed to zero (no covariate effect) (18).

RESULTS

Table I presents the type I error rate for detecting a false covariate using PLMEMs and NONMEM. Type I error rates were different among the methods ($p < 0.0001$). Type I error

Table I. Type I Error Rate for the Various Methods

Number of subjects	Partially linear mixed effects model using the LRT			Partially linear mixed effects model using Wald's test			NONMEM using the LRT			ANOVA using EBE for CL		
	2	4	6	2	4	6	2	4	6	2	4	6
50	2.1	8.3	13.6	11.7	25.3	35.6	6.5	8.8	9.5	4.3	4.0	4.4
75	1.8	7.2	13.4	10.4	24.7	34.3	4.9	8.4	10.5	4.5	4.0	5.4
100	1.6	6.5	12.6	11.0	24.3	33.1	6.1	7.8	10.6	4.7	5.1	4.0
150	2.3	6.3	10.2	11.0	25.0	33.2	6.7	8.8	8.9	5.2	3.8	3.8
250	1.3	5.0	10.1	12.8	25.1	34.0	7.0	9.0	10.1	5.3	4.4	3.5

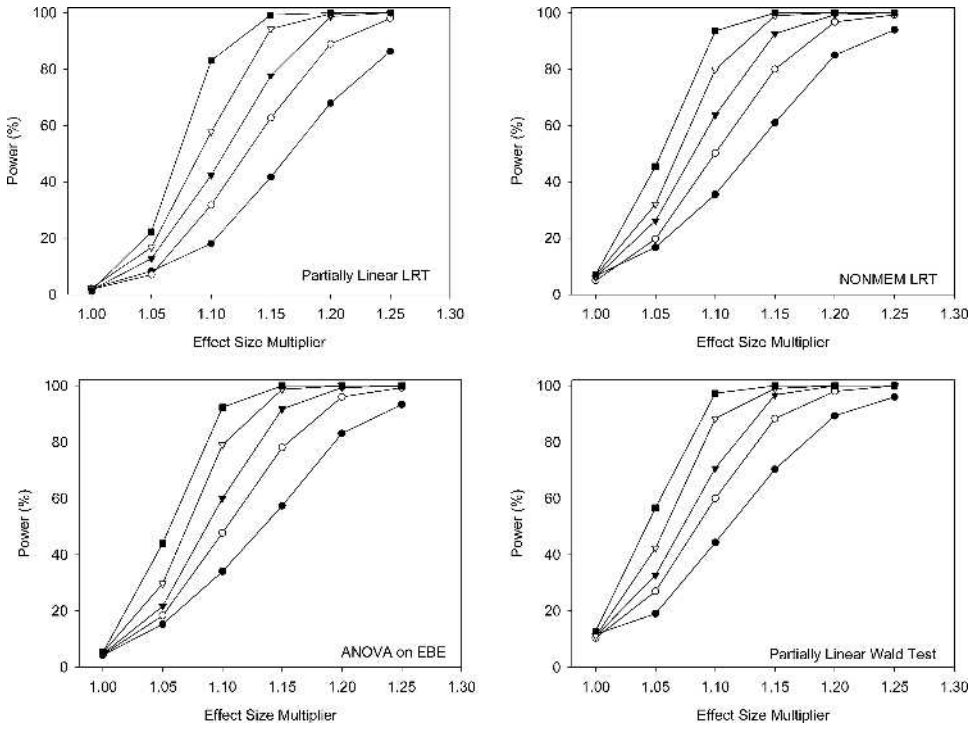

Fig. 6. Power curves for partially linear model covariate screening using the LRT test (top left), NONMEM screening using LRT (top right), covariate screening using the EBE for CL derived under the reduced NONMEM model (bottom left), and partially linear model screening using Wald test (bottom right) when 2 samples per patient were collected using sample sizes of 50 (solid circles), 75 (open circles), 100 (solid upside down triangle), 150 (open upside down triangle), or 250 (solid squares) subjects.

