Paper AS06

Mixed Model Repeated Measures (MMRM)

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ABSTRACT

This specialized Mixed Models procedure analyzes results from repeated measures designs in which the outcome (response) is continuous and measured at fixed time points. The procedure uses the standard mixed model calculation engine to perform all calculations. However, the user-interface has been simplified to make specifying the repeated measures analysis much easier. These designs that can be analyzed by this procedure include

• Repeated-measures designs

• Cross-over/ Parallel designs

• Designs with covariates.

This paper gives an abbreviated coverage of mixed models in general focuses on response measurements assumed to be normally distributed. Non-normal data are also frequently collected in a repeated measures layout.

INTRODUCTION

Repeated measures refer to multiple measurements taken from the same experimental unit, such as serial evaluation over time on the same patient. Special attention is given to these types of measurements because they cannot be considered independent. In particular, the analysis must make provisions for the correlation structure.

Most clinical studies require outpatients to return to the clinic for multiple visits during the trial with response measurements made at each. This is the most common example of repeated measures, sometimes called 'longitudinal' data. These repeated response measurements can be used to characterized a response profile overtime. One of the main questions the researcher asks is whether the mean response profile for one treatment group is the same as for another treatment group or placebo group. This situation might arise, e.g. when trying to determine if the onset of effect or rate of improvement due to a new treatment is faster than a competitor's treatment. Comparison of response profiles can be tested with a single F-test from a repeated measures analysis.

SYNOPSIS

In general, you have g independent groups of patients each of whom are subjected to repeated measurements of the same response variable, y, at t equally spaced time period. Letting n_i represent no of patients in Group (i = 1, 2, ….. , g), the layout for g=3 groups each shown in table below. In comparative trials, the groups often represent different parallel treatment groups or do levels of drug.

Layout for a Repeated Measures Design with 3 Groups

Group	Patient	----------Time---------- 1	2	…	t
1	1	y_{111}	y_{112}	…	y_{11t}
	2	y_{121}	y_{122}	…	y_{12t}
	…	…	…	…	…
	n_1	y_{1n11}	y_{1n12}	…	y_{1n1t}
2	1	y_{211}	y_{212}	…	y_{21t}
	2	y_{221}	y_{222}	…	y_{22t}
	…	…	…	…	…
	n_2	y_{2n21}	y_{2n22}	…	y_{2n2t}
3	1	y_{311}	y_{312}	…	y_{31t}
	2	y_{321}	y_{322}	…	y_{32t}
	…	…	…	…	…
	n_3	y_{3n31}	y_{3n32}	…	y_{3n3t}

There are number of analytical approaches for handling repeated measures. First, examine a 'univariate' method that uses the same ANOVA concepts. A 'multivariate' method, which treats repeated measurements as a multivariate response vector, may also be used many circumstances. Also can use modelling techniques that use the MIXED and GENMODE procedures in SAS, which are often preferable if there are missing data.

THE 'UNIVARIATE' APPROACH

Consider the group, patient, and time effects shown in table above as three factors in an ANOVA. We can examine the variability within and among these factors, noting that the time effect represents correlated measurements. Notice that the response might vary among groups, among patients within groups, and among the different measurement times. Therefore, include group effect, a patient (within – group) effect, and time effect as sources of variation in the ANOVA. In addition, the repeated measures analysis using a univariate approach includes the group-by-time interaction.

As with other ANOVA methods, assumption of normality of the response measurements and variance homogeneity among groups are considered to be satisfied. In addition, the univariate ANOVA requires that each pair of repeated measures has the same correlations, this feature is known as 'compound symmetry'.

A significant interaction means that changes in response over time differ among groups, i.e. significant difference in response profiles. When profiles are similar among groups (i.e. no group-by-time interaction), tests for the group effect measure the deviation from the hypothesis of equality of mean responses among groups, 'averaged' over time. This test using the repeated measures analysis method might be more sensitive to detecting group differences than using a one-way ANOVA to compare groups at a single time point. However, the group effect might not be meaningful if there is significant time effect. The time effect is a measure of deviation from the hypothesis of equality of mean responses among the measurement times for all groups combined. Repeated measures ANOVA also provides a test of this hypothesis.

In simplest case of repeated measure ANOVA, which is presented here, the group and the evaluation time are cross-classified main effects. However, the samples are not independent among the group-time cells, because measurement over time that are taken from the same patient are correlated. To account for this correlation, the patient effect must be included as a source of variation in the ANOVA.

With $N = n_1+n_2+\ldots+n_g$, the repeated measures ANOVA summary table takes the following form:

