

Discussion of the Comparison of GLM and MIXED Procedures for a Repeated Measures Design: One Within and One Between Subjects Factor

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Abstract

Many SAS[®] users are familiar with statistical procedures referred to as 'repeated measures analyses of variance'. Those who use these techniques may also be frustrated in determining the appropriate SAS code and in understanding the calculations involved in the results. In this paper, I first introduce the special characteristics of the required data structure for repeated measures analyses. Next, I present a medical example involving a single within subjects factor and a single between subjects factor. Four variations of this example are presented, including 1) no missing data & equal size groups, 2) no missing data & unequal size groups, 3) missing data & equal size groups and 4) missing data & unequal size groups. In addition, four sets of SAS code are discussed as options for analysis of the data: using 1) Proc GLM as a split plot design, 2) Proc GLM with the "repeated" statement, 3) Proc MIXED with the "repeated" statement and 4) Proc MIXED with the "random" statement. Finally, I compare and discuss the resulting differences from the four sets of SAS code.

Introduction

As a consulting biostatistician, I often have repeated measures data to analyze and have struggled through projects with concerns that remain unresolved. The concerns deal with questions such as, "Did I code my analysis appropriately?" and "Precisely how and what is my code testing?". I hope this paper addresses the appropriateness of some options for coding the analysis of repeated measures data and explains what the code is testing. However, I admit that some aspects of 'how' the code is testing hypotheses still remain a mystery to me.

Repeated measures analysis of variance is used for data involving multiple measurements on the experimental units. The experimental units can be people, animals, objects, etc. In general, analysis of variance refers to a wide set of statistical techniques and models for making inferences about the group means of a particular response variable. Repeated measures analysis of variance refers to a subset of these techniques appropriate when each experimental unit is observed more than once under possibly different times, settings or conditions. The response variable in repeated measures analysis of variance may be continuous, discrete or categorical and the same holds for the explanatory variables. In the example presented here, I assume the response variable has a multivariate normal distribution and I consider categorical explanatory variables.

The general mathematical assumptions in analysis of variance (ANOVA) include 1) the response variable has a normal distribution within each group, 2) the distributions of the response variable have equal variances in each group and 3) the observations are independent, random samples. Repeated measures data have two characteristics which cause difficulty when trying to analyze with standard ANOVA techniques. In particular, measurements on the same subjects tend to be correlated and the variability within a subject may not be constant across the levels of the repeated factor. Thus, the last two assumptions for ANOVA rarely hold for data with repeated measures. Fortunately, univariate and multivariate approaches exist to account for these special characteristics of the data structure.

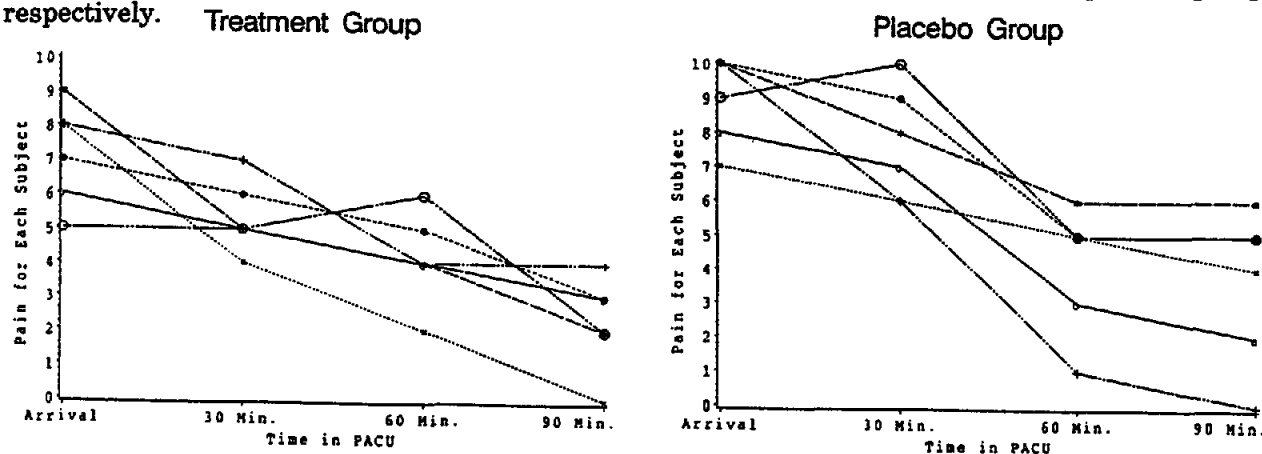
In the univariate approach, there is a single response variable and included in the model is a random factor for subjects. This model handles cases in which the measures from the same subject have equal variance and a common correlation, a structure known as compound symmetry. It also handles a somewhat more general structure called sphericity or circularity in the variances and correlations. Unfortunately, sphericity (and compound symmetry) are difficult to assume in most repeated measures data. Violating this assumption increases the chance of making a type I error (i.e., incorrectly finding a treatment effect).

