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Modeling Development of Sleep–Wake Behaviors: I. Using the Mixed General Linear Model[1,2]

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HOLDITCH-DAVIS, D., L. J. EDWARDS AND R. W. HELMS. *Modeling development of sleep–wake behaviors: I. Using the mixed general linear model.* PHYSIOL BEHAV **63**(3) 311–318, 1998.—The purpose of this paper is to demonstrate the use of the mixed general linear model (MixMod) for modeling development of sleep–wake behaviors in preterm infants. The mixed general linear model allows the concurrent identification of both group and individual developmental patterns in longitudinal data sets with inconsistently timed data, irregularly timed data, and randomly missing values. This statistical technique is well suited to data from preterm infants because these infants enter and leave longitudinal studies at varying times depending on their health status. One sleep organizational variable—the regularity of respiration in quiet sleep—obtained from a study of 37 preterm infants was used as an example. Seven infant characteristics were used as covariates. The various steps involved in conducting a mixed model analysis of this variable are illustrated. The strengths and limitations of this technique are discussed. © 1998 Elsevier Science Inc.

Mixed general linear model Longitudinal studies Development Preterm infants Sleep–wake states

THE development of sleeping and waking during early infancy, and particularly in preterm infants, has long been of interest to researchers and clinicians. As a result, a number of studies have been conducted that examined the sleeping and waking of premature infants before term (6–8,12,13,20,26–30,36). However, none of these studies was able to use a true longitudinal design. Preterm infants are born at different gestational ages, become healthy enough to study at different chronological ages, and become well enough to go home at different times. Thus, preterm infants enter and leave studies at different times and spend varying amounts of time in the study. Preterms are extremely heterogeneous with respect to demographic and medical factors that might affect state development. To date, researchers have not been able to appropriately model the developmental trajectories of these infants.

Most researchers have used one of three simple approaches: studying different infants at each age (6,31), studying the same infants at only two ages (11), or studying infants repeatedly but just descriptively comparing means at each age (4,12,25). Although these approaches were the best available at the time the studies were conducted, none of them provides an adequate estimation of individual developmental trajectories. The cross-sectional approach, by averaging over individuals, may markedly distort developmental processes (39). Studying only two time points allows the determination of whether there are differences between the two ages but not the overall developmental rate or pattern. By ignoring subjects and descriptively comparing means at each age, individual differences are overlooked. In this analysis, each observation on a subject is treated as if it is independent of all other observations on the subject, resulting in inflated Type I error rates.

The first statistically appropriate method for estimating individual developmental trajectories in preterm infants with differing numbers of observations was a two-stage linear regression procedure, developed by Kraemer, Korner, and Hurwitz (22), which is closely related to logistical regression. Transformed data are regressed on postconceptional or chronological age for each infant, producing an intercept and slope for each infant. The impacts of gestational age, birthweight, and chronological age on state development were determined by correlating these variables with the intercepts and slopes. This approach has been used in a number of studies (1,19–21), but it has limitations. Separate regressions are calculated for each subject so no estimation of the population

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development pattern is obtained. In addition, it is not possible to incorporate the effects of other variables, such as illness severity, race, or sex, into the initial regression.

In a previous paper (13), we examined the development of sleeping and waking during the preterm period using a combination of the previously described techniques. Multiple regression of the amount of each state over age and illness severity, ignoring individuals, was conducted for the group using $p < 0.01$ to correct for the inflated Type I error rate. Slopes from individual developmental trajectories were compared using signed ranked tests. However, these analyses, though the best we could conduct at the time, were complex and did not allow us to compare individual trajectories with the pattern of the group.