ANOVA SUMMARY FOR REPEATED-MEASURES DESIGN

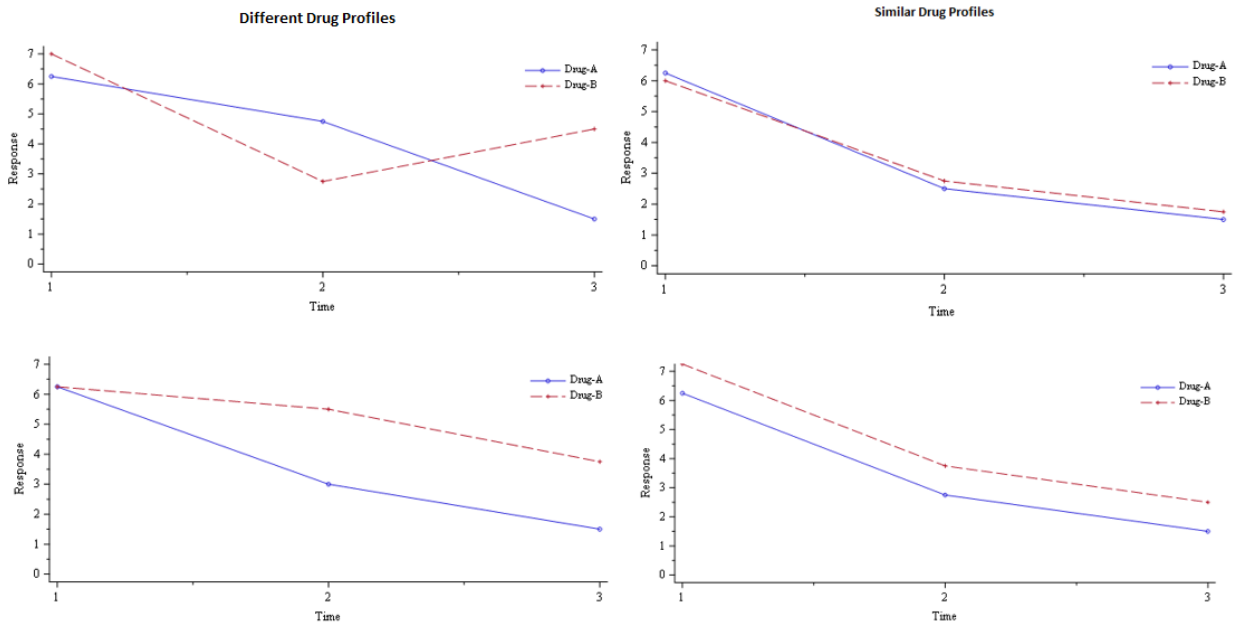
SOURCE	df	SS	MS	F
GROUP	g-1	SSG	MSG	F_G=MSG/MSP(G)
PATIENT (within GROUP)	N-g	SSP(G)	MSP(G)	--
TIME	t-1	SST	MST	F_T=MST/MSE
GROUP-by-TIME	(g-1)(t-1)	SSGT	MSGT	F_{GT}=MSGT/MSE
Error	(N-g)(t-1)	SSE	MSE	--
Total	Nt-1	TOT(SS)		

For the balanced layout ($n_1=n_2=\ldots=n_g$), the sums of squares can be computed in a manner similar to that used for the two-way ANOVA. These computations are shown in this paper in next examples. As usual, the mean squares (MS) are found by dividing the sums of squares(SS) By the corresponding degrees of freedom.

Variation from patient-to-patient is one type of random error, as estimated by the mean square for Patient (within Group). If there is no difference among groups, the between –groups variation merely reflects patient-to-patient variation. Therefore, under the null hypothesis of no Group effect, MSG and MSP(G) are independent estimates of the among-patient variability, so that F_G has the F-distribution with g-1 upper and N-g lower degrees of freedom. If there is no Time effect, the mean square for Time (MST) is an estimate of within-patient variability, as is the error variation, MSE. The ration of these independent estimates (F_T) is the F-statistic used to test the hypothesis of no Time effect. Similarly, the interaction means square, which is also a measure of within-patient variation udder H_0, is compared to the MSE to test for a significant Group-by-Time interaction.

Sample response profiles are shown in Figure below for 2 drug groups and 3 time periods. As depicted, treatment differences depend on time if the profiles differ.

FIGURE 1 Sample Profiles of Drug Response



EXAMPLES

The Following example is used to demonstrate the 'univariate' approach to the repeated measures ANOVA in the balanced case.

EXAMPLE 1 – Arthritic Discomfort Following Vaccine

A pilot study was conducted in 8 patients to evaluate the effect of new vaccine on discomfort due to arthritic outbreaks. Four patients were randomly assigned to receive an active vaccine, and 4 patients were to receive a placebo. The patients were asked to return to the clinic monthly to report discomfort level for preceding month using a scale of 0(no discomfort) to 10 (maximum discomfort). Eligibility criteria required patients to have a rating of at least an 8 in the month prior to vaccination. The rating data are shown in Table below. Is there any evidence of a difference in response profiles between the active and placebo vaccines?

Raw Data for Example 1

Vaccine	Patient Number	Visit: Month 1	Month 2	Month 3
Active	101	6	3	0
	103	7	3	1
	104	4	1	2
	107	8	4	3
Placebo	102	6	5	5
	105	9	4	6
	106	5	3	4
	108	6	2	3

With only one group (g=1) in Table above, the repeated measures layout is identical to that of a randomized block design. In this case, the analysis is carried out as Two Way ANOVA model by using the repeated factor as the Group effect and Patient as the blocking factor.

ANOVA	Active Group				Placebo Group			
Source	df	SS	MS	F	df	SS	MS	F
PATIENT	3	11.667	3.889	3.41	3	13.667	4.556	5.12*
VISIT	2	48.500	24.250	21.29*	2	18.667	9.333	10.50*
Error	6	6.833	1.139		6	5.333	0.889	
Total	11	67.000			11	37.667		

*Significant (p<0.05)

SSE for the repeated measures ANOVA is 6.833+5.333=12.167 based on 6+6=12 degrees of freedom. Similarly, the SS(Patient) can added to obtain the SS(Patient(Vaccine)) for the repeated measures ANOVA, 11.667+13.667=25.333 with 3+3=6 degrees of freedom. Notice that the sums of squares for the time effect (SS(Visit)) are not additive.

Solution

Using a 'univariate' repeated measures ANOVA, we can identify Vaccine as the Group effect and Visit as the Time effect. The Patient-within-Vaccine and Vaccine-by-Visit effects are also included in the ANOVA. The sum of squares for each of these sources can be computed in the same manner. First, obtain the marginal means, as shown in table below.