More general methods have been developed to handle different structures for the variances and correlations. The multivariate approach takes the repeated measures on each subject as multiple response variables, allowing any structure for the variances and correlations². Although all subjects

are assumed to have the same structure, when the sphericity structure holds, the univariate approach is more powerful than the multivariate approach, but with other structures, the reverse can be true.

Example

Consider the following hypothetical example in a medical setting involving post-operative pain. Assume twelve subjects were randomized to receive either a treatment or a placebo injection, which is administered in addition to the usual anesthetic prior to surgery. Pain after surgery is measured on a visual analog scale from 0 = 'no pain' to 10 = 'unbearable pain'. The response variable, pain, is measured multiple times in the post-administrative care unit (PACU), including 'at arrival', '30 minutes', '60 minutes' and '90 minutes'. It is of interest to examine if there is a statistically significant effect due to treatment group or time on the amount of pain after surgery. Note that although a small sample is used and pain is not measured here on a continuum, for ease of illustration, I assume pain is continuous and has a multivariate normal distribution. The complete data set is graphed below as the pain score for each subject across time in the PACU, for the treatment and placebo groups, respectively.



Four Variations of Example

The four variations of this example to be considered here include,

- 1) *no missing data and equal size groups* - all 12 subjects have pain measurements at all four time points,
- 2) *no missing data and unequal size groups* - all subjects have their pain measured at all four time points, but the numbers of subjects in the groups are not the same (i.e., six subjects in one group and four in the other),
- 3) *missing data and equal size groups* - all 12 subjects are involved in the study, but a small fraction of the pain measurements are missing, and
- 4) *missing data and unequal size groups* - a small fraction of the pain measurements are missing and the numbers of subjects in the groups are not the same.

Four Sets of SAS Code

Four sets of SAS code are presented as possible approaches to examine the effects due to treatment group and time on the amount of pain after surgery. The four sets of code are given as follows:

1. **Proc GLM as a split plot design**

```
proc glm;
  class id group time;
  model pain=group id(group) time group*time;
  test h=group e=id(group);
```
2. **Proc GLM with the "repeated" statement**

```
proc glm;
  class group;
  model t1-t4=group /nouni;
  repeated time 4 /print;
```

3. **Proc MIXED with the "repeated" statement**

```
proc mixed;
    class id group time;
    model pain=group time group*time;
    repeated/type=cs sub=id;
```
4. **Proc MIXED with the "random" statement**

```
proc mixed;
    class id group time;
    model pain=group time group*time;
    random id(group);
```

The first set of SAS code uses the GLM procedure to model the data as a split-plot design. The univariate model is stated as follows:

$$y_{ijk} = \mu + \tau_i + \gamma_k + (\tau\gamma)_{ik} + \zeta_{j(i)} + \varepsilon_{ijk},$$

where y_{ijk} represents the $(ijk)^{th}$ observation, μ represents the overall mean pain, τ_i represents the group effect, γ_k represents the time effect, $(\tau\gamma)_{ik}$ represents the group by time interaction effect, $\zeta_{j(i)}$ represents the effect of subject nested within group and ε_{ijk} represents the random error component. Split-plot designs require random assignment of the level of the repeated factor to the whole plot, which helps ensure that the assumption of compound symmetry is met³. With repeated measures data (as opposed to data from a split-plot design), the more general sphericity assumption is sufficient. The expected mean squares are required to construct tests of hypotheses and can be requested by including a "random" statement for subjects nested within group. Estimable functions of the model parameters can be used to determine the hypotheses being tested by the code and can be requested by including an 'e' option in the "model" statement.