The purpose of this paper is to demonstrate the usefulness of the mixed general linear model, a more appropriate statistical procedure, for modeling development of sleep–wake behaviors during the preterm period by reanalyzing the quiet sleep respiration regularity score variable, a measure of the organization of quiet sleep. Although the mixed general linear model has been known to biostatisticians for over 10 years and has been used in a few studies of clinical populations (14,16,18,32,37), it has not been previously used in studies of preterm infant behavior. The mixed general linear model allows the concurrent identification of both group and individual developmental patterns in longitudinal data sets with covariates that vary over time, inconsistently timed data (assessment schedules vary from subject to subject), irregularly timed data (varying time intervals between assessments), and randomly missing values. Since the mixed model has not been previously used in studies of preterm behavior, full-scale assessment of the development of sleep and waking that includes time-dependent covariates has not been accomplished. Despite the advantages of the mixed model, this model has not been widely used for longitudinal analyses of the physiological or behavioral development of any clinical population probably because most researchers are unfamiliar with it and because most investigators using it have utilized the mixed model only for group analyses and have ignored its ability to identify individual developmental patterns (40).

METHODS

Subjects

Subjects used in this example were 31 preterm infants who either weighed less than 1500 g at birth or required mechanical ventilation; 27 had both of these problems. They were part of a sample of 37 infants that has been previously described in detail (13). Due to technical problems with the recordings used to obtain the variable for this report, data were not obtained on six infants.

Procedures

Infant behaviors, including sleep–wake states and respiratory patterns, were observed once a week from approximately 1900 to 2300 hours. These observations began as soon as the infant's medical condition was no longer critical and consent was obtained from the parents. Infants left the study on transfer to a community hospital, discharge home, or reaching term age. Thus, the ages at which subjects entered the study and the length of time they were studied varied. A total of 116 observations were conducted on the 31 subjects between 29 and 39 weeks postconceptional age (P-C Age). The number of observations for each subject ranged from 1 to 9 with a mean of 3.6.

During the observations, the occurrences of infant behaviors were recorded every 10 s (13). The infant's respiration was recorded on a Gulton chart recorder with a piezoelectric sensor pad whenever the infant was asleep, so that the regularity of respiration could be identified.

Variables Used for Data Analysis

Quiet sleep respiration regularity score. The respiration tape was scored visually for three levels of respiration regularity during quiet sleep—very regular, regular, and irregular (16,33). Reliability was determined by rescoring five respiration tapes more than 6 months after they were originally scored. The percentage of exact agreements ranged from 80.4% to 93.6% for these variables, averaging 88.5% overall. A measure of the overall regularity of respiration, and thus the organization of quiet sleep, was calculated by summing two times the percent of very regular respiration plus the percent of regular respiration minus the percent of irregular respiration. This quiet sleep respiration regularity score had a possible range of -100 to $+200$.

Infant characteristics. Eight infant characteristics were used to model the development of sleeping and waking states. Each dependent variable was regressed over postconceptional age. The other variables were used as covariates.

Five variables—gestational age at birth, birthweight, race, sex, and days of mechanical ventilation—remained stable within each subject. The gestational age at birth of each infant was calculated from the obstetric estimated date of confinement which had been determined either by the date of the mother's last menstrual period or by an ultrasound examination, assuming that this gestational age agreed within 2 weeks with the results of a simplified version of the Dubowitz examination (5,9) conducted by a pediatrician on admission. If the obstetrical dates were unreliable, the gestational age from the Dubowitz was used. Race, sex, birthweight, and number of days on mechanical ventilation were determined from the medical record.

The other three variables—postconceptional age (in weeks), chronological age (in days), and theophylline treatment—were assessed at each observation. Whether or not the infant was receiving theophylline was determined for each observation because theophylline has been found to alter the sleeping and waking states of premature infants (34).

Infant characteristics variables were generally measured using the actual data. Race was scored as either white or minority. (There was one Native American infant in the minority group, and the rest were black.) Since the distribution of the number of days of mechanical ventilation was highly skewed (mean 10.3, median 5), infants receiving 1 day or less of mechanical ventilation were scored as receiving 1 day and then the natural logarithm of each subject's score was used in analyses.