Marginal Summary statistics for Example 1

Vaccine	Patient Number	Visit: Month 1	Visit: Month 2	Visit: Month 3	Mean
Active	101	6	3	0	3.000
	103	7	3	1	3.667
	104	4	1	2	2.333
	107	8	4	3	5.000
	Mean (SD)	6.25 (1.71)	2.75 (1.26)	1.50 (1.29)	3.500
Placebo	102	6	5	5	5.333
	105	9	4	6	6.333
	106	5	3	4	4.000
	108	6	2	3	3.667
	Mean (SD)	6.50 (1.73)	3.50 (1.29)	4.50 (1.29)	4.833
	Combined Mean	6.375	3.125	3.000	4.167

The Vaccine sum of squares is proportional to the sum of squared deviations of the group means from the overall mean:
SS(Vaccine)=(12.(3.500-4.167)2) + (12.(4.833-4.167)2) = 10.667
This is based on 1 degree of freedom because there are two levels of the Group Factor Vaccine.
The sum of squares for Visit, based on 2 degrees of freedom, and the Vaccine-by-Visit interaction, also with 2 degrees of freedom, can be computed as follows:
SS(Visit)=(8.(6.375-4.167)2) + (8.(3.125 -4.167)2 + (8.3.000 -4.167)2) =58.583

SS(Vaccine-by-Visit)= (4 .(6.25 -4.167)2) +
(4 .(2.75 -4.167)2) +
(4 .(1.50 -4.167)2) +
(4 .(6.50 -4.167)2) +
(4 .(3.50 -4.167)2) +
(4 .(4.50 -4.167)2) -
SS(Vaccine) –SS(Visit)
= 77.833 – 10.667 -58.583 = 8.583

The Patient within Vaccine sum of squares is found by summing the squared deviations between the mean response for each patient and the Vaccine Group mean within which that patient is nested. Within each Vaccine Group, the factor Patient has 3 degrees of freedom, so for the two vaccine groups, we have 6 degrees of freedom for Patient(Vaccine).

SS(Patient(Vaccine)) = (3 .(3.000 -3.500)2) +
(3 .(3.667 -4.500)2) +
(3 .(2.333 - 3.500)2) +
(3 .(5.000 - 3.500)2) +
(3 .(5.333 -4.833)2) +
(3 .(4.000 -4.833)2) +
(3 .(3.667 -4.833)2)
=25.333

The total sum of squares (based on 23 degrees of freedom) is found by summing the squared deviations of each observation from the overall mean.

SS(Total) = ((6 – 4.167)2) +
((3 – 4.167)2) +
((0 – 4.167)2) +
((7 – 4.167)2) +
…. +
((3 – 4.167)2)
= 115.3333

Finally, because this is balanced layout, the error sum of squares (SSE) can be found by subtraction.

SSE = SS(Total)
- SS(Vaccine)
- SS(Patient(Vaccine))
- SS(Visit)
- SS(Vaccine-by-Visit)

=115.333-10.667-25.333-58.583 -8.583
=12.167

The ANOVA table can now be completed as shown in table below. The mean squares for each effect are found by dividing the sum of squares (SS) by the degrees of freedom. The F-tests are the ratios of the effect mean squared to the appropriate error associated with that effect.

ANOVA Summary of Example 1

SOURCE	df	SS	MS	F
Vaccine	1	10.667	10.667	2.53
Patient(Vaccine)	6	25.333	4.222	--
Visit	2	58.583	29.292	28.89*
Vaccine-by-Visit	2	8.583	4.292	4.23*
Error	12	12.167	1.014	--
Total	23	115.333		

* Significant ($p<0.05$)

The F-test for the Vaccine effect is the ratio of the mean square of the mean square for Vaccine to the mean square for Patient(Vaccine), F=10.667/4.222 =2.53. The F-values for Visit and Vaccine-by-Visit are found by the ratios of the mean squared for these effects to the mean square error (MSE =1.014).

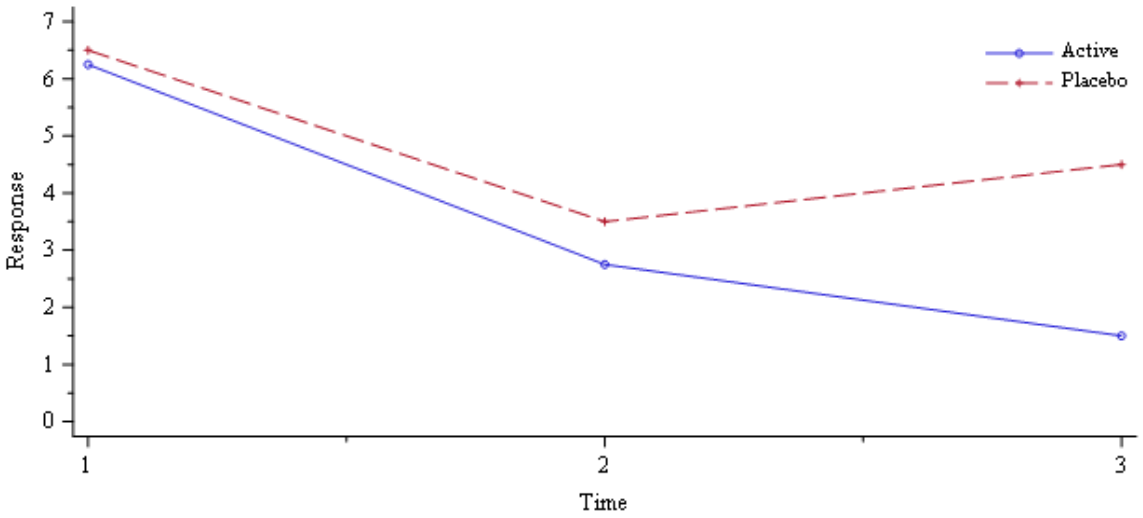
The null hypothesis of similar response profiles over time for each of the vaccine groups is tested by the Vaccine-by-Visit interaction. For two treatment groups as used in this example, the hypothesis can be expressed as the simultaneous equality of Treatment Group differences at each time point. That is, if Δ_j represents the difference in mean responses between the

active and placebo groups at Month j (e.g. $\Delta_j = \mu_{1j} - \mu_{2j}$) for j = 1,2,3 then the test summary can be expressed as

Null hypothesis $H_0 : \Delta_1 = \Delta_2 = \Delta_3$
Alt. hypothesis : H_A = not H_0
test statistic : F= MS(Vaccine-by-Visit) / MSE =4.23
decision rule : reject H_0 if F > F^2_{12} (0.05) = 3.89
conclusion : Because 4.23 > 3.89, reject H_0 and conclude that there is a significant Vaccine Group-by-Visit interaction.

