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Modelling covariance structure in the analysis of repeated measures data

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SUMMARY

The term 'repeated measures' refers to data with multiple observations on the same sampling unit. In most cases, the multiple observations are taken over time, but they could be over space. It is usually plausible to assume that observations on the same unit are correlated. Hence, statistical analysis of repeated measures data must address the issue of covariation between measures on the same unit. Until recently, analysis techniques available in computer software only offered the user limited and inadequate choices. One choice was to ignore covariance structure and make invalid assumptions. Another was to avoid the covariance structure issue by analysing transformed data or making adjustments to otherwise inadequate analyses. Ignoring covariance structure may result in erroneous inference, and avoiding it may result in inefficient inference. Recently available mixed model methodology permits the covariance structure to be incorporated into the statistical model. The MIXED procedure of the SAS® System provides a rich selection of covariance structures through the RANDOM and REPEATED statements. Modelling the covariance structure is a major hurdle in the use of PROC MIXED. However, once the covariance structure is modelled, inference about fixed effects proceeds essentially as when using PROC GLM. An example from the pharmaceutical industry is used to illustrate how to choose a covariance structure. The example also illustrates the effects of choice of covariance structure on tests and estimates of fixed effects. In many situations, estimates of linear combinations are invariant with respect to covariance structure, yet standard errors of the estimates may still depend on the covariance structure. Copyright © 2000 John Wiley & Sons, Ltd.

1. INTRODUCTION

Statistical linear mixed models state that observed data consist of two parts, fixed effects and random effects. Fixed effects define the expected values of the observations, and random effects define the variance and covariances of the observations. In typical comparative experiments with repeated measures, subjects are randomly assigned to treatment groups, and observations are made

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at multiple time points on each subject. Basically, there are two fixed effect factors, treatment and time. Random effects result from variation between subjects and from variation within subjects. Measures on the same subject at different times almost always are correlated, with measures taken close together in time being more highly correlated than measures taken far apart in time. Observations on different subjects are often assumed independent, although the validity of this assumption depends on the study design. Mixed linear models are used with repeated measures data to accommodate the fixed effects of treatment and time and the covariation between observations on the same subject at different times. Cnaan *et al.* [1] extensively discussed the use of the general linear mixed model for analysis of repeated measures and longitudinal data. They presented two example analyses, one using BMDP 5V [2] and the other using PROC MIXED of the SAS System [3]. Although Cnaan *et al.* discussed statistical analyses in the context of unbalanced data sets, their description of modelling covariance structure also applies to balanced data sets.

The objectives of repeated measures studies usually are to make inferences about the expected values of the observations, that is, about the means of the populations from which subjects are sampled. This is done in terms of treatment and time effects in the model. For example, it might be of interest to test or estimate differences between treatment means at particular times, or differences between means at different times for the same treatment. These are inferences about the fixed effects in the model.

Implementation of mixed models ordinarily occurs in stages. Different data analysts may use different sequences of stages. Ideally, different data sets would be used to choose model form and to estimate parameters, but this is usually not possible in practice. Here we present the more realistic situation of choosing model form using data to be analysed. We prefer a four stage approach, which is similar to recommendations of others, such as Diggle [4] and Wolfinger [5]. The first stage is to model the mean structure in sufficient generality to ensure unbiasedness of the fixed effect estimates. This usually entails a saturated parameter specification for fixed effects, often in the form of effects for treatment, time, treatment-by-time interaction, and other relevant covariables. The second stage is to specify a model for the covariance structure of the data. This involves modelling variation between subjects, and also covariation between measures at different times on the same subject. In the third stage, generalized least squares methods are used to fit the mean portion of the model. In the fourth stage the fixed effects portion may be made more parsimonious, such as by fitting polynomial curves over time. Then, statistical inferences are drawn based on fitting this final model.

In the present paper, we illustrate the four-stage process, but the major focus is on the second stage, modelling the covariance structure. If the true underlying covariance structure were known, the generalized least squares fixed effects estimates would be the best *linear unbiased estimates* (BLUE). When it is unknown, our goal is to estimate it as closely as possible, thus providing more efficient estimates of the fixed effects parameters. The MIXED procedure in the SAS® system [3] provides a rich selection of covariance structures from which to choose. In addition to selecting a covariance structure, we examine the effects of choice of covariance structure on tests of fixed effects, estimates of differences between treatment means, and on standard errors of the differences between means.

2. EXAMPLE DATA SET

A pharmaceutical example experiment will be used to illustrate the methodology. Objectives of the study were to compare effects of two drugs (A and B) and a placebo (P) on a measure of

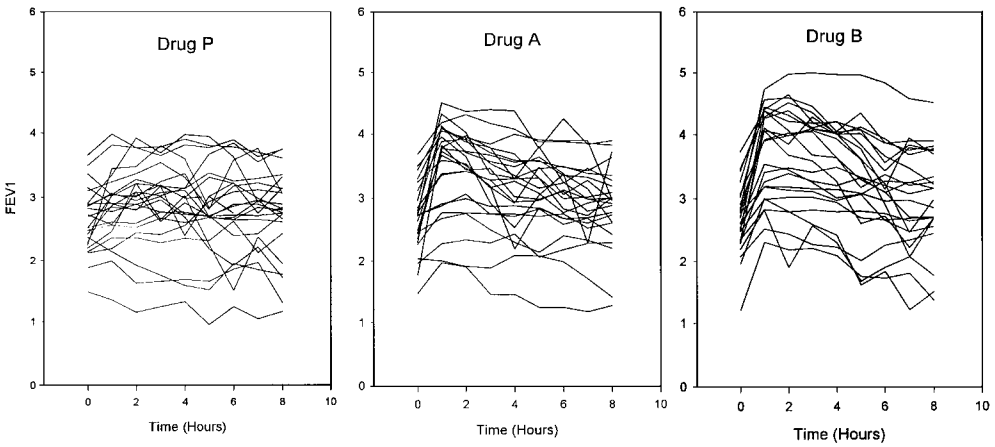

Figure 1. FEV1 repeated measures for each patient.

Table I. REML covariance and correlation estimates for FEV1 repeated measures data.

			Unstructured				
Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Time 7	Time 8
0.226	0.216	0.211	0.204	0.175	0.163	0.128	0.168
0.893	0.259	0.233	0.243	0.220	0.181	0.156	0.195
0.880	0.908	0.254	0.252	0.219	0.191	0.168	0.204
0.784	0.892	0.915	0.299	0.240	0.204	0.190	0.226
0.688	0.807	0.813	0.822	0.286	0.232	0.204	0.247
0.675	0.698	0.745	0.735	0.855	0.258	0.214	0.245
0.516	0.590	0.643	0.670	0.733	0.812	0.270	0.233
0.642	0.701	0.742	0.755	0.845	0.882	0.820	0.299

Variances on diagonal, covariances above diagonal, correlations below diagonal.

respiratory ability, called FEV1. Twenty-four patients were assigned to each of the three treatment groups, and FEV1 was measured at baseline (immediately prior to administration of the drugs), and at hourly intervals thereafter for eight hours. Data were analysed using PROC MIXED of the SAS System, using baseline FEV1 as a covariable. An SAS data set, named FEV1UN1, contained data with variables DRUG, PATIENT, HR (hour), BASEFEV1 and FEV1. Data for individual patients are plotted versus HR in Figure 1 for the three treatment groups. The drug curves appear to follow a classic pharmacokinetic pattern and thus might be analysed using a non-linear mean model. However, we will restrict our attention to models of the mean function which are linear in the parameters. Estimates of between-patient variances within drug group at each hour are printed

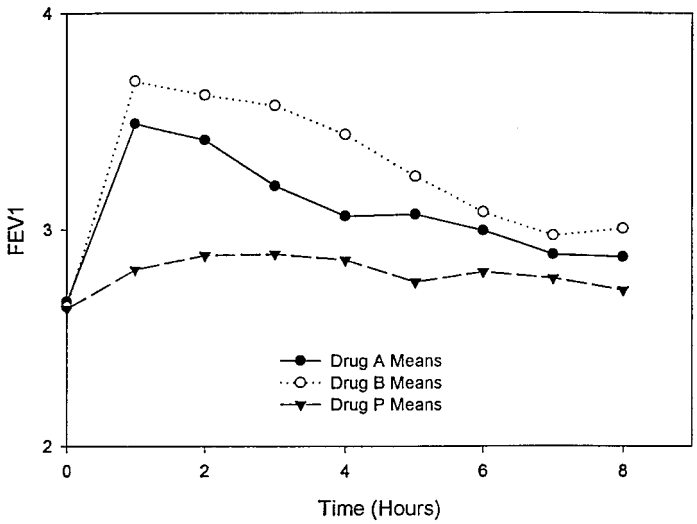

Figure 2. FEV1 repeated measures means for each drug.

in the diagonal of the matrix of Table I. It appears from these plots and variance estimates that variances between patients within drug groups are approximately equal across times. Therefore, an assumption of equal variances seems reasonable.

Treatment means are plotted versus HR in Figure 2. The graph shows that means for the three treatment groups are essentially the same at HR = 0 (baseline). At HR = 1 the mean for drug B is larger than the mean for drug A, and both of the drug means are much larger than the placebo mean. Means for drugs A and B continue to be larger than the placebo means for subsequent hours, but the magnitudes of the differences decrease sharply with time. It is of interest to estimate differences between the treatment group means at various times, and to estimate differences between means for the same treatment at different times.

Covariances and correlations are printed above and below the diagonal, respectively, of the matrix in Table I. The correlations between FEV1 at HR = 1 and later times are in the first column of the matrix. Correlations generally decrease from 0.893 between FEV1 at HR = 1 and HR = 2 down to 0.642 between FEV1 at HR = 1 and HR = 8. Similar decreases are found between FEV1 at HR = 2 and later times, between FEV1 at HR = 3 and later times etc. In short, correlations between pairs of FEV1 measurements decrease with the number of hours between the times at which the measurements were obtained. This is a common phenomenon with repeated measures data. Moreover, magnitudes of correlations between FEV1 repeated measures are similar for pairs of hours with the same interval between hours. Scatter plots of FEV1 for each hour versus FEV1 at each other hour are presented in Figure 3. These are similar to the 'draftsman's' plots as described by Dawson *et al.* [6]. The trends of decreasing correlations with increasing interval between measurement times is apparent in the plots. That is, points are more tightly packed in plots for two measures close in time than for measures far apart in time.

As a consequence of the patterns of correlations, a standard analysis of variance as prescribed in Milliken and Johnson [7] is likely not appropriate for this data set. Thus, another type of analysis must be used.