Statistical Analysis

The analysis in this paper was performed using a technique for longitudinal data analysis, called mixed general linear models (MixMod) or random regression models (2,3,10,17,23,35,37). In the MixMod, the regression of each subject is represented by its deviation from the group regression. The MixMod has three components: the fixed effects component, the random effects component, and the random error component. The fixed effects and random error components are analogous to the corresponding components of a standard multiple regression. The fixed effects component represents a population regression line. The random effects component for each subject is the difference between that subject's regression and the population regression and is a measure of how that subject differs from the population regression. Thus, each subject has its own random regression line which is defined

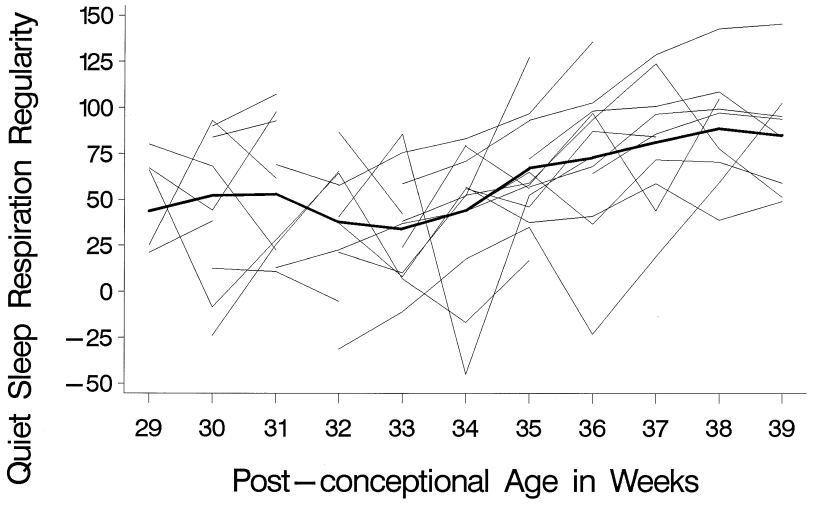

FIG. 1. Raw quiet sleep respiration regularity scores for individuals (thin lines) and the group mean (thick line) at each postconceptional age.

by the sum of the fixed effects component and the subject's random effects components. Because each subject has its own regression line, the MixMod can accommodate mistimed data and data missing ''completely at random'' without requiring that missing data be estimated. (See the Appendix for statistical information about the mixed model.)

RESULTS

The analysis of quiet sleep respiration regularity score will be described as an example of the use of the mixed general linear model. Quiet sleep organization is known to increase over the preterm period so this variable should serve as an effective example (6,7,12,13,20,26,29), and the development of this variable, unlike most of the other sleep–wake behaviors, is affected by covariates (15). The mean value of the quiet sleep respiration regularity score over the 116 observations was 59.7 (SD 38.7, range −44.9 to +145.5, median +59.1). Figure 1 presents the raw data for this variable. Although examination of the weekly means suggests that this variable undergoes systematic development over the preterm period, the investigator is almost overwhelmed by the variability apparent in this figure. Subjects enter and leave the study at different points in time. As a result, some subjects have as many as nine observations and others as few as one. There is also a large amount of intrasubject variation. However, this sort of data is typical for preterm infants.

It is difficult to identify a statistically appropriate analysis technique for this sort of data. One could use the approach of Kraemer, Korner, and Hurwitz (22), but that would involve calculating separate regressions for each subject, and no estimate of the population pattern would be obtained. In addition, in studies with significantly more subjects than the 31 in this analysis, calculating separate regressions for each subject might be impractical.

The mixed general linear model presents an analytical solution to the problems inherent in this type of data. A single equation is used to calculate developmental patterns for both the group and individuals. Figure 2 illustrates the results of calculating a MixMod for the quiet sleep regularity score and shows how this statistic enables the systematic aspects of the data in Fig. 1 to become apparent. In the rest of the results section, we will summarize the steps involved in calculating the MixMod.

Preliminary Examination of Variables

Normality of the variable. The first step in analyzing data with the MixMod, like all statistics, is conducting a preliminary examination of the data. The Shapiro–Wilk test of normality for the quiet sleep regularity score was nonsignificant ($W = 0.98$, $p = 0.43$), indicating that this variable was normally distributed. Skewness was −0.263, and kurtosis −0.105.