Because Vaccine Group differences are based on the study month, further analyses can be performed by depicting the mean responses over time in graphical format (see figure2 below) and by using linear contrasts to compare differences between vaccine groups in the changes from successive time points. A one-way ANOVA can also be used to test for the Vaccine effect at each visit.
The manual computing methods demonstrated in Example 1 apply only to balanced designs (i.e., same number of patients per group). For the balanced case, the repeated measures ANOVA error sum of squares (SSE) is simply the sum of the SSE's found by using two-way ANOVA's (with no interaction) within each treatment group.

FIGURE 2: Sample Profiles of Drug Response



In the ANOVA summary table, the Visit effect is also seen to be significant. This means that the average responses for the combined vaccine groups differ among evaluation months. The F-test for the Vaccine effect can be interpreted as a comparison of mean response between vaccine groups, 'averaged' over all measurement times. With a non-significant F-value of 2.53 for the Vaccine effect and a significant interaction. Further analyses, as suggested above, are required prior to forming any conclusions with regard to the main effects.

SAS Analysis of Example 1
In the SAS code for analyzing the data for Example 1, PROC TRANSPOSE in SAS is applied to the input data set ARTHR is to get a new format with 1 observation per record in the data set DISCOM.

```
DATA ARTHR;
    INPUT VACGRP $  PAT MO1 MO2 MO3 ;
    DATALINES;
ACT 101 6 3 0
ACT 103 7 3 1
ACT 104 4 1 2
ACT 107 8 4 3
PBO 102 6 5 5
PBO 105 9 4 6
PBO 106 5 3 4
PBO 108 6 2 3
;
run;

proc sort data = ARTHR;
 by VACGRP  PAT;
run;
```

```
proc transpose data =ARTHR out=tra;
 by VACGRP  PAT;
 var  MO1 MO2 MO3 ;
run;

data DISCOM;
 set tra;
 score = col1;
 month = strip(_name_);
 visit = input(substr(month,3,1),best.) ;
run;
```

PROC GLM in SAS is applied to this data set by using a MODEL statement that includes effects for Vaccine Group (VACGRP), Time (VISIT), and Patient (PAT) nested within Vaccine Group. Test effects must be designated as class variables in the CLASS statement. The interaction is specified in the MODEL, statement as VACGRP*VISIT.

```
PROC GLM DATA = DISCOM;
    CLASS VACGRP PAT VISIT;
    MODEL SCORE = VACGRP PAT(VACGRP) VISIT VACGRP*VISIT/SS3;
    RANDOM PAT(VACGRP);
    TEST H=VACGRP E=PAT(VACGRP);
    TITLE3 'Univariate Approach';
    QUIT;
RUN;
```

Output 1-

UNIVARIATE APPROACH

The GLM Procedure

Class Level Information		
Class	**Levels**	**Values**
VACGRP	**2**	ACT PBO
PAT	**8**	101 102 103 104 105 106 107 108
VISIT	**3**	1 2 3

Number of Observations Read	24
Number of Observations Used	24

Dependent Variable: SCORE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	103.1666667	9.3787879	9.25	0.0003
Error	12	12.1666667	1.0138889		
Corrected Total	23	115.3333333			

R-Square	Coeff Var	Root MSE	SCORE Mean
0.894509	24.16609	1.006920	4.166667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VACGRP	1	10.66666667	10.66666667	10.52	0.0070
PAT(VACGRP)	6	25.33333333	4.22222222	4.16	0.0171
VISIT	2	58.58333333	29.29166667	28.89	<.0001
VACGRP*VISIT	2	8.58333333	4.29166667	4.23	0.0406

Source	Type III Expected Mean Square
VACGRP	Var(Error) + 3 Var(PAT(VACGRP)) + Q(VACGRP,VACGRP*VISIT)
PAT(VACGRP)	Var(Error) + 3 Var(PAT(VACGRP))
VISIT	Var(Error) + Q(VISIT,VACGRP*VISIT)
VACGRP*VISIT	Var(Error) + Q(VACGRP*VISIT)

Dependent Variable: SCORE

Tests of Hypotheses Using the Type III MS for PAT(VACGRP) as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
VACGRP	1	10.66666667	10.66666667	2.53	0.1631

The MSE verifies the computation 1.014. Notice that SAS automatically computers F-values for each model effect by using the MSE as the denominator, unless otherwise specified. The programmer must tell SAS to test the Group effect (VACGRP) against the Patient-within-Group (PAT(VACGRP)) 'error' by using the TEST statement. The resulting F-value of 2.53 is the appropriate F-test for the null hypothesis of no Vaccine Group effect. The F-value for VACGRP, which is shown in the output, is not meaningful for a repeated measures ANOVA. The F-value 4.16 for PAT(VACGRP) can also be ignored because this tests for a difference among patients within each group and will option be inconsequentially significant. The significant Vaccine-by-Visit interaction suggests that the difference between treatments is time-related, as was previously discovered.

The 'Multivariate' Approach

The methodology demonstrated in Example 1 is a 'univariate' approach to repeated measures analysis and, as noted, requires the assumption of compound symmetry. This assumption is often not valid, especially if the trial is lengthy or time points are inequality spaced, because measurements taken farther apart in time might be less correlated that those taken close together. Erroneous conclusions might result if the 'univariate' approach is used under violation of the assumption of compound symmetry.