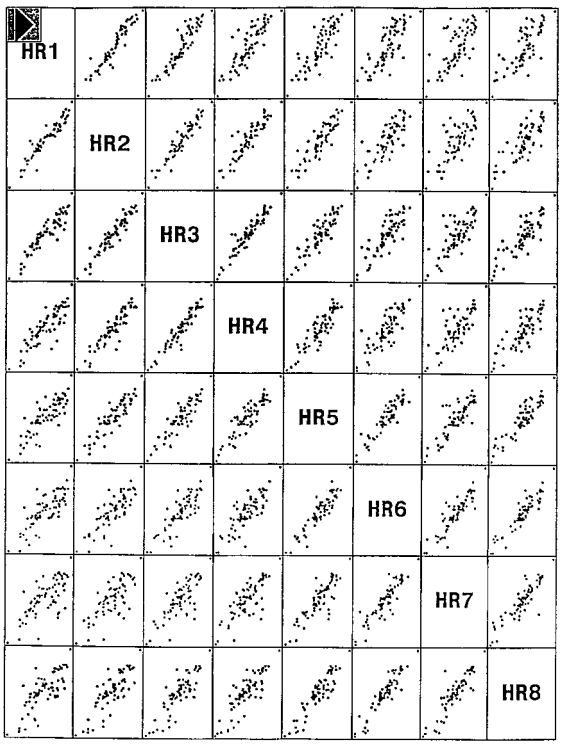

Figure 3. Scatter plots of FEV1 repeated measures at each hour versus each other hour.

3. LINEAR MIXED MODEL FOR REPEATED MEASURES

In this section we develop the general linear mixed model to a minimally sufficient level that will allow the reader to effectively begin using PROC MIXED of the SAS System. The development here is consistent and somewhat overlapping with that of Cnann *et al.* [2], but is needed for completeness. We assume a completely randomized design for patients in g treatment groups, with n_i subjects assigned to group i. Thus, we assume data on different subjects are independent. For simplicity, we assume there are t measurements at the same equally spaced times on each subject. We choose to work in this nicely balanced situation so that we can illustrate the basic issues of modelling covariance structure without complications introduced by unbalanced data.

Let Y_{ijk} denote the value of the response measured at time k on subject j in group $i, i = 1, \ldots, g$, $j = 1, \ldots, n_i$, and $k = 1, \ldots, t$. Throughout this paper, we assume all random effects are normally distributed. The fixed effect portion of the general linear mixed model specifies the expected value of Y_{ijk} to be $E(Y_{ijk}) = \mu_{ijk}$. The expected value, μ_{ijk}, usually is modelled as a function of treatment, time, and other fixed effects covariates. The random effect portion of the model specifies the covariance structure of the observations. We assume that observations on different subjects are independent, which is legitimate as a result of the completely randomized design. Thus, $\text{cov}(Y_{ijk}, Y_{i'j'l}) = 0$ if $i \neq i'$ or $j \neq j'$. Also, we assume that variances and covariances of measures on a single subject are the same within each of the groups. However, we allow for the possibility

that variances are not homogeneous at all times, and that covariance between observations at different times on the same subject are not the same at all pairs of times. A general covariance structure is denoted as $\text{cov}(Y_{ijk}, Y_{ijl}) = \sigma_{k,l}$, where $\sigma_{k,l}$ is the covariance between measures at times k and l on the same subject, and $\sigma_{k,k} = \sigma_k^2$ denotes the variance at time k. This is sometimes called 'unstructured' covariance, because there are no mathematical structural conditions on the variances and covariances.

Let $\mathbf{Y}_{ij} = (Y_{ij1}, Y_{ij2}, \ldots, Y_{ijt})'$ denote the vector of data at times $1, 2, \ldots, t$ on subject j in group i. Then, in matrix notation, the model can be written

$$\mathbf{Y}_{ij} = \mu_{ij} + \varepsilon_{ij}$$

where $\mu_{ij} = (\mu_{ij1}, \mu_{ij2}, \ldots, \mu_{ijt})'$ is the vector of means and $\varepsilon_{ij} = (\varepsilon_{ij1}, \varepsilon_{ij2}, \ldots, \varepsilon_{ijt})'$ is the vector of errors, respectively, for subject j in group i. Matrix representations of the expectation and variance of $\mathbf{Y}_{ij}$ are $E(\mathbf{Y}_{ij}) = \mu_{ij}$ and $V(\mathbf{Y}_{ij}) = \mathbf{V}_{ij}$, where $\mathbf{V}_{ij}$ is the $t \times t$ matrix with $\sigma_{k,l}$ in row k, column l. We assume that $\mathbf{V}_{ij}$ is the same for all subjects (that is, for all i and j), but we continue to use the subscripts ij to emphasize that we are referring to the covariance matrix for a single subject.

We represent the vector of data for all subjects as $\mathbf{Y} = (\mathbf{Y}'_{11}, \ldots, \mathbf{Y}'_{1n}, \mathbf{Y}'_{21}, \ldots, \mathbf{Y}'_{2n}, \ldots, \mathbf{Y}'_{g1}, \ldots, \mathbf{Y}'_{gn})'$, and similarly for the vectors of expected values and errors to get $E(\mathbf{Y}) = \mu = (\mu'_{11}, \ldots, \mu'_{1n}, \mu'_{21}, \ldots, \mu'_{2n}, \ldots, \mu'_{g1}, \ldots, \mu'_{gn})'$ and $\varepsilon = (\varepsilon'_{11}, \ldots, \varepsilon'_{1n}, \varepsilon'_{21}, \ldots, \varepsilon'_{2n}, \ldots, \varepsilon'_{g1}, \ldots, \varepsilon'_{gn})'$. Then we have the model

$$\mathbf{Y} = \mu + \varepsilon \tag{1}$$

and

$$V(\mathbf{Y}) = \mathbf{V} = \text{diag}\{\mathbf{V}_{ij}\}$$

where $\text{diag}\{\mathbf{V}_{ij}\}$ refers to a block-diagonal matrix with $\mathbf{V}_{ij}$ in each block.

A univariate linear mixed model for the FEV1 repeated measures data is

$$Y_{ijk} = \mu + \lambda x_{ij} + \alpha_i + d_{ij} + \tau_k + (\alpha\tau)_{ik} + e_{ijk} \tag{2}$$

where μ is a constant common to all observations, λ is a fixed coefficient on the covariate $x_{ij} = \text{BASEFEV1}$ for patient j in drug group i, α_i is a parameter corresponding to drug i, τ_k is a parameter corresponding to hour k, and $(\alpha\tau)_{ik}$ is an interaction parameter corresponding to drug i and hour k; d_{ij} is a normally distributed random variable with mean zero and variance σ_d^2 corresponding to patient j in drug group i, and e_{ijk} is a normally distributed random variable with mean zero and variance σ_e^2, independent of d_{ij}, corresponding to patient j in drug group i at hour k. Then

$$\begin{aligned} E(Y_{ijk}) &= \mu_{ijk} = \mu + \lambda x_{ij} + \alpha_i + \tau_k + (\alpha\tau_{ik}) \\ V(Y_{ijk}) &= \sigma_d^2 + \sigma_e^2 \end{aligned} \tag{3}$$

and

$$\text{cov}(Y_{ijk}, Y_{ijl}) = \sigma_d^2 + \text{cov}(e_{ijk}, e_{ijl})$$

The model (2), written in matrix rotation, is

$$\mathbf{Y} = \mathbf{X}\beta + \mathbf{ZU} + \mathbf{e} \tag{4}$$

where $\mathbf{X}$ is a matrix of known coefficients of the fixed effect parameters $\mu, \lambda, \alpha_i, \tau_k$, and $(\alpha\tau_{ik}), \beta$ is the vector of fixed effect parameters, $\mathbf{Z}$ is a matrix of coefficients (zeros and ones) of the random patient effects $d_{ij}, \mathbf{U}$ is the vector of random effects d_{ij}, and $\mathbf{e}$ is the vector of the errors e_{ijk}. In relation to model (1), $\mu = \mathbf{X}\beta$ and $\varepsilon = \mathbf{ZU} + \mathbf{e}$.

Model (4) for the FEV1 data is a special case of the general linear mixed model

$$\mathbf{Y} = \mathbf{X}\beta + \mathbf{ZU} + \mathbf{e} \tag{5}$$

in which no restrictions are necessarily imposed on the structures of $\mathbf{G} = V(\mathbf{U})$ and $\mathbf{R} = V(\mathbf{e})$. We assume only that $\mathbf{U}$ and $\mathbf{e}$ are independent, and obtain

$$V(\mathbf{Y}) = \mathbf{ZGZ}' + \mathbf{R}. \tag{6}$$

Equation (6) expresses the structure of $V(\mathbf{Y})$ as a function of $\mathbf{G}$ and $\mathbf{R}$. In many repeated measures applications, $\mathbf{ZGZ}'$ represents the between-patient portion of the covariance structure, and $\mathbf{R}$ represents the within-patient portion. By way of notation, sub-matrices of $\mathbf{X}, \mathbf{Z}, \mathbf{R}$ and $\mathbf{e}$ corresponding to subject j in drug group i will be denoted by $\mathbf{X}_{ij}, \mathbf{Z}_{ij}, \mathbf{R}_{ij}$ and $\mathbf{e}_{ij}$, respectively.

More details on implementation of the model for statistical inference are presented in the Appendix.

In order to apply the general linear mixed model (5) using PROC MIXED in the SAS System, the user must specify the three parts of the model: $\mathbf{X}\beta, \mathbf{ZU}$ and $\mathbf{e}$. Specifying $\mathbf{X}\beta$ is done in the same manner as with PROC GLM, and presents no new challenges to PROC MIXED users who are familiar with GLM. However, specifying $\mathbf{ZU}$ and $\mathbf{e}$ entails defining covariance structures, which may be less familiar concepts. Several covariance structures are discussed in Section 4.

4. COVARIANCE STRUCTURES FOR REPEATED MEASURES

Modelling covariance structure refers to representing $V(\mathbf{Y})$ in (6) as a function of a relatively small number of parameters. Functional specification of the covariance structure for the mixed model is done through $\mathbf{G}$ and $\mathbf{R}$ of (5), often only in terms of $\mathbf{R}_{ij}$. We present six covariance structures that will be fitted to the FEV1 data. Since observations on different patients are assumed independent, the structure refers to the covariance pattern of measurements on the same subject. For most of these structures, the covariance between two observations on the same subject depends only on the length of the time interval between measurements (called the lag), and the variance is constant over time. We assume the repeated measurements are equally spaced so we may define the lag for the observations Y_{ijk} and Y_{ijl} to be the absolute value of $k - l$, that is $|k - l|$. For these structures, the covariance can be characterized in terms of the variance and the correlations expressed as a function of the lag. We generically denote the correlation function $\text{corr}_{\text{XXX}}(\text{lag})$, where XXX is an abbreviation for the name of a covariance structure.