Although in this case the dependent variable was normally distributed, it is unclear how necessary this is. Very little is known about the extent to which MixMod estimation and hypothesis testing procedures are robust to nonnormality (24). Standard multiple regression analyses are generally quite robust to certain types of nonnormality but are sensitive to distributions with long tails or great skewness. Informal studies suggest that MixMod estimates of parameters and the distribution of the test statistic inherit a relative insensitivity to nonnormality from their standard regression counterparts. Also, unlike standard multiple regressions, the MixMod is relatively unaffected by heteroscedasticity (inhomogeneous variances) since the MixMod explicitly includes both within- and between-subject heteroscedasticity.

Covariates. Prior to the analysis, postconceptional age was ''centered'' at its mean value (34 weeks), averaged over subjects and over time points within subjects. Thus, postconceptional age ranged from −5 (29 weeks) to +5 (39 weeks). The eight infant characteristics were intercorrelated. In general, these relationships were small to moderate with the following exceptions. Postconceptional age was highly correlated with chronological age at observation ($r = 0.77$) and days of mechanical ventilation ($r = 0.65$). Chronological age at observation was highly correlated with days of mechanical ventilation ($r = 0.83$), birthweight ($r = -0.64$), and gestational age at birth ($r = 0.69$). Mechanical ventilation was also highly correlated with gestational age at birth ($r = 0.72$). These findings would be expected in preterm infants since smaller birthweight and lower gestational age at birth increase the risk of illness and prolonged

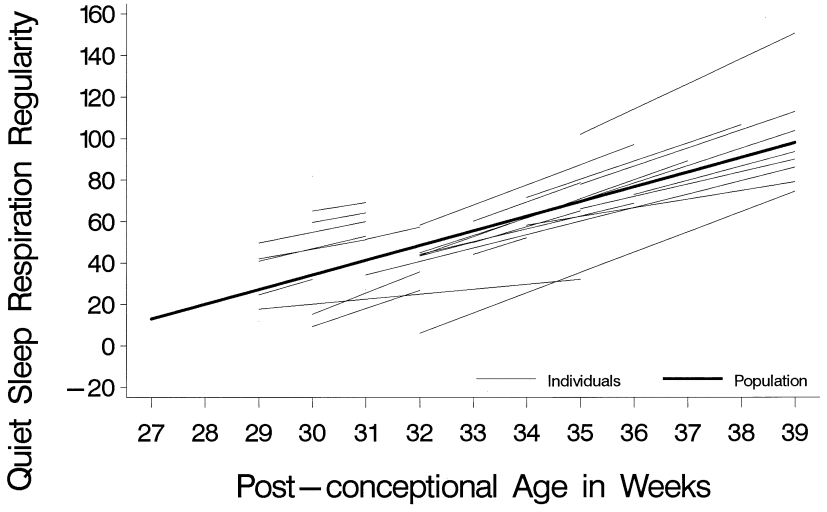

FIG. 2. Predicted regression of quiet sleep respiration regularity scores over postconceptional age for individuals (thin lines) and the group (thick line).

hospitalization. A decision was made on theoretical grounds that postconceptional age would be the basic time frame over which each dependent variable was regressed because postconceptional age directly reflects biological maturation. The other infant characteristics were retained in the model only if they contributed significantly to the regression in addition to the variance they share with postconceptional age.