A 'multivariate' approach can sometimes be used to circumvent this situation. This approach used the repeated measurements as multivariate response vectors, and is more robust when the assumption of compound symmetry is violated.
The SAS code needed to analyze Example 1 using the 'multivariate' approach is show next. This analysis used the data set ARTHR, which is in the correct format for multivariate analysis. Notice that the ODS statement is used to customize the output.

SAS Code for Example 1 –'Multivariate' Approach

```
ods exclude
    partialcorr
    errorsscp;

PROC GLM DATA = ARTHR;
CLASS VACGRP;
MODEL  MO1  MO2  MO3  = VACGRP /SS3;
REPEATED VISIT PROFILE /PRINTE SUMMARY;
TITLE3 'Multivariate Approach';
QUIT;
RUN;
```

The MODEL statement is specified using the responses at each time point (MO1, MO2, MO3) as dependent variables (i.e. included before the equal sign(=)) The REPEATED statement specifies that these dependent invariables are repeated observations and provides SAS with a name for the repeated observations and provides SAS with a name for the repeated factor, in this case, VISIT. The PROFILE option is used to compare successive changes among groups. When the PRINTE option is specified in the REPEATED statement, the SAS output includes a sphericity test (indicated as Mauchly's Criterion) applied to a set of orthogonal components. Sphericity is an important assumption of a repeated-measures ANOVA. It is the condition where the variances of the differences between all possible pairs of within-subject conditions (i.e., levels of the independent variable) are equal.

OUTPUT 2-

MULTIVARIATE APPROACH

The GLM Procedure

Class Level Information		
Class	**Levels**	**Values**
VACGRP	2	ACT PBO

Dependent Variable: MO1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.12500000	0.12500000	0.04	0.8439
Error	6	17.75000000	2.95833333		
Corrected Total	7	17.87500000			

R-Square	Coeff Var	Root MSE	MO1 Mean
0.006993	26.98009	1.719981	6.375000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VACGRP	1	0.12500000	0.12500000	0.04	0.8439

Dependent Variable: MO2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1.12500000	1.12500000	0.69	0.4372
Error	6	9.75000000	1.62500000		
Corrected Total	7	10.87500000			

R-Square	Coeff Var	Root MSE	MO2 Mean
0.103448	40.79216	1.274755	3.125000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VACGRP	1	1.12500000	1.12500000	0.69	0.4372

Dependent Variable: MO3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	18.00000000	18.00000000	10.80	0.0167
Error	6	10.00000000	1.66666667		
Corrected Total	7	28.00000000			

R-Square	Coeff Var	Root MSE	MO3 Mean
0.642857	43.03315	1.290994	3.000000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VACGRP	1	18.00000000	18.00000000	10.80	0.0167

The GLM Procedure
Repeated Measures Analysis of Variance

Repeated Measures Level Information			
Dependent Variable	MO1	MO2	MO3
Level of VISIT	1	2	3

Sphericity Tests				
Variables	DF	Mauchly's Criterion	Chi-Square	Pr > ChiSq
Transformed Variates	2	0.8962875	0.5474703	0.7605
Orthogonal Components	2	0.9547758	0.2313939	0.8907

MANOVA Test Criteria and Exact F Statistics for the Hypothesis of no VISIT Effect
H = Type III SSCP Matrix for VISIT
E = Error SSCP Matrix

S=1 M=0 N=1.5

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.10207029	21.99	2	5	0.0033
Pillai's Trace	0.89792971	21.99	2	5	0.0033
Hotelling-Lawley Trace	8.79716981	21.99	2	5	0.0033
Roy's Greatest Root	8.79716981	21.99	2	5	0.0033

MANOVA Test Criteria and Exact F Statistics for the Hypothesis of no VISIT*VACGRP Effect
H = Type III SSCP Matrix for VISIT*VACGRP
E = Error SSCP Matrix

S=1 M=0 N=1.5

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.44444444	3.12	2	5	0.1317
Pillai's Trace	0.55555556	3.12	2	5	0.1317
Hotelling-Lawley Trace	1.25000000	3.12	2	5	0.1317
Roy's Greatest Root	1.25000000	3.12	2	5	0.1317

Tests of Hypotheses for Between Subjects Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VACGRP	1	10.66666667	10.66666667	2.53	0.1631
Error	6	25.33333333	4.22222222		

Univariate Tests of Hypotheses for Within Subject Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F	Adj Pr > F	
						G - G	H-F-L
VISIT	2	58.58333333	29.29166667	28.89	<.0001	<.0001	<.0001
VISIT*VACGRP	2	8.58333333	4.29166667	4.23	0.0406	0.0433	0.0406
Error(VISIT)	12	12.16666667	1.01388889				

Greenhouse-Geisser Epsilon	0.9567
Huynh-Feldt-Lecoutre Epsilon	1.3941

Analysis of Variance of Contrast Variables
VISIT_N represents the nth successive difference in VISIT

Contrast Variable: VISIT_1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean	1	84.50000000	84.50000000	46.09	0.0005
VACGRP	1	0.50000000	0.50000000	0.27	0.6202
Error	6	11.00000000	1.83333333		

Contrast Variable: VISIT_2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean	1	0.12500000	0.12500000	0.07	0.8005
VACGRP	1	10.12500000	10.12500000	5.65	0.0550
Error	6	10.75000000	1.79166667		

The SAS output includes a sphericity test (indicated as Mauchly's Criterion) applied to a set of orthogonal components. For this example, the sphericity tests are not significant. The 'multivariate' approach is known to produce overly conservative results when the compound symmetry assumption is satisfied. 'multivariate' tests shown as Wilks, Pillai, Hoteling, and Roy (see output 2) with p-value of 0.0033 for VISIT*VACGRP interaction indicates that response profiles differ between groups. The output confirms this, as both the VISIT and the VISIT*VACGRP effects have less significance (i.e. greater p-values) than obtained by the 'univariate' approach, namely 0.0033 vs. 0.0001 for VISIT and 0.1317 vs. 0.0406 for VISIT*VACGRP. In fact, the interaction depicted by a plot of the means (shown in Figure 2) goes undetected when the 'multivariate' approach is used.