4.1. Simple (SIM)

$$\text{cov}(Y_{ijk}, Y_{ijl}) = 0 \text{ if } k \neq l, \quad V(Y_{ijk}) = \sigma^2_{\text{SIM}}$$

Simple structure specifies that the observations are independent, even on the same patient, and have homogeneous variance $V(Y_{ijk}) = \sigma^2_{\text{SIM}}$. The correlation function is $\text{corr}_{\text{SIM}}(\text{lag}) = 0$. Simple structure is not realistic for most repeated measures data because it specifies that observations on

the same patient are independent. In terms of model (5), $\mathbf{G}=\mathbf{0}$ and $\mathbf{R}_{ij}=\sigma^2_{\mathrm{SIM}}\mathbf{I}$, where $\mathbf{I}$ is an identity matrix. For the model (3), simple structure would be obtained with $d_{ij}=0$ (equivalently, $\sigma^2_d=0$), $\mathrm{cov}(e_{ijk},e_{ijl})=0$ for $k\neq l$, and $V(e_{ijk})=\sigma^2_{\mathrm{SIM}}$.

4.2. Compound Symmetric (CS)

$$\mathrm{cov}(Y_{ijk},Y_{ijl})=\sigma^2_{\mathrm{CS,b}} \text{ if } k\neq l, \quad V(Y_{ijk})=\sigma^2_{\mathrm{CS,b}}+\sigma^2_{\mathrm{CS,w}}$$

Compound symmetric structure specifies that observations on the same patient have homogeneous covariance $\sigma^2_{\mathrm{CS,b}}$, and homogeneous variance $V(Y_{ijk})=\sigma^2_{\mathrm{CS,b}}+\sigma^2_{\mathrm{CS,w}}$. The correlation function is

$$\mathrm{corr}_{\mathrm{CS}}(\mathrm{lag})=\sigma^2_{\mathrm{CS,b}}/(\sigma^2_{\mathrm{CS,b}}+\sigma^2_{\mathrm{CS,w}})$$

Notice that the correlation does not depend on the value of lag, in the sense that the correlations between two observations are equal for all pairs of observations on the same subject. Compound symmetric structure is sometimes called 'variance components' structure, because the two parameters $\sigma^2_{\mathrm{CS,b}}$ and $\sigma^2_{\mathrm{CS,w}}$ represent between-subjects and within-subjects variances, respectively. This mix of between- and within-subject variances logically motivates the form of $V(Y_{ij})$ in many situations and implies a non-negative correlation between pairs of within-subject observations. It can be specified in one of two ways through $\mathbf{G}$ and $\mathbf{R}$ in (5). One way is to define $\mathbf{G}=\sigma^2_{\mathrm{CS,b}}\mathbf{I}$, and $\mathbf{R}=\sigma^2_{\mathrm{CS,w}}\mathbf{I}$. In terms of the univariate model (3), we would have $\sigma^2_d=\sigma^2_{\mathrm{CS,d}}$, $\mathrm{cov}(e_{ijk},e_{ijl})=0$ for $k\neq l$, and $V(e_{ijk})=\sigma^2_{\mathrm{CS,w}}$. The other way to specify compound symmetric structure is to define $\mathbf{G}=\mathbf{0}$, and define $\mathbf{R}_{ij}$ to be compound symmetric; for example, $\mathbf{R}_{ij}=\sigma^2_{\mathrm{CS,w}}\mathbf{I}+\sigma^2_{\mathrm{CS,b}}\mathbf{J}$, where $\mathbf{J}$ is a matrix of ones. In terms of the univariate model (3), we would have $\sigma^2_d=0$, $\mathrm{cov}(e_{ijk},e_{ijl})=\sigma^2_{\mathrm{CS,b}}$ for $k\neq l$, and $V(e_{ijk})=\sigma^2_{\mathrm{CS,b}}+\sigma^2_{\mathrm{CS,w}}$. The second formulation using only the $\mathbf{R}$ matrix is more general, since it can be defined with negative within-subject correlation as well.

4.3. Autoregressive, order 1 (AR(1))

$$\mathrm{cov}(Y_{ijk},Y_{ijl})=\sigma^2_{\mathrm{AR(1)}}\rho^{|k-l|}_{\mathrm{AR(1)}}$$

Autoregressive (order 1) covariance structure specifies homogeneous variance $V(Y_{ijk})=\sigma^2_{\mathrm{AR(1)}}$. It also specifies that covariances between observations on the same patient are not equal, but decrease toward zero with increasing lag. The correlation between the measurements at times k and l is given by the exponential function

$$\mathrm{corr}_{\mathrm{AR(1)}}(\mathrm{lag})=\rho^{\mathrm{lag}}_{\mathrm{AR(1)}}$$

Thus, observations on the same patient far apart in time would be essentially independent, which may not be realistic. Autoregressive structure is defined in model (5) entirely in terms of $\mathbf{R}$, with $\mathbf{G}=\mathbf{0}$. The element in row k, column l of $\mathbf{R}_{ij}$ is denoted to be $\sigma^2_{\mathrm{AR(1)}}\rho^{|k-l|}_{\mathrm{AR(1)}}$. In terms of the univariate model (3), we would have $\sigma^2_d=0$, and $\mathrm{cov}(e_{ijk},e_{ijl})=\sigma^2_{\mathrm{AR(1)}}\rho^{|k-l|}_{\mathrm{AR(1)}}$.

4.4. Autoregressive with random effect for patient (AR(1)+RE)

$$\mathrm{cov}(Y_{ijk},Y_{ijl})=\sigma^2_{\mathrm{AR(1)+RE,b}}+\sigma^2_{\mathrm{AR(1)+RE,w}}\rho^{|k-l|}_{\mathrm{AR(1)+RE}}$$

Autoregressive with random effect for patient covariance structure specifies homogeneous variance $V(Y_{ijk}) = \sigma^2_{\mathrm{AR(1)+RE,b}} + \sigma^2_{\mathrm{AR(1)+RE,w}}$. The correlation function is

$$\mathrm{corr}_{\mathrm{AR(1)+RE}}(\mathrm{lag}) = (\sigma^2_{\mathrm{AR(1)+RE,b}} + \sigma^2_{\mathrm{AR(1)+RE,w}}\rho^{\mathrm{lag}}_{\mathrm{AR(1)+RE}})/(\sigma^2_{\mathrm{AR(1)+RE,b}} + \sigma^2_{\mathrm{AR(1)+RE,w}})$$

Autoregressive plus random effects structure specifies that covariance between observations on the same patient comes from two sources. First, any two observations share a common contribution simply because they are on the same subject. This is the $\sigma^2_{\mathrm{AR(1)+RE,b}}$ portion of the covariance, and results from defining a random effect for patients. Second, the covariance between observations decreases exponentially with lag, but decreases only to $\sigma^2_{\mathrm{AR(1)+RE,b}}$. This is the autoregressive contribution to the covariance, $\sigma^2_{\mathrm{AR(1)+RE,w}}\rho^{|k-l|}$. In terms of model (5), AR(1)+RE is represented with $\mathbf{G} = \sigma^2_{\mathrm{AR(1)+RE,b}}\mathbf{I}$ and autoregressive $\mathbf{R}_{ij}$. In terms of the univariate model (3), we would have $\sigma^2_d = \sigma^2_{\mathrm{AR(1)+RE,b}}$, and $\mathrm{cov}(e_{ijk}, e_{ijl}) = \sigma^2_{\mathrm{AR(1)+RE,w}}\rho^{|k-l|}$. The AR(1)+RE covariance structure actually results from a special case of the model proposed by Diggle [4].

4.5. *Toeplitz (TOEP)*

$$\mathrm{cov}(Y_{ijk}, Y_{ijl}) = \sigma_{\mathrm{TOEP},|k-l|}, \qquad V(Y_{ijk}) = \sigma^2_{\mathrm{TOEP}}$$

Toeplitz structure, sometimes called 'banded', specifies that covariance depends only on lag, but not as a mathematical function with a smaller number of parameters. The correlation function is $\mathrm{corr}(\mathrm{lag}) = \sigma_{\mathrm{TOEP},|\mathrm{lag}|}/\sigma^2_{\mathrm{TOEP}}$. In terms of model (5), TOEP structure is given with $\mathbf{G} = \mathbf{0}$. The elements of the main diagonal of $\mathbf{R}$ are σ^2_{TOEP}. All elements in a sub-diagonal $|k-l| = \mathrm{lag}$ are $\sigma_{\mathrm{TOEP},|k-l|}$, where k is the row number and l is the column number.

4.6. *Unstructured (UN)*

$$\mathrm{cov}(Y_{ijk}, Y_{ijl}) = \sigma_{\mathrm{UN},kl}$$

The 'unstructured' structure specifies no patterns in the covariance matrix, and is completely general, but the generality brings the disadvantage of having a very large number of parameters. In terms of model (5), it is given with $\mathbf{G} = \mathbf{0}$ and a completely general $\mathbf{R}_{ij}$.

5. USING THE MIXED PROCEDURE TO FIT LINEAR MIXED MODELS

We now turn to PROC MIXED for analyses of the FEV1 data which fit the mean model (3) and accommodate structures defined on the covariance matrix. We assume the reader has some familiarity with the SAS System, and knows how to construct SAS data sets and call SAS procedures.

The general linear mixed model (5) may be fit by using the MODEL, CLASS, RANDOM and REPEATED statements in the MIXED procedure. The MODEL statement consists of an equation which specifies the response variable on the left side of the equal sign and terms on the right side to specify the fixed effects portion of the model, $\mathbf{X}\beta$. Readers familiar with the GLM procedure

in SAS will recognize the RANDOM and REPEATED statements as being available in GLM, but their purposes are quite different in MIXED. The RANDOM statement in MIXED is used to specify the random effects portion, **ZU**, including the structure of $V(\mathbf{U}) = \mathbf{G}$. The REPEATED statement in MIXED is used to specify the structure of $V(\mathbf{e}) = \mathbf{R}$. Also, the MODEL statement in MIXED contains only fixed effects, but in GLM it contains both fixed and random effects. The CLASS statement, however, has a similar purpose in MIXED as in GLM, which is to specify classification variables, that is, variables for which indicator variables are needed in either **X** or **Z**. The CLASS statement in MIXED also is used to identify grouping variables, for example, variables that delineate the submatrices of block diagonal **G** or **R**.