TABLE 1

PREDICTOR VARIABLES IN THE PRELIMINARY SCREENING REGRESSION ANALYSES FOR QUIET SLEEP RESPIRATION REGULARITY SCORE

Term	Coefficient	SE	*p* Level
	Initial Screening Procedure		
Intercept	3.951	53.393	0.941
Postconceptional Age	−2.474	8.401	0.770
Mechanical Ventilation	−12.895	8.529	0.025
Birthweight	0.050	0.024	0.043
Theophylline	5.454	7.900	0.493
Gestational Age	8.260	8.529	0.337
Chronological Age	1.756	1.211	0.153
Race	−6.228	12.056	0.608
Sex	13.319	10.782	0.222
	Second Screening Procedure		
Intercept	61.996	13.255	0.000
Postconceptional Age	2.368	3.848	0.541
Mechanical Ventilation	−2.380	5.224	0.650
Birthweight	0.038	0.019	0.050
P-C Age × Mechanical Ventilation	2.391	1.555	0.130
P-C Age × Birthweight	−0.000	0.008	0.977

Note: Italicized variables were retained for the subsequent analysis.

Screening Analyses

To achieve a parsimonious model, the main effects were fit first in a preliminary MixMod regression that required a $p < 0.15$ for an infant characteristic to be retained. The intercept and postconceptional age effects would be retained regardless of their probability level. As Table 1 indicates, all of the infant characteristics, except birthweight and mechanical ventilation, were eliminated at this stage.

In a second step, the screening regression was repeated with the two remaining infant characteristics, intercept, postconceptional age, and the interactions between postconceptional age and the remaining infant characteristics (see Table 1). This time one infant characteristic—birthweight—and one interaction—mechanical ventilation × postconceptional age—met the 0.15 probability level. A theoretical decision had been made to retain the main effect if the interaction reached a *p* level of 0.15. Thus, intercept, postconceptional age, and the main effect of mechanical ventilation were also retained in the model. These variables were used in the final MixMod analyses.

Final Analyses

In a MixMod analysis, the computer uses an iterative process to fit the best estimates for the fixed effects (the group regression of intercept, postconceptional age, birthweight, mechanical ventilation, race, and the interaction between postconceptional age and mechanical ventilation) and for the random components (the deviations of each individual's intercept and postconceptional age effect from the group regression). We decided on theoretical grounds to limit the random components to intercept and postconceptional age. However, additional random effects could be fitted if a variable that has a value at each age, such as theophylline, was expected to have differing influences on different individuals. Thus, our random effects component of the MixMod, or individual subjects' deviations, was limited to the deviation of an individual subject's intercept from the group intercept and the deviation of an individual subject's slope from the slope of the population regression with regard to postconceptional age.

We again utilized a model reduction procedure in which all

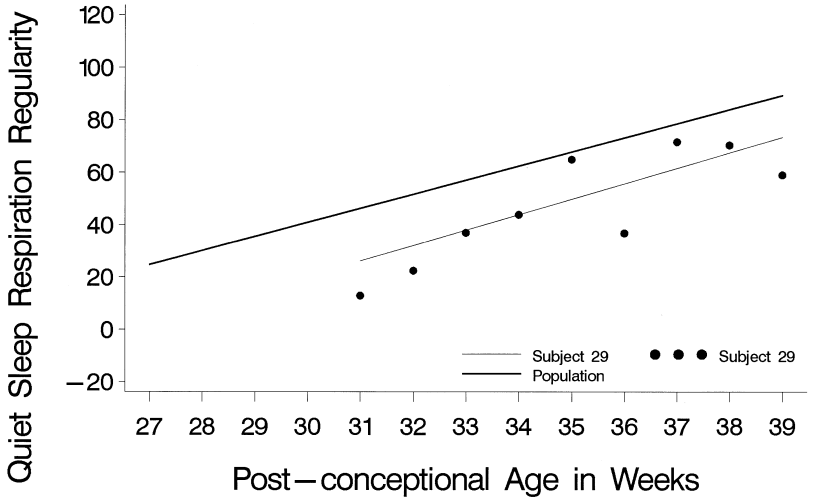

FIG. 3. Predicted regression of quiet sleep respiration regularity score for the group (thick line) and for Subject 29 (thin line). Subject 29's actual data points are indicated by dots.

variables not achieving $p = 0.05$ in an initial MixMod, except intercept and postconceptional age, were removed, and MixMod was repeated. Two variables were found to contribute significantly in the group analysis, or fixed effects components, of the final MixMod analysis. (Since this is a secondary analysis of already published data, statistical ''significance'' and p values should be interpreted as descriptive terms. However, in analysis situations appropriate for hypothesis testing, they would be interpreted in the same way as p values of more familiar statistics.)