FIGURE-2 Response Profiles (Example 1)
Four multivariate methods due to Wilks, Pillai, Hoteling, and Roy (see output 2) are used to obtain the F-test statistic for these within-patient effects. Notice that the test for the group effect (VACGRP) is the same as in the 'univariate' analysis.

This example illustrates the method by which the 'multivariate' approach can be conducted, although it is not the best approach for analyzing the data in this case because of the small sample sizes and consistency with compound symmetry. Output 2 shows how the 'multivariate' approach provides a series of one-way ANOVA's at each time point (dependent variables MO1, MO2 and MO3), and how changes across time points can be compared. A simple execution of the ODS statement is SAS to customize the output is also shown.
Additional factors can also be used in the repeated measures analysis.

MISSING DATA

Analyses of repeated measures using the general linear model (GLM) in SAS can be disconcerting in the presence of missing data. If the 'multivariate' approach is used, only 1 missing value from each of several subjects can lead to the exclusion of large amounts of data, not only lowering the power of the statistical tests, but also removing information from the analysis that could be valuable in interpreting the trends of the data.

PROC MIXED in SAS handles missing values more efficiently than PROC GLM, allowing the inclusion of subjects with missing data. In addition, the assumption of compound symmetry is not required with PROC MIXED. In fact, the analyst can specify a model that uses the most appropriate correlation patterns among pairs of measurements across time.

By using PROC MIXED, which more efficiently handles missing values. The SAS code and output follow.
A new data set called UNIALZ, the PROC MIXED uses the CLASS and MODEL, statements similar to PROC GLM. The main difference is that you can specify a covariance structure rather than being bound by the assumption of compound symmetry. A vast array of different user-select covariance structures is available is SAS.
Initially, the 'unstructured' covariance model, which allows SAS to estimate a working covariance matrix from the estimated correlations among study visits, is used. The TYPE =option in the REPEATED statement specifies UN (for unstructured). Notice that, in PROC MIXED the PAT(TREAT) effect is specified in the SUBJECT =option in the REPEATED statement rather than in the MODEL statement.

Example 2-

```
DATA ALZHMRS;
    INPUT TREAT $ PAT ADAS02 ADAS04 ADAS06 ADAS08 ADAS10 ADAS12;
    DATALINES;
L  1  22  30   .  33  28  30
L  5  34  35  46  37  31  35
L  8  40  41  41  46  52  48
L 12  24   .  21  28  30  27
L 13  29  26  29  26   .  36
L 15  31  36  41  46  52  57
L 19  22  27  28  24  27  28
L 21  43  49  42  48  48  46
L 24  18  28  29   .  25  28
L 28  25  24  27  18  21  22
L 31  37  35  35  38  42   .
L 34  24  27  28  24  27  25
L 37  45  50  58  59  60  58
L 40  33  32  35  30  31  35
L 44  34  37  43  44  39  38
L 47  25  27  29  28  31   .
L 51  30   .  36  32  34  38
L 54  23   .  33  28  32  32
L 57  35  37  39  38  41  43
L 59  44  48  48  45  50  52
L 63  28  30  32  31  35  32
L 67  24  22  23  24  27  30
L 68   .  49  51  48  55  54
L 73  26  28  30  27  30  33
L 76  30  32  35  35  36  38
L 78  40  42  44  43  45  46
H  2  31  36  35  31  31  31
H  6  24  27  28  21  27  26
H  7  31  31  39  37  41   .
H 14  45  48  46  52  48  42
```

```
H 17  24  28  26  23  24  29
H 20  21  32  39  36  33  30
H 22  32  34  45  42  37  32
H 25  18  22  26  26  27  24
H 27  51  47   .  43  43  43
H 33  20  22  29  24  29  30
H 38  41  34  37  29  35  33
H 42  24  35  39  32  24   .
H 45  23   .  33  36  33  30
H 50  25  28  25  28  28  30
H 52  31  34   .  33  34  35
H 56  27  31  26  33  33  34
H 60  37  43  39  42  43  36
H 62  41  42  51  45  46  51
H 66  35  33  34  35  36  41
H 69  30  31  27  34  33  36
H 72  54  60  55  58   .  65
H 75  35  37  39  41  39  44
H 79  18  21  19  19  20  27
H 80  40  35  33  39  38  41
P  3  31  36  37  41  39  44
P  4  20  26  32  35  25  29
P  9  33  33  29  33  39  41
P 10  35  39  40  38  40  38
P 11  26  24  31  42  50   .
P 16  44  48  44  37  36  47
P 18  25  31  21  27  41  32
P 23  28  34  26  26  36  35
P 26  27   .  28  35  40   .
P 29  20  30  30  27  33  29
P 30  49   .  43  48  44  53
P 32  26  29  31  30  35  38
P 35  30  33  41   .  41  44
P 36  31  34  44  44  50  56
P 39  42  46  36  43  48  48
P 41  31  30  31   .  41  38
P 43  27  22  36  45  54  60
P 46  24  37  41  31  36  44
P 48  33  31  38  41  31   .
P 49  27  30  36  36  32  33
P 53  35  34  45  44  38  40
P 55  39  40  38  44  43  44
P 58  32  34  40  45  36  38
P 61  45  50   .  54  50  53
P 64  21  23  31  34  27  27
P 65  26  30  37  37  30  32
P 70  53  50  55  57   .   .
P 71  32  34  27  30  36  35
P 74   .  50  52  56  52  54
P 77  24  32  31  37  35  30
;
RUN;

 PROC SORT DATA =ALZHMRS ;
  BY  TREAT PAT ADAS02 ADAS04 ADAS06 ADAS08 ADAS10 ADAS12;
 RUN;

 PROC TRANSPOSE DATA = ALZHMRS OUT=TRA;
   BY  TREAT PAT ;
   VAR ADAS02 ADAS04 ADAS06 ADAS08 ADAS10 ADAS12;
 RUN;
```

```
 DATA UNIALZ;
   SET TRA;
   ADASCOG=COL1;
   MONTH=INPUT(SUBSTR(_NAME_,5,2),BEST12.);
   RUN;

ODS SELECT MIXED.RCORR
           MIXED.DIMENSIONS
           MIXED.FITSTATISTICS
           MIXED.TESTS3;

PROC MIXED DATA = UNIALZ;
    CLASS TREAT MONTH PAT;
    MODEL ADASCOG = TREAT MONTH TREAT*MONTH;
    REPEATED / TYPE=UN SUBJECT=PAT(TREAT) RCORR;
    TITLE4 'PROC MIXED USING UNSTRUCTURED COVARIANCE (UN)';
RUN;
```