In the FEV1 data, PATIENT and DRUG are clearly classification variables, and must be listed in the CLASS statement. The variable HR (hour) could be treated as either a continuous or a classification variable. In the first stage of implementing the linear mixed model, the mean structure $E(\mathbf{Y}) = \mathbf{X}\beta$ usually should be fully parameterized, as emphasized by Diggle [4]. Underspecifying the mean structure can result in biased estimates of the variance and covariance parameters, and thus lead to an incorrect assessment of covariance structure. Therefore, unless there are a very large number of levels of the repeated measures factor, we usually specify the repeated measures factor as a classification variable. Thus, we include the variable HR in the CLASS statement

```
class drug patient hr;
```

On the right side of the MODEL statement, we list terms to specify the mean structure (3)

```
model fev1 = basefev1 drug hr drug * hr
```

Executing the statements

```
proc mixed data = fev1uni;
    class drug patient hr;                    (7)
    model fev1 = basefev1 drug hr drug * hr;
```

would provide an ordinary least squares fit of the model (3). Results would be equivalent to those obtained by executing the CLASS and MODEL statements in (7) using PROC GLM. All tests of hypotheses, standard errors, and confidence intervals for estimable functions would be computed with an implicit assumption that $V(\mathbf{Y}) = \sigma^2\mathbf{I}$, that is, that $\mathbf{G} = \mathbf{0}$ and that $\mathbf{R} = \sigma^2\mathbf{I}$.

Specifying the MODEL statement in (7) is basically stage 1 of our four-stage process. Stage 2 is to select an appropriate covariance structure. The covariance structures described in Section 3 may be implemented in PROC MIXED by using RANDOM and/or REPEATED statements in conjunction with the statements (7). These statements cause PROC MIXED to compute *RE*sidual *M*aximum *L*ikelihood (REML, also known as *restricted* maximum likelihood) or *M*aximum *L*ikelihood (ML) (Searle *et al.* reference [8], chapter 6) estimates of covariance parameters for the specified structures.

Several options are available with the REPEATED and RANDOM statements, and would be specified following a slash (/). Following is a list of some of the options, and a brief description

of their functions:

TYPE = *structure type*.	Specifies the type of structure for **G** or **R**. Structure options are given in SAS Institute Inc. [3].
R and RCORR (REPEATED).	Requests printing of **R** matrix in covariance or correlation form.
G and GCORR (RANDOM).	Requests printing of **G** matrix in covariance or correlation form.
V and VCORR (RANDOM).	Requests printing of $\mathbf{V} = \mathbf{ZGZ}' + \mathbf{R}$ matrix in covariance or correlation form
SUBJECT = *variable name*.	Specifies variables whose levels are used to identify block diagonal structure in **G** or **R**. When used in conjunction with R, RCORR, G, GCORR, V, or VCORR options, only a sub-matrix for a single value of the variable is printed.

We now present statements to produce each of the covariance structures of Section 3. Basic output from these statements would include a table of estimates of parameters in the specified covariance structure and a table of tests of fixed effects, similar to an analysis of variance table. In each of the REPEATED statements, there is a designation 'SUBJECT = PATIENT (DRUG)'. This specifies that **R** is a block diagonal matrix with a sub-matrix for each patient. In this example, it is necessary to designate PATIENT (DRUG) because patients are numbered 1–24 in each drug. If patients were numbered 1–72, with no common numberings in different drugs, it would be sufficient to designate only 'PATIENT'. The options R and RCORR are used with the REPEATED statement and V and VCORR are used with the RANDOM statement to request printing of covariance and correlation matrices.

5.1. Simple

This is the default structure when no RANDOM or REPEATED statement is used, as in statements (7), or when no TYPE option is specified in a RANDOM or REPEATED statement. It can be specified explicitly with a REPEATED statement using a TYPE option:

```
proc mixed data = fev1uni; class drug patient hr;
    model fev1 = basefev1 drug hr drug * hr;                    (8)
    repeated/type = vc subject = patient(drug) r corr;
```

Note that in SAS version 6.12, the option 'simple' can replace 'vc' in the REPEATED statement.

5.2. Compound Symmetric

As noted in the previous section, compound symmetric covariance structure can be specified two different ways using **G** or **R**. Correspondingly, it can be implemented two different ways in the MIXED procedure, which would give identical results for non-negative within-subject correlation, except for labelling. The first way, setting $\mathbf{G} = \sigma^2_{\mathrm{CS,b}}\mathbf{I}$ and $\mathbf{R} = \sigma^2_{\mathrm{CS,w}}\mathbf{I}$, is implemented with the RANDOM statement:

```
proc mixed data = fev1uni; class drug patient hr;
    model fev1 = basefev1 drug hr drug * hr;                    (9)
    random patient(drug);
```


The RANDOM statement defines $\mathbf{G} = \sigma^2_{\mathrm{CS,b}}\mathbf{I}$ and the absence of a REPEATED statement (by default) defines $\mathbf{R} = \sigma^2_{\mathrm{CS,w}}\mathbf{I}$. The second way, setting $\mathbf{G} = \mathbf{0}$ and $\mathbf{R}_{ij} = \sigma^2_{\mathrm{CS,w}}\mathbf{I} + \sigma^2_{\mathrm{CS,b}}\mathbf{J}$, is implemented with a REPEATED statement using a SUBJECT and TYPE options. The following statements would specify compound symmetric structure for each individual patient, and print the $\mathbf{R}_{ij}$ submatrix for one patient in both covariance and correlation forms:

```
proc mixed data = fev1uni; class drug patient hr;
    model fev1 = basefev1 drug hr drug * hr;                    (10)
    repeated/type = cs subject = patient(drug) r rcorr;
```

The PROC MIXED output from statements (10) is shown in Figure 4, so that the reader can relate it to the parts we summarize in tables.

5.3. *Autoregressive, order 1*

This covariance structure would be specified for each patient using a REPEATED statement:

```
proc mixed data = fev1uni; class drug patient hr;
    model fev1 = basefev1 drug hr drug * hr;                    (11)
    repeated/type = ar(1) subject = patient(drug) r rcorr;
```

5.4. *Autoregressive with random effect for patient*

This covariance structure involves both **G** and **R**, and therefore requires both a RANDOM and a REPEATED statement:

```
proc mixed data = fev1uni; class drug patient hr;
    model fev1 = basefev1 drug hr drug * hr;
    random patient(drug);                                       (12)
    repeated/type = ar(1) subject = patient(drug);
```

The RANDOM statement defines $\mathbf{G} = \sigma^2_{\mathrm{AR(1)+RE:b}}\mathbf{I}$; and the REPEATED statement defines $\mathbf{R}_{ij}$ to be autoregressive, with parameters $\sigma^2_{\mathrm{AR(1)+RE,w}}$ and $\rho_{\mathrm{AR(1)+RE}}$.

Notice that we have no R and RCORR options in the REPEATED statement in (12). Covariance and correlation estimates that would be printed by R and RCORR options in (12) would not be directly comparable with the other covariances and correlations for other structures that are defined by REPEATED statements without a RANDOM statement. Covariance and correlation estimates that would be printed by R and RCORR options in the REPEATED statement in (12) would pertain only to the **R** matrix. Estimates for AR(1)+RE structure which are comparable to covariances and correlations for other structures must be based on covariances of the observation vector **Y**, that is, on $V(\mathbf{Y}) = \mathbf{ZGZ}' + \mathbf{R}$. This could be printed by using V and VCORR options in the RANDOM statement in (12). However, the entire $\mathbf{ZGZ}' + \mathbf{R}$ matrix, of dimension 576×576, would be printed. Alternatively, the statements (13) could be used, which are the same as (12) except for the RANDOM statement, but would print only $\mathbf{Z}_{ij}\mathbf{GZ}'_{ij} + \mathbf{R}_{ij}$, of dimension 8×8.

```
proc mixed data = fev1uni; class drug patient hr;
    model fev1 = basefev1 drug hr drug * hr;
    random int/subject = patient(drug) v vcorr;                 (13)
    repeated/type = ar(1) subject = patient(drug);
```


Effects of Three Drugs on FEV1
Compound Symmetric with BaseFEV1 Covariable

Covariance Parameter Estimates (REML)

Cov Parm	Subject	Estimate
CS	PATIENT(DRUG)	0.20625696
Residual		0.06312683

Model Fitting Information for FEV1

Description	Value
Observations	576.0000
Res Log Likelihood	-173.645
Akaike's Information Criterion	-175.645
Schwarz's Bayesian Criterion	-179.957
-2 Res Log Likelihood	347.2902
Null Model LRT Chi-Square	569.6449
Null Model LRT DF	1.0000
Null Model LRT P-Value	0.0000

Effects of Three Drugs on FEV1
Compound Symmetric with BaseFEV1 Covariable

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
BASEFEV1	1	68	76.42	0.0001
DRUG	2	68	7.24	0.0014
HR	7	483	38.86	0.0001
DRUG*HR	14	483	7.11	0.0001

Figure 4. Basic PROC MIXED output for compound symmetric covariance structure.

Executing statements (13) results in the covariance and corresponding correlation estimates for AR(1)+RE structure shown in Table II. The RANDOM statement in (13) defines **ZU** in (5) equivalent to the RANDOM statement in (12), but from an 'individual subject' perspective rather than a 'sample of subjects' perspective. The RANDOM statement in (12) basically defines columns of **Z** as indicator variables for different patients. The RANDOM statement in (13), with the 'int/sub = patient(drug)' designation, defines a set of ones as 'intercept' coefficients for each patient.

Table II. REML variance, covariance and correlation estimates for five covariance structures for FEV1 repeated measures.

Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Time 7	Time 8
1. *Simple*							
0.267	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2. *Compound Symmetric*							
0.269	0.206	0.206	0.206	0.206	0.206	0.206	0.206
1.0	0.766	0.766	0.766	0.766	0.766	0.766	0.766
3. *Autoregressive* (1)							
0.266	0.228	0.195	0.167	0.143	0.123	0.105	0.090
1.0	0.856	0.733	0.629	0.538	0.461	0.394	0.338
4. *Autoregressive* (1) *with random effect for patient*							
0.268	0.230	0.209	0.198	0.192	0.189	0.187	0.186
1.0	0.858	0.780	0.739	0.716	0.705	0.698	0.694
5. *Toeplitz* (*banded*)							
0.266	0.228	0.216	0.207	0.191	0.183	0.169	0.158
1.0	0.858	0.811	0.777	0.716	0.686	0.635	0.593

Variances and covariances in top line; correlations in bottom line.