The intercept at 34 weeks postconceptional age was the first predicted component. It had a value of 62.571 (SE 4.878, $F(1, 58) = 164.61$, $p < 0.001$). Postconceptional age contributed significantly to the regression with a coefficient of 7.09 (SE 1.563, $F(1, 58) = 20.62$, $p < 0.001$), indicating that the organization of quiet sleep increased over the preterm period. Birthweight also had a significant effect with a coefficient of 0.046 (SE 0.017, $F(1, 58) = 7.41$, $p < 0.01$). Infants with larger birthweights had slightly greater quiet sleep respiration regularity scores. Thus, the estimated regression for quiet sleep respiration regularity score was

$$\text{QS Resp. Reg. Score} = 62.571 + [7.085 \times (\text{P-C Age})] + [0.046 \times (\text{Birthweight})]$$

where postconceptional age ranges from −5 to +5. Figure 3 presents this group regression (or fixed effects component) of quiet sleep respiration regularity score over postconceptional age with birthweight set at the mean value of 995 g.

The MixMod also identifies random components, or each subject's deviations from the group regression. An individual subject's intercept is the sum of the population intercept and the subject's random intercept increment. In the MixMod, the regression line for the kth subject is treated as random. The randomness comes from the assumption that the intercept and slope increments for the kth subjects are random variables from a joint normal distribution with zero means.

For example, Subject 29 had a random intercept coefficient of −8.795 and a random postconceptional age component of −0.574. Thus, the ''predicted'' regression for this subject who had a birthweight of 770 g is

$$\text{QS Resp. Reg. Score} = 62.571 - 8.803 + [(7.085 - 0.573) \times (\text{P-C Age})] + [0.046 \times 770]$$

Figure 3 shows this subject's predicted regression as compared to the group regression and to the subject's actual data. A subject's actual data do not lie precisely on a straight line. Each data value deviates from the individual's ''true'' line by a small amount. This error is assumed to be random variables from a normal distribution with mean of 0.

Figure 2 shows the dispersion of all subjects' predicted regression lines around the group regression. The variances of the actual data around the individual intercepts and slopes (postconceptional age effect) for each variable in the MixMod analyses give a measure of the amount of dispersion. The standard deviation of the random intercepts was 19.75 (as compared to a group coefficient of 62.571) (see Fig. 2). The standard deviation of the random slopes was 4.47 as compared to the estimated population slope of 7.085. The correlation between the individual random intercepts and slopes was very low (0.063), indicating there was no systematic relationship between random slopes and intercepts and that the individual regression lines for quiet sleep respiration regularity score generally parallel the group regression line.

DISCUSSION

Our example demonstrated that the MixMod analyses can be useful for determining the development of sleep-related behaviors, such as quiet sleep regularity score, over the preterm period. This statistical technique has the advantage that each subject is represented in the model so that subjects with differing starting ages and different lengths of time in the study can be included in the same analysis without estimating missing values or violating assumptions. Using the MixMod, we found that the organization of the quiet sleep increased over age and that demographic characteristics and medical complications have relatively small impact on this developmental change. Thus, the MixMod analyses had similar findings to that of our earlier multiple regression analysis with the same data (13), but the MixMod is a statistically appropriate method for analyzing this type of data.

Unlike the standard multiple regression, the MixMod analyses also permitted examination of developmental patterns of individual infants. The individual developmental patterns generally paralleled the group regression lines for organization of sleep states. There was no significant correlation between individual slopes and intercepts, but examination of the graphs (Fig. 2) does show a fair amount of interindividual variation in slopes. Thus, differences between individual and group developmental patterns in different states can be explored with the MixMod but not with a standard multiple regression.