Output-3

PROC MIXED USING UNSTRUCTURED COVARIANCE (UN)

The Mixed Procedure

Dimensions	
Covariance Parameters	21
Columns in X	28
Columns in Z	0
Subjects	80
Max Obs per Subject	6

Estimated R Correlation Matrix for PAT(TREAT) 2 H						
Row	Col1	Col2	Col3	Col4	Col5	Col6
1	1.0000	0.9005	0.7703	0.8002	0.7763	0.7773
2	0.9005	1.0000	0.8179	0.7995	0.7521	0.7261
3	0.7703	0.8179	1.0000	0.8738	0.7223	0.7418
4	0.8002	0.7995	0.8738	1.0000	0.8628	0.8273
5	0.7763	0.7521	0.7223	0.8628	1.0000	0.9206
6	0.7773	0.7261	0.7418	0.8273	0.9206	1.0000

Fit Statistics	
-2 Res Log Likelihood	2649.9
AIC (Smaller is Better)	2691.9
AICC (Smaller is Better)	2694.1
BIC (Smaller is Better)	2741.9

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
TREAT	2	77	0.94	0.3967
MONTH	5	77	21.55	<.0001
TREAT*MONTH	10	77	2.08	0.0357

Output 3 shows a significant (p= 0.0357) Treatment-by-Month interaction using the unstructured covariance. The section entitled Fit Statistics provides an indication of relative goodness of fit, smaller values suggesting a better fit. You can specify other covariance structures by using the same MODEL statement in PROC MIXED and compare the fit statistics among them. To illustrate this, re-run the analysis for autoregressive (AR(1)), Toeplitz(TOEP), first order autoregressive moving average (ARMA(1,1)), and compound symmetric (CS) covariance structures by replacing the REPEATED statement with the following. When there are no missing values, PROC MIXED, using the compound symmetric covariance structure (TYPE=CS), produces the same results as PROC GLM in the 'univariate' approach. The covariance structure specified in PROC MIXED will model the variance assumptions at different time points and the patterns of correlations among the time points. The unstructured approach (TYPE=UN) makes no assumption at all about the relationship in the correlations among visits. Measurements taken closer together in time, however, often tend to be more highly correlated those measurements taken over lengthily periods of time.

As seen previously, the compound symmetric structure (TYPE=CS) assumes the same correlation, say ρ $(0< \rho<1)$, between each pair of time points.

In the first-order autoregressive structure (TYPE =AR(1)), measurements taken at adjacent time points (e.g. consecutive visits) have the same correlation such as ρ. The correlation of ρ^2 is assigned to measurements that are 2 visits apart; ρ^3, to measurements that are 3 visits apart, etc.

The autoregressive moving average (TYPE =ARMA(1,1)) is similar, except the entries that involve powers of ρ are multiplied by a constant, γ $(0< \gamma<1)$.

The Toeplitz structure (TYPE=TOEP) is more general. It assigns a correlation of ρ_1 to measurements taken from consecutive visits; a different correlation, ρ_2, two measurements that are taken 2 visits apart; ρ_3, to measurements that are taken 3 visits apart, etc. Each of these (expect the unstructured) assumes equal variances at all measurements times.

SAS code for Example 3 (PROC MIXED) (continued)

```
...
REPEATED / TYPE=AR(1) SUBJECT=PAT(TREAT);
TITLE4 'PROC MIXED Using First-Order Auto-Regressive Covariance(AR(1))';

...
REPEATED / TYPE=ARMA(1,1) SUBJECT=PAT(TREAT);
TITLE4 'PROC MIXED Using First-Order Auto-Regressive Moving Average Covariance(ARMA(1,1))';

...
REPEATED / TYPE=TOEP SUBJECT=PAT(TREAT);
TITLE4 'PROC MIXED Using Toeplitz Covariance (TOEP)';
...
REPEATED / TYPE=CS SUBJECT=PAT(TREAT);
TITLE4 'PROC MIXED Using Compound Symmetric Covariance(CS)';
```

Output-4

PROC MIXED USING First-Order Auto-Regressive COVARIANCE (AR(1))

Fit Statistics	
-2 Res Log Likelihood	2698.2
AIC (Smaller is Better)	2702.2
AICC (Smaller is Better)	2702.2
BIC (Smaller is Better)	2706.9

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
TREAT	2	77	0.94	0.3952
MONTH	5	359	11.21	<.0001
TREAT*MONTH	10	359	1.43	0.1671