5.5. *Toeplitz*

This structure can be specified in terms of $\mathbf{R}$ with $\mathbf{G} = \mathbf{0}$, and therefore requires only a REPEATED statement:

$$\begin{aligned} &\text{proc mixed data} = \text{fev1uni; class drug patient hr;} \\ &\quad \text{model fev1} = \text{basefev1 drug hr drug} * \text{hr;} \\ &\quad \text{repeated/type} = \text{toep subject} = \text{patient(drug) r rcorr;} \end{aligned} \tag{14}$$

5.6. *Unstructured*

This structure can be specified in terms of $\mathbf{R}$ with $\mathbf{G} = \mathbf{0}$, and therefore requires only a REPEATED statement:

$$\begin{aligned} &\text{proc mixed data} = \text{fev1uni; class drug patient hr;} \\ &\quad \text{model fev1} = \text{basefev1 drug hr drug} * \text{hr;} \\ &\quad \text{repeated/type} = \text{un subject} = \text{patient(drug) r rcorr;} \end{aligned} \tag{15}$$

Parameter estimates in the covariance and correlation matrices for the various structures (excepting 'unstructured') are:

$$\text{SIM} \qquad \hat{\sigma}^2_{\text{SIM}} = 0.267$$

CS	$\hat{\sigma}^2_{\mathrm{CS,b}} = 0.206$ $\hat{\sigma}^2_{\mathrm{CS,w}} = 0.063$
AR(1)	$\hat{\sigma}^2_{\mathrm{AR(1)}} = 0.266$ $\hat{\rho}\hat{\sigma}_{\mathrm{AR(1)}} = 0.856$
AR(1) + RE	$\hat{\sigma}^2_{\mathrm{AR(1)+RE,b}} = 0.185$ $\hat{\sigma}^2_{\mathrm{AR(1)+RE,w}} = 0.083$ $\hat{\rho}_{\mathrm{AR(1)+RE}} = 0.540$
TOEP	$\hat{\sigma}^2_{\mathrm{TOEP}} = 0.266$ $\hat{\sigma}_{\mathrm{TOEP,1}} = 0.228$ $\hat{\sigma}_{\mathrm{TOEP,2}} = 0.216$ $\hat{\sigma}_{\mathrm{TOEP,3}} = 0.207$ $\hat{\sigma}_{\mathrm{TOEP,4}} = 0.191$ $\hat{\sigma}_{\mathrm{TOEP,5}} = 0.183$ $\hat{\sigma}_{\mathrm{TOEP,6}} = 0.169$ $\hat{\sigma}_{\mathrm{TOEP,7}} = 0.158$
UN	(parameter estimates shown in Table I).

The covariance and correlation matrices resulting from statements (8), (10), (11), (13) and (14) are summarized in Table II. Rather than printing the entire matrices, covariances and correlations are displayed as a function of lag for SIM, CS, AR(1), AR(1)+RE and TOEP structures. Covariances and correlations resulting from (15) are printed in Table I.

6. COMPARISON OF FITS OF COVARIANCE STRUCTURES

We discuss covariance and correlation estimates in Table II for the structured covariances in comparison with those in Table I for the unstructured covariances. First, simple and compound symmetric estimates in Table II clearly do not reflect the trends in Table I. Autoregressive estimates in Table II show the general trend of correlations decreasing with length of time interval, but the values of the correlations in the autoregressive structure are too small, especially for long intervals. Thus, none of SIM, CS or AR(1) structures appears to adequately model the correlation pattern of the data. The AR(1)+RE correlations in Table II show good agreement with TOEP estimates in Table II and UN estimates in Table I. Generally, we prefer a covariance model which provides a good fit to the UN estimates, and has a small number of parameters. On this principle, AR(1)+RE is preferable.

The correlogram (Cressie, reference [9], p. 67) is a graphical device for assessing correlation structure. It is basically a plot of the correlation function. Correlation plots are shown in Figure 5 based on estimates assuming UN, CS, AR(1), AR(1)+RE and TOEP structures. Plots for CS, AR(1), AR(1)+RE and TOEP may be considered correlogram estimates assuming these structures. Of these correlations which are a function only of lag, the TOEP structure is the most general, and thus is used as the reference type in Figure 5. These plots clearly show that the fit of the AR(1)+RE structure agrees with TOEP and is superior to the fits of CS and AR(1).

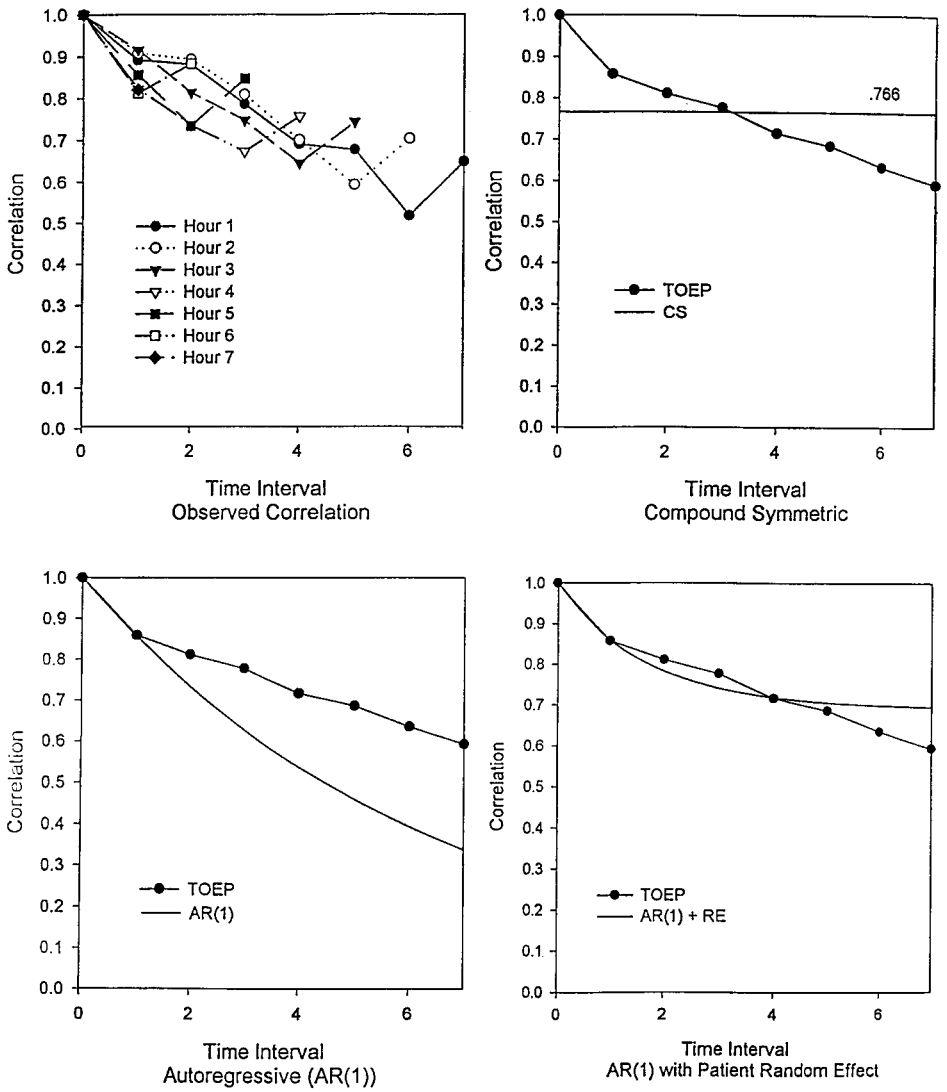

Figure 5. Plots of correlation estimates and correlograms.

Akaike's information criterion (AIC) [10] and Schwarz's Bayesian criterion (SBC) [11] are indices of relative goodness-of-fit and may be used to compare models with the same fixed effects but different covariance structures. Both of these criteria apply rather generally for purposes of model selection and hypothesis testing. For instance, Kass and Wassermann [12] have shown that the SBC provides an approximate Bayes factor in large samples. Formulae for their computation are

$$\mathrm{AIC} = L(\hat{\theta}) - q$$
$$\mathrm{SBC} = L(\hat{\theta}) - (q/2)\log(N^*)$$

Table III. Akaike's information criterion (AIC) and Schwarz's Bayesian criterion (SBC) for six covariance structures.

	Structure name	AIC	SBC
1.	Simple	−459.5	−461.6
2.	Compound symmetric	−175.6	−179.9
3.	Autoregressive (1)	−139.5	−143.8
4.	Autogressive (1) with random effect for patients	−126.5	−132.9
5.	Toeplitz (banded)	−121.9	−139.2
6.	Unstructured	−110.1	−187.7

where $L(\hat{\theta})$ is the maximized log-likelihood or restricted log-likelihood (REML), q is the number of parameters in the covariance matrix, p is the number of fixed effect parameters and N^* is the total number of 'observations' (N for ML and $N - p$ for REML, where N is the number of subjects).

Models with large AIC or SBC values indicate a better fit. However, it is important to note that the SBC criterion penalizes models more severely for the number of estimated parameters than does AIC. Hence the two criteria will not always agree on the choice of 'best' model. Since our objective is parsimonious modelling of the covariance structure, we will rely more on the SBC than the AIC criterion.

AIC and SBC values for the six covariance structures are shown in Table III. 'Unstructured', has the largest AIC, but AR(1)+RE, 'autoregressive with random effect for patient', has the largest SBC. Toeplitz ranks second in both AIC and SBC. The discrepancy between AIC and SBC for the UN structure reflects the penalty for the large number of parameters in the UN covariance matrix. Based on inspection of the correlation estimates in Tables I and III, the graphs of Figure 5, and the relative values of SBC, we conclude that AR(1)+RE, 'autoregressive with random effect for patient', is the best choice of covariance structure.

7. EFFECTS OF COVARIANCE STRUCTURE ON TESTS OF FIXED EFFECTS, ESTIMATES OF FIXED EFFECTS AND STANDARD ERRORS OF ESTIMATES

In Section 6 we compared the correlation and covariance matrices produced by five choices of covariance structure. In this section we examine the effects of choices of covariance structure on tests and estimates of fixed effects. First, we examine the table of tests for fixed effects specified in the MODEL statements. Then we select a set of 15 comparisons among means and use the ESTIMATE statement to illustrate effects of covariance structure on estimates of linear combinations of fixed effects.

Table IV contains values of F tests for fixed effects that are computed by the MIXED procedure for each of the covariance structures specified in (8), (10), (11), (13), (14) and (15). The F values differ substantially for SIM, CS and AR(1) structures. These are the structures that did not provide good fits in Section 6. Failure of SIM to recognize between-patient variation results in the excessively large F values for BASEFEV1 and DRUG, which are between patient effects. Using CS structure produces essentially the same results that would be obtained by using a univariate split-plot type analysis of variance (Milliken and Johnson, reference [7], chapter 26). It results in excessively large F values for HR and DRUG $*$ HR. This is a well-known phenomenon of

Table IV. Values of F tests for fixed effects for six covariance structures.