The next set of SAS code uses the GLM procedure with the "repeated" statement and uses the multivariate model. The model is stated in matrix form as follows:

$$\mathbf{y}_i = \boldsymbol{\mu} + \boldsymbol{\tau}_i + \boldsymbol{\varepsilon}_i,$$

where $\mathbf{y}_i$ represents the (1×4) vector of subjects' pain observations, $\boldsymbol{\mu}$ represents the (1×4) vector of overall mean pain scores, $\boldsymbol{\tau}_i$ represents the (1×4) vector for the group effect, and $\boldsymbol{\varepsilon}_i$ represents the (1×4) vector for the random error component. This code actually generates output for testing the sphericity assumption and offers adjustments to the hypothesis tests of interest if this assumption does not hold. This output is obtained by including a 'printe' option in the "repeated" statement. It models the repeated measures (i.e., pain at the four time points) on each subject as a multivariate response. However, if there is a missing observation at any of the time points, all the data for that subject is excluded from the analysis with this set of SAS code.

The third set of SAS code uses the MIXED procedure with the "repeated" statement. The univariate model is stated as follows:

$$y_{ijk} = \mu + \tau_i + \gamma_k + (\tau\gamma)_{ik} + \varepsilon_{ijk},$$

where y_{ijk} represents the $(ijk)^{th}$ observation, μ represents the overall mean pain, τ_i represents the group effect, γ_k represents the time effect, $(\tau\gamma)_{ik}$ represents the group by time interaction effect and ε_{ijk} represents the random error component. By using the "repeated" statement, the intra-subject structure of variances and correlations can be specified. Note that a compound symmetry structure is specified here for the sake of comparison of results of the four sets of code. The 'sub=id' option in the "repeated" statement tells SAS to construct a structure for the variances and correlations on an intrasubject basis¹.

The last set of SAS code uses the MIXED procedure with the "random" statement. The univariate model is stated as follows:

$$y_{ijk} = \mu + \tau_i + \gamma_k + (\tau\gamma)_{ik} + \zeta_{j(i)} + \varepsilon_{ijk},$$

where y_{ijk} represents the $(ijk)^{th}$ observation, μ represents the overall mean pain, τ_i represents the group effect, γ_k represents the time effect, $(\tau\gamma)_{ik}$ represents the group by time interaction effect, $c_{j(i)}$ represents the effect of subject nested within group and ϵ_{ijk} represents the random error component. A compound symmetry model is fit by using this "random" statement. The only difference from the third set of SAS code is that the denominator degrees of freedom is smaller since a portion of the total degrees of freedom are partitioned out for the random component of the model.

In addition to the differences in the four sets of SAS code outlined above, the two procedures differ in the general testing and estimation techniques. The GLM procedure uses the more common F-statistics that are ratios of mean squares, whereas the MIXED procedure gives F-statistics that are of the Wald-type quadratic form⁴. The GLM procedure uses the method-of-moments estimates for variance components and the MIXED procedure uses maximum-likelihood (ML) or restricted/residual maximum-likelihood (REML) estimates¹.

The method-of-moments estimates used in the GLM procedure are obtained by equating the expected values to the sample moments. The equations formed by equating the calculated sums of squares to their expectations are solved for the variance components. Variance components are the variances associated with the sources of variation or randomness in the data. The ML estimates used in the MIXED procedure are obtained by iteratively searching over the parameter space for parameter values that maximize a function related to the chance of observing the data collected. The function maximized is called the likelihood function. The REML estimates, also used in the MIXED procedure, are obtained by constructing the likelihood function in two parts - one involving the fixed effects, the other free of the fixed effects - and then the maximum likelihood estimates of the variance components are obtained from the portion of the model that is free of fixed estimates.