One of the strengths of the MixMod analysis was that we were able to include seven infant characteristics in the screening regression, which is more than could be appropriately included in a standard multiple regression that would need to be limited to only one observation for each of the 31 subjects in order not to violate statistical assumptions. With the MixMod, investigators can statistically identify the effects of other variables on developmental patterns and determine which of these variables most directly influences state development. We found that most of the infant characteristics had either no effect on the development of quiet sleep organization or such small effects that they did not enter into the regression.

Another advantage of the MixMod was that it avoided the biases having repeated measures on the same subjects would introduce into a standard multiple regression. An analysis using standard multiple regression requires fitting separate models to each individual. Serial observations on a single subject are in most cases correlated. Standard multiple regression techniques assume that observations are independent, i.e., that there is no correlation from one observation to the next. Thus, when one uses multiple regression, one has to ignore the correlations between observations. This not only violates model assumptions but also leads to biased estimation. Also, to compare fixed group effects, such as sex, race, or medical complications, some type of meta-analytic technique must be used to summarize the results in each group since the models would have been fit on an individual basis. Such techniques often are not robust and add further noise to the results. The mixed model simultaneously fitted group and individual subject effects. It also easily adjusted for correlated observations through the inclusion covariances in estimation results.

Standard repeated-measures analyses assume the data are regularly timed. In addition, missing values on a subject are usually handled by completely removing the subject from the analyses or estimating the missing value. If 5% or more of the subjects are removed or 5% or more of the data estimated, severe bias in estimation and hypothesis testing is introduced. The mixed model did not require regularly timed observations, and all nonmissing values were used in the analysis.

Although there were a number of advantages of the mixed model, it also had limitations. Assessing model assumptions (distributional assumptions, linearity, outliers) through ''residual'' analysis can be very difficult and is the subject of ongoing theoretical and practical research. Second, finding easy to use software for the proper implementation of the mixed model can be difficult for investigators. Although SAS Proc Mixed is available and relatively easy to use, the software presently has convergence problems even under simple modeling scenarios. Hence, specialized software must be used to address nonconvergence problems. Also, residual analysis techniques for the MixMod can only be implemented using specialized software. The analysis in this paper overcame these limitations by using specialized software.

In conclusion, the findings of this study demonstrate the potential usefulness of the MixMod technique for determination of developmental patterns in preterm infants. This statistical technique is able to deal with the problems of subjects entering and leaving the study at different times, an inevitable characteristic of a longitudinal study with preterm infants. Moreover, it permits the concurrent determination of individual developmental patterns and comparison of the individuals' regressions with the group. It also can be used to examine a wide variety of demographic factors and medical complications so that specific hypotheses about maturational and environmental effects on development of states can be tested. The MixMod technique deserves a wider utilization in studies of preterm infants and should also be strongly considered for use with any population, including clinical populations who are medically ill, neurologically impaired, or elderly, in which an irregular pattern of data collection or loss of subjects due to death or physical incapacity is to be expected by the nature of the population under study.

APPENDIX

It may be useful to discuss the formulation of the mixed general linear model in more detail. In general, the mixed model equation for a random regression model with continuous outcome measure, i.e., continuous dependent variable, is typically expressed for an individual as

$$\mathbf{Y}_k = \mathbf{X}_k\boldsymbol{\beta} + \mathbf{Z}_k\mathbf{d}_k + \mathbf{e}_k$$

where $\mathbf{Y}_k$ is the $n_k \times 1$ vector of observations from the kth subject, $\mathbf{X}_k$ is the $\mathbf{n}_k \times q$ matrix of ''fixed effect'' regressor values (including a column of 1s for the interceptor) for the kth subject, $\boldsymbol{\beta}$ is the $q \times 1$ vector of population regression coefficients, or ''fixed effect'' coefficients, $\mathbf{Z}_k$ is the $n_k \times p$ matrix of ''random effect'' regressor values (including a column of 1s for the intercept term) for the kth subject, and $\mathbf{d}_k$ is the $p \times 1$ vector of ''random effect'' parameters for the kth subject. $\mathbf{e}_k$ is the $\mathrm{n}_k \times 1$ vector of ''random error'' terms for the kth subject. Note that $\boldsymbol{\beta}$ has no subscript; as in a multiple regression, one $\boldsymbol{\beta}$ vector serves for all subjects.