Structure name	BaseFEV1	DRUG	HR	DRUG * HR
1. Simple	490.76	46.50	9.20	1.69
2. Compound symmetric	76.42	7.24	38.86	7.11
3. Autoregressive (1)	90.39	8.40	7.39	2.46
4. Autoregressive (1) with random effect for patient	75.93	7.28	17.10	3.94
5. Toeplitz (banded)	76.31	7.30	13.75	3.82
6. Unstructured	92.58	7.25	13.72	4.06

performing univariate analysis of variance when CS (actually, Hyunh–Feldt [13]) assumptions are not met. It is basically the reason for making the so-called Hyunh–Feldt [13] and Greenhouse–Geisser [14] adjustments to ANOVA p-values as done by the REPEATED statement in PROC GLM [15]. F values for tests of HR and DRUG * HR using AR(1) structure are excessively small due to the fact that AR(1) underestimates covariances between observations far apart in time, and thereby overestimates variances of differences between these observations. Results of F tests based on AR(1)+RE, TOEP and UN covariance are similar for all fixed effects. All of these structures are adequate for modelling the covariance, and therefore produce valid estimates of error.

Now, we investigate effects of covariance structure on 15 linear combinations of fixed effects, which are comparisons of means. The first seven comparisons are differences between hour 1 and subsequent hours in drug A; these are within-subject comparisons. In terms of the univariate model (2), they are estimates of

$$\mu_{\mathrm{A}.1} - \mu_{\mathrm{A}.k} = \tau_1 - \tau_k + (\alpha\tau)_{\mathrm{A}1} - (\alpha\tau)_{\mathrm{A}k} \tag{16}$$

for $k = 2, \ldots, 8$.

The next eight comparisons are differences between drugs A and B at hours 1 to 8; these are between-subject comparisons at particular times. In terms of the univariate model (3), they are estimates of

$$\mu_{\mathrm{A}.k} - \mu_{\mathrm{B}.k} = \lambda(\bar{X}_{\mathrm{A}} - \bar{X}_{\mathrm{B}}) + \alpha_{\mathrm{A}} - \alpha_{\mathrm{B}} + (\alpha\tau)_{\mathrm{A}k} - (\alpha\tau)_{\mathrm{B}k} \tag{17}$$

for $k = 1, \ldots, 8$.

The ESTIMATE statement in the MIXED procedure can be used to compute estimates of linear combinations of fixed effect parameters. It is used for this purpose in essentially the same manner as with the GLM procedure. With MIXED, the ESTIMATE statement can be used for the more general purpose of computing estimates of linear combinations of fixed and random effectes, known as *B*est *L*inear *U*nbiased *P*redictors (BLUPs) [16].

The following ESTIMATE statements in (18) can be run in conjunction with the PROC MIXED statements (7)–(15) to obtain estimates of the differences (16). Coefficients following 'hr' in (18) specify coefficients of τ_k parameters in (16), and coefficients following 'drug * hr' in (18) specify coefficients of $(\alpha\tau)_{ik}$ parameters in (16):

```
estimate 'hr1–hr2 drgA' hr 1  −1 0 0 0 0 0 0 drug * hr 1  −1 0 0 0 0 0 0;
estimate 'hr1–hr3 drgA' hr 1 0  −1 0 0 0 0 0 drug * hr 1 0  −1 0 0 0 0 0;
```


Table V. Estimates and standard errors for six covariance structures: within-subject comparisons across time.

		Standard errors					
Parameter	Estimate*	Simple	CS	AR(1)	AR(1)+RE	Toeplitz (banded)	Unstructured
hr1–hr2 drug A	0.0767	0.1491	0.0725	0.0564	0.0564	0.0562	0.0470
hr1–hr3 drug A	0.2896	0.1491	0.0725	0.0769	0.0700	0.0647	0.0492
hr1–hr4 drug A	0.4271	0.1491	0.0725	0.0908	0.0764	0.0704	0.0698
hr1–hr5 drug A	0.4200	0.1491	0.0725	0.1012	0.0796	0.0794	0.0822
hr1–hr6 drug A	0.4942	0.1491	0.0725	0.1093	0.0813	0.0836	0.0811
hr1–hr7 drug A	0.6050	0.1491	0.0725	0.1158	0.0822	0.0900	0.1002
hr1–hr8 drug A	0.6154	0.1491	0.0725	0.1211	0.0827	0.0951	0.0888

*Parameter estimates are the same regardless of variance structure for these contrasts.

```
estimate 'hr1–hr4 drgA' hr 1 0 0  −1 0 0 0 0 drug*hr 1 0 0  −1 0 0 0 0;
estimate 'hr1–hr8 drgA' hr 1 0 0 0  −1 0 0 0 drug*hr 1 0 0 0  −1 0 0 0;
estimate 'hr1–hr5 drgA' hr 1 0 0 0 0  −1 0 0 drug*hr 1 0 0 0 0  −1 0 0;
estimate 'hr1–hr6 drgA' hr 1 0 0 0 0 0  −1 0 drug*hr 1 0 0 0 0 0  −1 0;
estimate 'hr1–hr7 drgA' hr 1 0 0 0 0 0 0  −1 drug*hr 1 0 0 0 0 0 0  −1;
```
(18)

Results from running these ESTIMATE statements with each of the six covariance structures in (18) appear in Table V. The estimates obtained from (18) are simply differences between the two drug A means for each pair of hours, that is, the estimate labelled 'hr1-hrk drgA' is $\bar{Y}_{\mathrm{A}.1} - \bar{Y}_{\mathrm{A}.k}$, or in terms of the model (2), $\tau_1 - \tau_k + (\alpha\tau)_{\mathrm{A}1} - (\alpha\tau)_{\mathrm{A}k} + \bar{e}_{\mathrm{A}.1} - \bar{e}_{\mathrm{A}.k}$, for $k = 2, \ldots, 8$. Because the covariable BASEFEV1 is a subject-level covariate, it cancels in this comparison. Consequently, the estimates are all the same for any covariance structure due to the equivalence of generalized least squares (GLS) and ordinary least squares (OLS) in this setting. This will not happen in all cases, such as when the data are unbalanced, when the covariate is time-varying, or when polynomial trends are used to model time effects. In this example, the data are balanced and hour is treated as a discrete factor. See Puntanen and Styan [17] for general conditions when GLS estimates are equal to OLS estimates.

Even though all estimates of differences from statements (18) are equal, each of the six covariance structures results in a different standard error estimate (Table V). Note that the 'simple' standard error estimates are always larger than those from the mixed model. The general expression for the variance of the standard error estimate is

$$V(Y_{\mathrm{A}.1} - Y_{\mathrm{A}.k}) = [\sigma_{1,1} + \sigma_{k,k} - 2\sigma_{1,k}]/24 \tag{19}$$

where $\sigma_{k,l} = \mathrm{cov}(Y_{ijk}, Y_{ijl})$. For structured covariances, $\sigma_{k,l}$ will be a function of k, l, and a small number of parameters.

Standard error estimates printed by PROC MIXED are square roots of (19), with $\sigma_{k,l}$ expressions replaced by their respective estimates, assuming a particular covariance structure. We now discuss effects of the assumed covariance structure on the standard error estimates.

Structure number 1, 'simple', treats the data as if all observations are independent with the same variance. This results in equal standard error estimates of

$$\begin{aligned} 0.14909825 &= (2\,\hat{\sigma}^2_{\mathrm{SIM}}/24)^{1/2} \\ &= (2(0.267/24))^{1/2} \end{aligned}$$

for all differences between time means in the same drug. These are incorrect because SIM structure clearly is inappropriate for two reasons. First, the SIM structure does not accommodate between-patient variation, and second, it does not recognize that measures close together in time are more highly correlated than measures far apart in time.

Structure number 2, 'compound symmetric', acknowledges variation as coming from two sources, between- and within-patient. This results in standard error estimates of

$$\begin{aligned} 0.07252978 &= (2\,\hat{\sigma}^2_{\mathrm{CS,w}}/24)^{1/2} \\ &= (2(0.063/24))^{1/2} \end{aligned}$$

being functions only of the within-patient varience component estimate. However, compound symmetry does not accommodate different standard errors of differences between times as being dependent on the length of the time interval. Consequently, the standard error estimates based on the compound symmetric structure also are invalid.

Structure number 3, 'autoregressive', results in standard errors of estimates of differences between times which depend on the length of the time interval. For example, the standard error estimate for the difference between hours 1 and 8 (lag=7) is

$$\begin{aligned} 0.121 &= (2\,\hat{\sigma}^2_{\mathrm{AR(1)}}(1-\hat{\rho}^7_{\mathrm{AR(1)}})/24))^{1/2} \\ &= (2(0.266(1-0.856^7)/24))^{1/2} \end{aligned}$$

and similarly for other lags. The standard error estimates are 0.121 for the difference between hours 1 and 8 etc., down to 0.056 for the difference between hours 1 and 2. If the autoregressive structure were correct, then these estimates of standard errors should be in good agreement with those produced by TOEP covariance. The TOEP standard error estimates range from 0.095 for the difference between hours 1 and 8 down to 0.056 for the difference between hours 1 and 2. Thus the autoregressive estimates are too large by approximately 30 per cent for long time intervals (for example, hours 1 to 8). This is because the autoregressive structure underestimates the correlation between observations far apart in time by forcing the correlation to decrease exponentially toward zero.

Next, we examine the standard errors provided by structure 4, 'autoregressive with random effect for patient'. The standard error estimate for the difference between hours 1 and 8 (lag = 7) is

$$\begin{aligned} 0.083 &= (2(\hat{\sigma}^2_{\mathrm{AR(1)+RE,w}}(1-\hat{\rho}^7_{\mathrm{AR(1)+RE}})/24))^{1/2} \\ &= (2(0.083(1-0.540^7)/24))^{1/2} \end{aligned}$$

and similarly for other lags. We see that these standard error estimates generally provide good agreement with the TOEP and UN standard error estimates. These three structures (TOEP, UN and AR(1)+RE) are all potential candidates, because they accommodate between-subject variance and decreasing correlation as the lag increases. The intuitive advantage of the AR(1)+RE estimates over the TOEP and UN estimates in this setting is that the standard errors of the AR(1)+RE estimates follow a smooth trend as a function of lag, whereas the TOEP and UN standard error estimates are more erratic, particularly so for the UN estimates. In all three strucutres, the standard errors for the larger time lags are larger than those for the smaller lags, reflecting the pattern seen in the data.