Also, depending on the code, the data must either be set up in the univariate or multivariate mode, as shown below.

For SAS Code #'s 1, 3 & 4 - univariate mode:

Patient#	Group	Time	Pain
1	1	T1	10
1	1	T2	8
1	1	T3	6
1	1	T4	6
2	1	T1	7
2	1	T2	6
2	1	T3	5
2	1	T4	4
3	1	T1	8
3	1	T2	7
3	1	T3	3
3	1	T4	2

For SAS Code #2 - multivariate mode:

Patient#	Group	T1	T2	T3	T4
1	1	10	8	6	6
2	1	7	6	5	4
3	1	8	7	3	2
4	1	9	10	5	5
5	1	10	9	5	5
6	1	10	6	1	0
7	2	9	5	4	2
8	2	8	4	2	0
9	2	6	5	4	3
10	2	5	5	6	2
11	2	7	6	5	3
12	2	8	7	4	4

where, Group 1 = Placebo, Group 2 = Treatment,
and T1 = Arrival, T2 = 30 Min, T3 = 60 Min, T4 = 90 Min.

Discussion of the Results

Results from the four sets of SAS code for each of the four variations are given in Table 1, using type III sums of squares. The first set of SAS code, which models the data as a split-plot design, may not be desirable here, since with repeated measures data, one cannot randomly assign the levels of time to the time interval. For example, you cannot take a 60 minute pain measurement before you take a 30 minute pain measurement, there is an inherent order to obtaining the measurements. Although this approach can produce reasonable results, if the assumption of compound symmetry is suspect, it will be a poor way to analyze repeated measures data. When the compound symmetry assumption is met, this analysis can be used for variations 1 and 2, but with missing data in variations 3 and 4, appropriate adjustments should be made after examining the expected mean squares and estimable functions³.

The next set of SAS code, using the GLM procedure with the "repeated" statement is undesirable, since it drops any subject with missing data from the analysis. In many settings, missing data are often inevitable. Keep in mind that you can always impute missing values from the remainder of the subject's data and then apply this code. Such imputation is acceptable for samples with small fractions of missing data.

The remaining two sets of SAS code involve the MIXED procedure. The p-values from the MIXED procedure provide more liberal results than the GLM procedure, particularly for small samples. I'm not exactly sure why this is true. However, appropriate adjustments to the F-tests are available⁵. In fact, Wolfinger⁶ is incorporating these adjustments to appear in the next version of the MIXED procedure.

As mentioned previously, some of the differences in the two procedures discussed here include 1) the GLM procedure uses the more common F-statistics that are ratios of mean squares, whereas the MIXED procedure gives F-statistics that are of the Wald-type quadratic form and 2) the GLM procedure uses the method-of-moments estimates for variance components and the MIXED procedure uses ML or REML estimates. One advantage of the way the MIXED procedure constructs the test statistics is that it allows for different types of structures for the variances and correlations¹. It is unclear to me how these test statistics are formed and why it allows for different types of structures for the variances and correlations. The advantage of the likelihood based approach used in the MIXED procedure is that it allows SAS to use all subjects' observations, regardless of missing data.

Summary

Although I only present two options with the MIXED procedure, there are numerous other appropriate ways to model this type of data with the MIXED procedure. Additionally, there are other ways in SAS for modeling this type of data including the "manova" statement in the GLM procedure. I struggle most with the complex determination of which method is appropriate in any situation.

This paper is not intended to offer a solution for all situations, nor to exhaust the possibilities for analysis of repeated measures data. This paper is intended to address the two questions outlined in my introduction: "Did I code my analysis appropriately?" and "Precisely how and what is my code testing?". I hope this paper offers a satisfactory answer to the first question and can serve as a guide for making decisions about appropriate SAS code for analysis of repeated measures data. I address some aspects of the second question, however, there are specific questions that remain unresolved. These include, 1) How can I choose the correct structure for the variances and correlations?, 2) How can sphericity be explained in layman terms?, 3) Why, particularly in small samples, do the p-values from the mixed procedure seem to be more liberal than those from the GLM procedure? and 4) How do F-statistics of the Wald-type quadratic form allow for different types of structures for the variances and correlations? For further related reading on the topic of the paper, see Wolfinger & Chang (1995)⁶, Schluchter (1988)⁷ and Jennrich & Schluchter (1986)⁸.