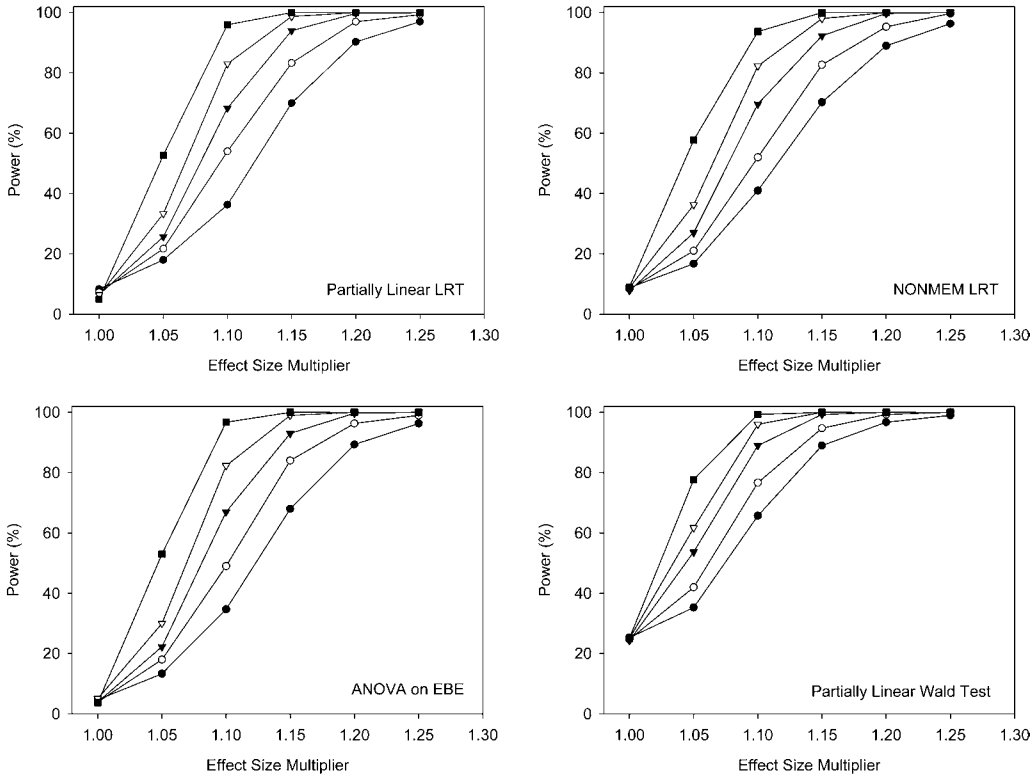

Fig. 7. Power curves for partially linear model covariate screening using the LRT test (top left), NONMEM screening using LRT (top right), covariate screening using the EBE for CL derived under the reduced NONMEM model (bottom left), and partially linear model screening using Wald test (bottom right) when 4 samples per patient were collected using sample sizes of 50 (solid circles), 75 (open circles), 100 (solid upside down triangle), 150 (open upside down triangle), or 250 (solid squares) subjects.

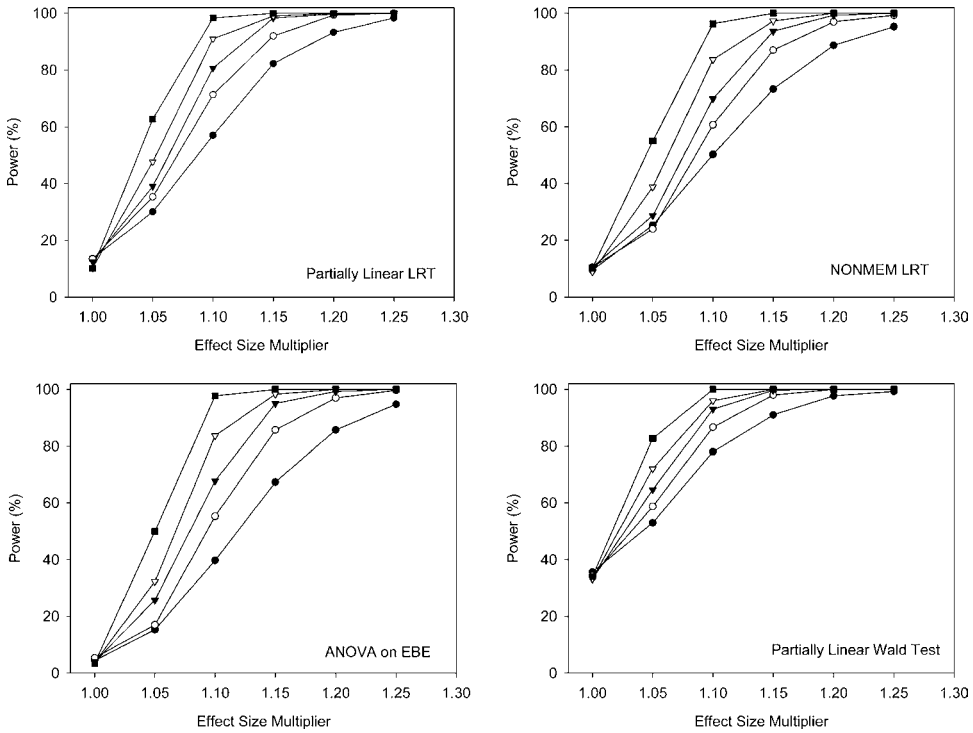

Fig. 8. Power curves for partially linear model covariate screening using the LRT test (top left), NONMEM screening using LRT (top right), covariate screening using the EBE for CL derived under the reduced NONMEM model (bottom left), and partially linear model screening using Wald test (bottom right) when 6 samples per patient were collected using sample sizes of 50 (solid circles), 75 (open circles), 100 (solid upside down triangle), 150 (open upside down triangle), or 250 (solid squares) subjects.

rate was dependent on sample size ($p < 0.001$) and number of observations per subject ($p < 0.0001$) using the LRT as a covariate screen within a partially linear model. Further, type I error rates were below nominal levels when only 2 observations per subject were available but where higher than nominal levels when 6 observations per subject were available. In contrast, type I error rates using Wald's test within the context of a partially linear model were always higher than nominal levels and were as high as 36%. Wald's test cannot be advocated under any conditions. Type I error rate was dependent on number of observations per subject ($p < 0.0001$) but not sample size using the LRT as a covariate screen within NONMEM and tended to increase above nominal levels as the number of observations per subject increased. Finally, type I error rate was not affected by either sample size or number of observations per subject using ANOVA on the EBEs for CL and were near nominal levels under all conditions.