The following ESTIMATE statements can be run in conjunction with PROC MIXED statements (7)–(15) to obtain estimates of the differences between drugs A and B at each hour, defined

Table VI. Estimates and standard errors for six covariance structures: between-subject comparisons.

Parameter	Simple	CS	AR(1)	AR(1)+RE	Toeplitz (Banded)	Unstructured
Estimates						
drg B–drg A hr1	0.2184	0.2184	0.2179	0.2182	0.2180	0.2188
drg B–drg A hr2	0.2305	0.2305	0.2300	0.2303	0.2301	0.2308
drg B–drg A hr3	0.3943	0.3943	0.3938	0.3941	0.3939	0.3946
drg B–drg A hr4	0.3980	0.3981	0.3975	0.3978	0.3976	0.3984
drg B–drg A hr5	0.1968	0.1968	0.1963	0.1966	0.1964	0.1971
drg B–drg A hr6	0.1068	0.1068	0.1063	0.1066	0.1064	0.1071
drg B–drg A hr7	0.1093	0.1093	0.1088	0.1091	0.1088	0.1096
drg B–drg A hr8	0.1530	0.1530	0.1525	0.1528	0.1526	0.1534
Standard errors						
drg B–drg A hr1	0.1491	0.1499	0.1489	0.1494	0.1490	0.1374
drg B–drg A hr2	0.1491	0.1499	0.1489	0.1494	0.1490	0.1471
drg B–drg A hr3	0.1491	0.1499	0.1489	0.1494	0.1490	0.1454
drg B–drg A hr4	0.1491	0.1499	0.1489	0.1494	0.1490	0.1578
drg B–drg A hr5	0.1491	0.1499	0.1489	0.1494	0.1490	0.1544
drg B–drg A hr6	0.1491	0.1499	0.1489	0.1494	0.1490	0.1465
drg B–drg A hr7	0.1491	0.1499	0.1489	0.1494	0.1490	0.1501
drg B–drg A hr8	0.1491	0.1499	0.1489	0.1494	0.1490	0.1579

in (17):

```
estimate 'drgA–drgB hr1' drug 1 −1 0 drug*hr 1 0 0 0 0 0 0 0 −1 0 0 0 0 0 0 0;
estimate 'drgA–drgB hr2' drug 1 −1 0 drug*hr 0 1 0 0 0 0 0 0 0 −1 0 0 0 0 0 0;
estimate 'drgA–drgB hr3' drug 1 −1 0 drug*hr 0 0 1 0 0 0 0 0 0 0 −1 0 0 0 0 0;
estimate 'drgA–drgB hr4' drug 1 −1 0 drug*hr 0 0 0 1 0 0 0 0 0 0 0 −1 0 0 0 0;
estimate 'drgA–drgB hr5' drug 1 −1 0 drug*hr 0 0 0 0 1 0 0 0 0 0 0 0 −1 0 0 0;
estimate 'drgA–drgB hr6' drug 1 −1 0 drug*hr 0 0 0 0 0 1 0 0 0 0 0 0 0 −1 0 0;
estimate 'drgA–drgB hr7' drug 1 −1 0 drug*hr 0 0 0 0 0 0 1 0 0 0 0 0 0 0 −1 0;
estimate 'drgA–drgB hr8' drug 1 −1 0 drug*hr 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 −1;
```

(20)

Results appear in Table VI. These estimates are the same for structures SIM and CS. They are simply differences between ordinary least squares means, adjusted for the covariable BASEFEV1. However, a simple expression for the variance of the estimates is not easily available. The standard errors differ for the two covariance structures, because simple structure does not recognize between-patient variation.

Estimates of drug differences for the four covariance structures other than 'Simple' and 'Compound symmetric' are all numerically different, though similar. Also, standard errors of the drug differences are not the same for covariance structures AR(1), AR(1)+RE, TOEP and UN, but the standard errors for the AR(1)+RE and TOEP structure are constant over the hours. This is desirable, because data variance are homogeneous over hours, and the adjustment for the covariable BASEFEV1 would be the same at each hour. However, the standard errors of drug differences for UN covariance vacillate between 0.137 and 0.158, a range of approximately 16 per cent. The standard errors are not constant because UN does not assume homogeneous variances. In the present

example, it is reasonable to assume homogeneous variances, and this should be exploited. Not doing so results in variable and inefficient standard error estimates.

The purpose of this section was to illustrate the practical effects of choosing a covariance structure. The results show that SIM, CS and AR(1) covariance structures are inadequate for the example data. These structure models basically provide ill-fitting estimates of the true covariance matrix of the data. In turn, the ill-fitting estimates of data covariance result in poor estimates of standard errors of certain differences between means, even if estimates of differences between means are equal across covariance structures. The structures AR(1)+RE, TOEP and UN are adequate, in the sense that they provide good fits to the data covariance. (This is always true of UN because there are no constraints to impose lack of fit.) These adequate structures incorporate the two essential features of the data covariance. One, observations on the same patient are correlated, and two, observations on the same patient taken close in time are more highly correlated than observations taken far apart in time. As a result, standard error estimates based on assumptions of AR(1)+RE, TOEP or UN covariance structures are valid, but because UN imposes no constraints or patterns, the standard error estimates are somewhat unstable.

8. MODELLING POLYNOMIAL TRENDS OVER TIME

Previous analyses have treated hour as a classification variable and not modelled FEV1 trends as a continuous function of hour. In Section 6, we fitted six covariance structures to the FEV1 data, and determined that AR(1)+RE provided the best fit. In Section 7, we examined effects of covariance structure on estimates of fixed effect parameters and standard errors. In this section, we treat hour as a continuous variable and model hour effects in polynomials to refine the fixed effects portion of the model. Then we use the polynomial model to compute estimates of differences analogous to those in Section 7.

Statements (21) fit the general linear mixed model using AR(1)+RE covariance structure to model random effects and third degree polynomials to model fixed effects of drug and hour. A previous analysis (not shown) that fitted fourth degree polynomials using PROC MIXED showed no significant evidence of fourth degree terms.

```
proc mixed data = fev1uni; class drug patient;
    model fev1 = basefev1 drug drug * hr drug * hr * hr drug * hr * hr * hr/htype = 1 3
          solution noint;                                                          (21)
    random patient(drug);
    repeated/type = ar(1) sub = patient(drug);
```

The MODEL statement in (21) is specified so that parameter estimates obtained from the SOLUTION option directly provide the coefficients of the third degree polynomials for each drug. The fitted polynomial equations, after inserting the overall average value of 2.6493 for BASEFEV1, are

$$\text{A:} \quad \text{FEV1} = 3.6187 - 0.1475\ \text{HR} + 0.0034\ \text{HR}^2 + 0.0004\ \text{HR}^3$$

$$\text{B:} \quad \text{FEV1} = 3.5793 + 0.1806\ \text{HR} - 0.0802\ \text{HR}^2 + 0.0061\ \text{HR}^3$$

$$\text{P:} \quad \text{FEV1} = 2.7355 + 0.1214\ \text{HR} - 0.0289\ \text{HR}^2 + 0.0017\ \text{HR}^3$$

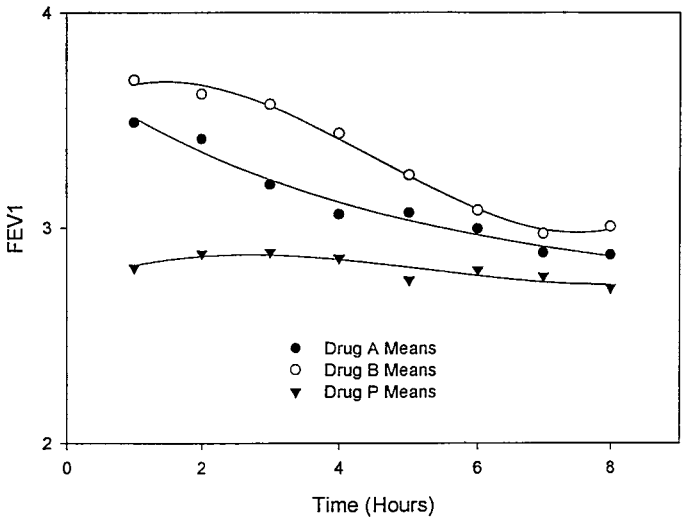

Figure 6. Plots of polynomial trends over hours for each drug.

The polynomial curves for the drugs are plotted in Figure 6.

Estimates of differences between hour 1 and subsequent hours in drug A based on the fitted polynomials may be obtained from the ESTIMATE statements (22):

```
estimate 'hr1–hr2 drga'drug * hr -1 drug * hr * hr -03 drug * hr * hr * hr -007;
estimate 'hr1–hr3 drga'drug * hr -2 drug * hr * hr -08 drug * hr * hr * hr -026;
estimate 'hr1–hr4 drga'drug * hr -3 drug * hr * hr -15 drug * hr * hr * hr -063;
estimate 'hr1–hr5 drga'drug * hr -4 drug * hr * hr -24 drug * hr * hr * hr -124;
estimate 'hr1–hr6 drga'drug * hr -5 drug * hr * hr -35 drug * hr * hr * hr -215;
estimate 'hr1–hr7 drga'drug * hr -6 drug * hr * hr -48 drug * hr * hr * hr -342;
estimate 'hr1–hr8 drga'drug * hr -7 drug * hr * hr -63 drug * hr * hr * hr -511;
```
(22)

Results from statements (22) appear in Table VII.

We see that standard errors of differences between hour 1 and subsequent hours in drug A using AR(1)+RE covariance and polynomial trends for hour are smaller than corresponding standard errors in Table V using AR(1)+RE covariance and hour as a classification variable. This is due to the use of the polynomial model which exploits the continuous trend over hours. If the polynomial model yields very different results, one would conclude it does not adequately represent the trend over time.