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Table 1
Summary of Results From Four sets of SAS code for Each of Four Variations

No Missing & Equal Size Groups:

Factor	GLM - Split Plot (Used 48/48 Obs.)		GLM - Repeated (Used 12/12 Obs.)		MIXED - Repeated (Used 48/48 Obs.)		MIXED - Random (Used 48/48 Obs.)	
	F	P-Value	F	P-Value	F	P-Value	F	P-Value
Group	$F_{1,10}=4.50$	0.0598	$F_{1,10}=4.50$	0.0598	$F_{1,40}=4.50$	0.0401	$F_{1,10}=4.50$	0.0598
Time	$F_{3,30}=39.66$	0.0001	$F_{3,6}=26.46$	0.0002	$F_{3,40}=39.66$	0.0000	$F_{3,30}=39.66$	0.0000
Inter.	$F_{3,30}=1.91$	0.1493	$F_{3,6}=2.37$	0.1463	$F_{3,40}=1.91$	0.1435	$F_{3,30}=1.91$	0.1493

No Missing & Unequal Size Groups:

Factor	GLM - Split Plot (Used 40/40 Obs.)		GLM - Repeated (Used 10/10 Obs.)		MIXED - Repeated (Used 40/40 Obs.)		MIXED - Random (Used 40/40 Obs.)	
	F	P-Value	F	P-Value	F	P-Value	F	P-Value
Group	$F_{1,8}=5.45$	0.0478	$F_{1,8}=5.45$	0.0478	$F_{1,32}=5.45$	0.0260	$F_{1,8}=5.45$	0.0478
Time	$F_{3,24}=28.54$	0.0001	$F_{3,6}=18.30$	0.0020	$F_{3,32}=28.54$	0.0000	$F_{3,24}=28.54$	0.0000
Inter.	$F_{3,24}=1.73$	0.1871	$F_{3,6}=3.77$	0.0782	$F_{3,32}=1.73$	0.1801	$F_{3,24}=1.73$	0.1871

Missing & Equal Size Groups:

Factor	GLM - Split Plot (Used 46/48 Obs.)		GLM - Repeated (Used 10/12 Obs.)		MIXED - Repeated (Used 46/48 Obs.)		MIXED - Random (Used 46/48 Obs.)	
	F	P-Value	F	P-Value	F	P-Value	F	P-Value
Group	$F_{1,10}=4.29$	0.0652	$F_{1,8}=2.20$	0.1763	$F_{1,38}=4.20$	0.0474	$F_{1,10}=4.20$	0.0676
Time	$F_{3,28}=35.43$	0.0001	$F_{3,6}=16.84$	0.0025	$F_{3,38}=36.25$	0.0001	$F_{3,28}=36.25$	0.0000
Inter.	$F_{3,28}=1.70$	0.1900	$F_{3,6}=1.34$	0.3457	$F_{3,38}=1.70$	0.1834	$F_{3,28}=1.70$	0.1898

Missing & Unequal Size Groups:

Factor	GLM - Split Plot (Used 38/40 Obs.)		GLM - Repeated (Used 8/10 Obs.)		MIXED - Repeated (Used 38/40 Obs.)		MIXED - Random (Used 38/40 Obs.)	
	F	P-Value	F	P-Value	F	P-Value	F	P-Value
Group	$F_{1,8}=5.25$	0.0512	$F_{1,6}=2.99$	0.1346	$F_{1,30}=5.26$	0.0290	$F_{1,8}=5.26$	0.0510
Time	$F_{3,22}=25.04$	0.0001	$F_{3,4}=10.71$	0.0221	$F_{3,30}=25.65$	0.0000	$F_{3,22}=25.65$	0.0000
Inter.	$F_{3,22}=1.50$	0.2413	$F_{3,4}=2.39$	0.2092	$F_{3,30}=1.53$	0.2281	$F_{3,22}=1.53$	0.2358