Figure 6, Fig. 7, and Fig. 8 present the power curves at detecting sex as a covariate on clearance when 2, 4, and 6 samples per subject, respectively, were collected. Wald's test within the context of partially linear model tended to have higher power than the other methods, but Wald's test also had significantly higher type I error rate. Hence, Wald's test cannot be advocated within the context of a partially linear model. Power curves for ANOVA of the EBEs of CL were below those of the LRT methods. Similar power curves were obtained using the LRT within NONMEM and within a partially linear mixed effects model with four or more observations per subject. With two observations per subject, the LRT within NONMEM appeared to be more powerful than the LRT using partially linear mixed effects models. Eighty percent power was achieved with 4 samples per subject and 50 subjects total when the effect size was approximately 1.07, 1.07, 1.08, and 1.05 for LRT using PLMEMs, LRT using NONMEM, ANOVA on the EBEs, and Wald's test using PLMEMs, respectively.

DISCUSSION

These results indicate that PLMEM using the LRT test appear about as powerful as the LRT within NONMEM at detecting important covariate effects. Type I error rates were similar between PLMEM and NONMEM using the LRT but were inflated (as high as 36%) based on PLMEM using Wald's test. Type I error rate tended to increase as the number of observations per subject increased for all LRT methods. Power curves were similar between the PLMEM and NONMEM LRT methods and were slightly more than the power curve using ANOVA on the EBEs of CL. Eighty percent power was achieved with 4 samples per subject and 50 subjects total when the effect size was approximately 1.07, 1.07, 1.08, and 1.05 for LRT using PLMEMs, LRT using NONMEM, ANOVA on the EBEs, and Wald's test using PLMEMs, respectively.

These conclusions must be couched in one very important regard. The same model used to simulate the data was used to model the data within NONMEM. In reality, the data generating model is unknown so that the results of this simulation represent the best-case scenario. Under real-world conditions, the power of NONMEM and PLMEMs at detecting a covariate is unknown in the face of model misspecifications. It

is anticipated that these models will not replace NONMEM-based covariate screening but will serve as ancillary methods for covariate identification, like tree-based methods (19).

PLMEMs are advantageous in that they effectively remove the need to develop a structural model that encompasses the time component in a compartmental model by using a set of spline basis functions that are treated as random effects. Second, because PLMEMs make use of linear mixed effects model algorithms, they are faster than covariate screening in NONMEM. For example, the NONMEM runtime in the simulation with 250 subjects having 6 samples per subject on a Pentium IV (1500 MHz) personal computer was ~38 s but was less than 3 s for the PLMEM. Third, PLMEMs may also be useful for pharmacodynamic modeling where the dependent variable is some effect and the independent variable is concentration. In this case, the knots may be chosen to cover the range of observed concentrations. Of course, much further research is needed on the utility of these models in the pharmacokinetic-pharmacodynamic setting.

PLMEMs, while certainly having some advantages, were not without their disadvantages. These disadvantages relate primarily to use of underlying spline basis functions, a criticism that would also apply to earlier published work on spline functions in population analysis. First, extension of PLMEMs to multiple dose regimens where data are available on multiple occasions is not entirely obvious. Second, PLMEMs are not meant to serve as a replacement for population models that explicitly model how a variable changes over time. Indeed, these latter models are highly useful for simulation as PLMEMs are at a disadvantage in that simulation is not possible. Also, population models that use an explicit function, such as a polyexponential equation, can be used to quantify how a covariate impacts a pharmacokinetic parameter (e.g., how age or weight affects clearance). All that can be interpreted with PLMEMs is that a covariate affects the dependent variable. Although it may be possible to estimate the magnitude of the effect of a covariate through examination of the fixed effect estimates in PLMEMs, their purpose is primarily to simply identify whether a covariate has an effect—without relying on an explicit underlying function.

In summary, PLMEMs represent a viable alternative to NONMEM-based covariate screening. PLMEMs are easy to program, both within SAS and NONMEM, and are advantageous in that they remove the need to explicitly model the time component in a pharmacokinetic model. Further research is needed in their use in population modeling.

APPENDIX:

SAS CODE USED TO MODEL DATA IN FIG. 4 Using Partially linear Mixed Model Framework (Quadratic Spline Basis Function)

```
data conc;
  infile "loess.csv" delimeter = ",";
  input subject time conc x y z;
  timeclss = time;
  lnconc = log(conc);
  array knot_{9};
  do k = 1 to 9;
        knot_{k} = max(0, time - (k-1)*3)**2;
  end;
proc mixed data=conc method=reml;
  class subject timeclss;
  model lnconc = time|time / outpred=reml;
  random knot_1 - knot_9 / type=toep(1);
  repeated timeclss / subject=subject type=sp(power)(time);
run;
quit;
```

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