In the same fashion as the usual multiple regression model, there are distributional assumptions for the random terms and techniques for estimating model parameters. Details regarding distributional assumptions and parameter estimation in the mixed model are given in the literature (2,3,10,17,23,35,37,38). An estimation equation for $\boldsymbol{\beta}$ is

$$\hat{\boldsymbol{\beta}} = (\mathbf{X}'\hat{\boldsymbol{\Sigma}}^{-1}\mathbf{X})^{-1}\mathbf{X}'\hat{\boldsymbol{\Sigma}}^{-1}\mathbf{Y}$$

where $\hat{\boldsymbol{\Sigma}} = \hat{\mathbf{V}}(\mathbf{Y})$ an estimate of the variance of the vector of all observations from subjects produced by vertically concatenating the $\mathbf{Y}_k$. This equation shows that one must know $\hat{\boldsymbol{\Sigma}}$ to compute $\boldsymbol{\beta}$. Because of the complicated method involved in doing the estimation, iterative methods are required to compute simultaneous estimates of all parameters.

The vector $\boldsymbol{\beta}$ is the primary (fixed effect) parameter. Much of the work of statistical analysis is performed via secondary (fixed effect) parameters of the form $\boldsymbol{\Theta} = \mathbf{C}\boldsymbol{\beta} - \boldsymbol{\Theta}_0$, where $\mathbf{C}$ and $\boldsymbol{\Theta}_0$ are a known, fixed $a \times q$ and $a \times 1$ vector, respectively. For an a priori secondary parameter Θ, an approximate F statistic for H_0: $\boldsymbol{\Theta} = \mathbf{0}$ vs H_a: $\boldsymbol{\Theta} \neq \mathbf{0}$ is

$$F = \hat{\boldsymbol{\Theta}}'[\mathbf{C}(\mathbf{X}'\hat{\boldsymbol{\Sigma}}^{-1}\mathbf{X})\mathbf{C}']^{-1}\hat{\boldsymbol{\Theta}}/a$$

The specific equation for the MixMod with multiple regressors, such as is the case in this study, is

$$y_{ki} = [\beta_0 + \beta_1(x_{1ki} - x_{10}) + \beta_2(x_{2ki} - x_{20}) + \ldots + \beta_q(x_{qki} - x_{q0})] + [d_{0k} + d_{1k}(z_{1ki} - z_{10}) + d_{2k}(z_{2ki} - z_{20}) + \ldots + d_{pk}(z_{pki} - z_{p0})] + e_{ki}$$

In this equation, β_0 is the intercept and β_1 to β_q, the slopes for the various predictor variables and covariates (postconceptional age, birthweight, mechanical ventilation, race, and the interaction between postconceptional age and mechanical ventilation).

An individual subject's intercept is the sum of the population intercept and the subject's random intercept increment. To achieve this, in the overall equation, we let the variable Z be the same as the first two columns in X (corresponding to intercept and slope), so that $z_{ki} = x_{ki}$ and $z_0 = x_0$. These terms can be rearranged to produce an equation for the regression line for the kth subject in which $\beta_0 + d_{0k}$ is the intercept, $\beta_1 + d_{1k}$ is the slope (postconceptional age effect), β_2 is the birthweight effect, and e_{ki} is the deviation about the line:

$$y_{ki} = [\beta_0 + \beta_1(x_{ki} - x_0)] + [d_{0k} + d_{1k}(z_{ki} - z_0)] + e_{ki}$$

Notice that d_{0k} is an intercept increment and d_{1k} is the slope increment. The regression for the kth subject is calculated by adding d_{0k} to the intercept of the population regression line (β_0) and d_{1k} to the slope of population regression line (β_1). In the MixMod, the regression line for the kth subject is treated as random.

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