Estimates of differences between drugs A and B at each hour may be obtained from the ESTIMATE statements (23):

```
estimate 'drga – drgb hr1' drug 1 -1 0
      drug * hr 1 -1 0 drug * hr * hr 1 -1 0 drug * hr * hr * hr 1 -1 0;
estimate 'drga – drgb hr2' drug 1 -1 0
      drug * hr 2 -2 0 drug * hr * hr 4 -4 0 drug * hr * hr * hr 8 -8 0;
```


Table VII. Estimates and standard errors for AR(1) + RE covariance structure and third degree polynomial model for hour.m

Parameter	Estimate	Standard error
Within-subject comparisons		
hr1–hr2 drug A	0.1346	0.0453
hr1–hr3 drug A	0.2577	0.0634
hr1–hr4 drug A	0.3669	0.0686
hr1–hr5 drug A	0.4599	0.0720
hr1–hr6 drug A	0.5344	0.0754
hr1–hr7 drug A	0.5880	0.0753
hr1–hr8 drug A	0.6183	0.0828
Between-subject comparisons		
drg B–drg A hr1	0.2108	0.1494
drg B–drg A hr2	0.3280	0.1429
drg B–drg A hr3	0.3463	0.1434
drg B–drg A hr4	0.2998	0.1408
drg B–drg A hr5	0.2228	0.1408
drg B–drg A hr6	0.1492	0.1434
drg B–drg A hr7	0.1132	0.1429
drg B–drg A hr8	0.1489	0.1494

```
estimate 'drga – drgb hr3' drug 1 –1 0
      drug*hr 3 –3 0 drug*hr*hr 9 –9 0 drug*hr*hr*hr 27 –27 0;
estimate 'drga – drgb hr4' drug 1 –1 0
      drug*hr 4 –4 0 drug*hr*hr 16 –16 0 drug*hr*hr*hr 64 –64 0;
estimate 'drga – drgb hr5' drug 1 –1 0
      drug*hr 5 –5 0 drug*hr*hr 25 –25 0 drug*hr*hr*hr 125 –125 0;
estimate 'drga – drgb hr6' drug 1 –1 0
      drug*hr 6 –6 0 drug*hr*hr 36 –36 0 drug*hr*hr*hr 216 –216 0;
estimate 'drga – drgb hr7' drug 1 –1 0
      drug*hr 7 –7 0 drug*hr*hr 49 –49 0 drug*hr*hr*hr 343 –343 0;
estimate 'drga – drgb hr8' drug 1 –1 0
      drug*hr 8 –8 0 drug*hr*hr 64 –64 0 drug*hr*hr*hr 512 –512 0;
```
(23)

Results from statements (23) appear in Table VII.

Standard errors for differences between drug A and drug B at hours 1 and 8 using the polynomial model are similar to standard errors for these differences using the model with hour as a classification variable. The standard errors of differences between drugs A and B at intermediate hours are less than the standard errors for respective differences using hour as a classification variable. Again, this is a phenomenon related to using regression models, and has very little to do with the covariance structure. It demonstrates that there is considerable advantage to refining the fixed effects portion of the model. We believe, however, that refining the fixed effects portion of the model should be done after arriving at a satisfactory covariance structure using a saturated fixed effects model.

9. SUMMARY AND CONCLUSIONS

One of the primary distinguishing features of analysis of repeated measures data is the need to accommodate the covariation of the measures on the same sampling unit. Modern statistical software enables the user to incorporate the covariance structure into the statistical model. This should be done at a stage prior to the inferential stage of the analysis. Choice of covariance structure can utilize graphical techniques, numerical comparisons of covariance estimates, and indices of goodness-of-fit. After covariance is satisfactorily modelled, the estimated covariance matrix is used to compute generalized least squares estimates of fixed effects of treatments and time.

In most repeated measures settings there are two aspects to the covariance structure. First is the covariance structure induced by the subject experimental design, that is, the manner in which subjects are assigned to treatment groups. The design typically induces covariance due to contribution of random effects. In the example of this paper, the design was completely randomized which results in covariance of observations on the same subject due to between-subject variation. If the design were randomized blocks, then there would be additional covariance due to block variation. When using SAS PROC MIXED, the covariance structure induced by the subject experimental design is usually specified in the RANDOM statement. Second is the covariance structure induced by the phenomenon that measures close in time are more highly correlated than measures far apart in time. In many cases this can be described by a mathematical function of time lag between measures. This aspect of covariance structure must be modelled using the REPEATED statement in PROC MIXED.

Estimates of fixed effects, such as differences between treatment means, may be the same for different covariance structures, but standard errors of these estimates can still be substantially different. Thus, it is important to model the covariance structure even in conditions when estimates of fixed effects do not depend on the covariance structure. Likewise, tests of significance may depend on covariance structure even when estimates of fixed effects do not.

The example in the present paper has equal numbers of subjects per treatment and no missing data for any subject. Having equal numbers of subjects per treatment is not particularly important as far as implementation of data analysis is concerned using mixed model technology. However, missing data within subjects can present serious problems depending on the amount, cause and pattern of missing data. In some cases, missing data can cause non-estimability of fixed effect parameters. This would occur in the extreme situation of all subjects in a particular treatment having missing data at the same time point. Missing data can also result in unstable estimates of variance and covariance parameters, though non-estimability is unlikely. The analyst must also address the underlying causes of missing data to assess the potential for introducing bias into the estimates. If the treatment is so toxic as to cause elimination of study subjects, ignoring that cause of missingness would lead to erroneous conclusions about the efficacy of the treatment. For more information on this topic, the reader is referred to Little and Rubin [18], who describe different severity levels of missingness and modelling approaches to address it.

Unequal spacing of observation times presents no conceptual problems in data analysis, but computation may be more complex. In terms of PROC MIXED, the user may have to resort to the class of covariance structures for spatial data to implement autoregressive covariance. See Littell *et al.* [15] for illustration.

Using regression curves to model mean response as functions of time can greatly decrease standard errors of estimators of treatment means and differences between treatment means at

particular times. This is true in any modelling situation involving a continuous variable, and is not related particularly to repeated measures data. This was demonstrated in Section 8 using polynomials to model FEV1 trends over time. In an actual data analysis application, pharmacokinetic models could be used instead. Such models usually are non-linear in the parameters, and thus PROC MIXED could not be used in its usual form. However, the NLINMIX macro or the new NLMIXED procedure could be used.

APPENDIX

The general linear mixed model specifies that the data vector $\mathbf{Y}$ is represented by the equation

$$\mathbf{Y} = \mathbf{X}\beta + \mathbf{Z}\mathbf{U} + \mathbf{e} \tag{24}$$

where $E(\mathbf{U}) = 0$, $E(\mathbf{e}) = 0$, $V(\mathbf{U}) = \mathbf{G}$ and $V(\mathbf{e}) = \mathbf{R}$. Thus

$$E(\mathbf{Y}) = \mathbf{X}\beta \tag{25}$$

We assume that $\mathbf{U}$ and $\mathbf{e}$ are independent, and obtain

$$V(\mathbf{Y}) = \mathbf{Z}\mathbf{G}\mathbf{Z}' + \mathbf{R} \tag{26}$$

Thus, the general linear mixed model specifies that the data vector $\mathbf{Y}$ has a multivariate normal distribution with mean vector $\mu = \mathbf{X}\beta$ and covariance matrix $\mathbf{V} = \mathbf{Z}\mathbf{G}\mathbf{Z}' + \mathbf{R}$.

Generalized least squares theory (Graybill, Reference [19], Chapter 6) states that the best linear unbiased estimate of β is given by

$$\mathbf{b} = (\mathbf{X}'\mathbf{V}^{-1}\mathbf{X})^{-1}\mathbf{X}'\mathbf{V}^{-1}\mathbf{Y} \tag{27}$$

and the covariance matrix of the sampling distribution of $\mathbf{b}$ is

$$V(\mathbf{b}) = (\mathbf{X}'\mathbf{V}^{-1}\mathbf{X})^{-1} \tag{28}$$

The BLUE of a linear combination $\mathbf{a}'\beta$ is $\mathbf{a}'\mathbf{b}$, and its variance is $\mathbf{a}'(\mathbf{X}'\mathbf{V}^{-1}\mathbf{X})^{-1}\mathbf{a}$. More generally, the BLUE of a set of linear combinations $\mathbf{A}'\beta$ is $\mathbf{A}'\mathbf{b}$, and its sampling distribution covariance matrix is $\mathbf{A}'(\mathbf{X}'\mathbf{V}^{-1}\mathbf{X})^{-1}\mathbf{A}$. Thus, the sampling distribution of $\mathbf{A}'\mathbf{b}$ is multivariate normal with mean vector $E(\mathbf{A}'\mathbf{b}) = \mathbf{A}'\beta$ and covariance matrix $\mathbf{A}'(\mathbf{X}'\mathbf{V}^{-1}\mathbf{X})^{-1}\mathbf{A}$. Inference procedures for the general linear mixed model are based on these principles. However, the estimate $\mathbf{b} = (\mathbf{X}'\mathbf{V}^{-1}\mathbf{X})^{-1}\mathbf{X}'\mathbf{V}^{-1}\mathbf{Y}$ and its covariance matrix $V(\mathbf{b}) = (\mathbf{X}'\mathbf{V}^{-1}\mathbf{X})^{-1}$ both are functions, of $\mathbf{V} = \mathbf{Z}\mathbf{G}\mathbf{Z}' + \mathbf{R}$, and in most all cases $\mathbf{V}$ will contain unknown parameters. Thus, an estimate of $\mathbf{V}$ must be used in its place.

Usually, elements of $\mathbf{G}$ will be functions of one set of parameters, and elements of $\mathbf{R}$ will be functions of another set. The MIXED procedure estimates the parameters of $\mathbf{G}$ and $\mathbf{R}$, using by default the REML method, or the ML method, if requested by the user. Estimates of the parameters are then inserted into $\mathbf{G}$ and $\mathbf{R}$ in place of the true parameter values to obtain $\hat{\mathbf{V}}$. In turn, $\hat{\mathbf{V}}$ is used in place of $\mathbf{V}$ to compute $\hat{\mathbf{b}}$ and $\hat{\mathbf{V}}(\hat{\mathbf{b}})$.

Standard errors of estimates of linear combinations are computed as $(\hat{\mathbf{V}}(\mathbf{a}'\hat{\mathbf{b}}))^{1/2} = (\mathbf{a}'(\hat{\mathbf{V}}(\hat{\mathbf{b}}))\mathbf{a})^{1/2}$. Statistics for tests of fixed effects are computed as $F = \mathbf{b}'\mathbf{A}(\hat{\mathbf{V}}(\hat{\mathbf{b}}))^{-1}\mathbf{A}'\mathbf{b}/\text{rank}(\mathbf{A})$. In some cases, the distributions of F are, in fact, F distributions, and in other cases they are only approximate. Degrees of freedom for the numerator of the F statistic are given by the rank of $\mathbf{A}$,

but computation of degrees of freedom for the denominator is a much more difficult problem. One possibility is a generalized Satterthwaite approximation as given by Fai and Cornelius [20]. The interested reader is also referred to McLean and Sanders [21] for further discussion on approximating degrees of freedom, and to Hulting and Harville [22] for some Bayesian and non-Bayesian perspectives on this issue. For more information on analysis of repeated measures data, see Diggle *et al.* [23] and Verbeke and Molenberghs [24].

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