PROC MIXED USING Auto-Regressive Moving Average Covariance ARMA(1,1)

Fit Statistics	
-2 Res Log Likelihood	2691.2
AIC (Smaller is Better)	2697.2
AICC (Smaller is Better)	2697.2
BIC (Smaller is Better)	2704.3

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
TREAT	2	77	0.90	0.4116
MONTH	5	359	14.02	<.0001
TREAT*MONTH	10	359	1.47	0.1478

PROC MIXED USING Toeplitz COVARIANCE (TOEP)

Fit Statistics	
-2 Res Log Likelihood	2671.1
AIC (Smaller is Better)	2683.1
AICC (Smaller is Better)	2683.3
BIC (Smaller is Better)	2697.4

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
TREAT	2	77	0.91	0.4077
MONTH	5	359	19.65	<.0001
TREAT*MONTH	10	359	2.08	0.0266

PROC MIXED USING Compound Symmetric Covariance (CS)

Fit Statistics	
-2 Res Log Likelihood	2743.5
AIC (Smaller is Better)	2747.5
AICC (Smaller is Better)	2747.5
BIC (Smaller is Better)	2752.2

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
TREAT	2	77	0.88	0.4172
MONTH	5	359	26.69	<.0001
TREAT*MONTH	10	359	2.30	0.0125

AIC stands for “Akaike’s Information Criterion”, which measures model fit. Based on Output 4, values for the AIC are consistent with the other fit statistics (AICC, BIC, -2 Res Log Likelihood). Toeplitz provides the best fit parameters (smallest values). The unstructured covariance is a close second based on these measures, and it is the preferred method based on the -2 Res Log Likelihood fit statistic. Both of these covariance structures result in a significant Treatment-by-Month interaction. Compound symmetry shows the worst fit and, as previously discovered, is not a good assumption.

TABLE Summary of PROC MIXED Results for Example 3 for Various Correlation Structures

Covariance Structure	SAS Name	AIC	-2 Res Log Likelihood	p-value for TREAT*MONTH
Unstructured	UN	2691.9	2649.9	0.0357
Autoregressive	AR(1)	2702.2	2698.2	0.1671
Autoregressive MA	ARMA(1,1)	2697.2	2691.2	0.1478
Toeplitz	TOEP	2683.1	2671.1	0.0266
Compound Symmetric	CS	2747.5	2743.5	0.0125

GEE Analysis

The use of generalized estimating equations (GEE) is another method for analyzing repeated measures data sets that have missing values. PROC GENMOD, which is used for GEE analysis in SAS, requires the specification of a 'working' correlation structure (such as compound symmetric (CS) or unstructured (UN)), which represents the correlations among the repeated measurements. The GEE modelling methodology will usually produce good estimates of the model parameters even if the correlation structure is not specified. In this sense, a GEE analysis is more robust than using the generalized linear modelling methods of PROC GLM or PROC MIXED.

The SAS statements that follow this section illustrate how to conduct a GEE analysis of the data in Example 2 by using the autoregressive(TYPE = AR(1)) working correlation matrix. The DIST option in the MODEL statement specifies the assumption of normal distribution of the data (DIST = NORMAL). In this case, the DIST option is not required because the normal distribution is the default. The TYPE3 option in the MODEL statement requests tests (analogous to the PROC GLM SAS Type III tests) for each of the effects given in the MODEL statement. This approach also results in a significant Treatment-by-Month interaction (p=0.0164).

SAS Code for Example 2 (PROC GENMOD)

```
ODS SELECT
     Genmod.Type3;

PROC GENMOD DATA = UNIALZ;
     CLASS TREAT MONTH PAT;
       MODEL ADASCOG = TREAT MONTH TREAT*MONTH /
                                        DIST = NORMAL TYPE3;
              REPEATED SUBJECT = PAT / TYPE = AR(1);
      TITLE4 'GEE Analysis Using PROC GENMOD';
      TITLE5 'Autoregressive Correlation (AR(1)) Working Correlation';
RUN;
```

Output-

GEE Analysis Using PROC GENMOD
Autoregressive Correlation (AR(1)) Working Correlation

Score Statistics For Type 3 GEE Analysis			
Source	**DF**	**Chi-Square**	**Pr > ChiSq**
TREAT	2	2.02	0.3639
MONTH	5	46.14	<.0001
TREAT*MONTH	10	21.76	0.0164

```
REPEATED SUBJECT =PAT / TYPE =CS;
```

Output

GEE Analysis Using PROC GENMOD
compound symmetric (CS) Correlation structure

Score Statistics For Type 3 GEE Analysis			
Source	**DF**	**Chi-Square**	**Pr > ChiSq**
TREAT	2	1.96	0.3745
MONTH	5	45.41	<.0001
TREAT*MONTH	10	21.28	0.0192

```
REPEATED SUBJECT =PAT /
TYPE=USER(1.0  0.9  0.8  0.7  0.7    0.7
          0.9  1.0  0.9  0.8  0.7    0.7
          0.8  0.9  1.0  0.9  0.8    0.7
          0.7  0.8  0.9  1.0  0.9    0.8
          0.7  0.7  0.8  0.9  1.0    0.9
          0.7  0.7  0.7  0.8  0.9    1.0);
```

Output

GEE Analysis Using PROC GENMOD
user-defined Correlation structure (USER())

Score Statistics For Type 3 GEE Analysis			
Source	**DF**	**Chi-Square**	**Pr > ChiSq**
TREAT	2	2.05	0.3582
MONTH	5	45.39	<.0001
TREAT*MONTH	10	21.44	0.0182

CONCLUSION

PROC MIXED is capable of handling missing values and unbalanced cases for repeated measures analysis, but it assumes values are missing at random (MAR). Generalized estimating equations (GEE) require the more restrictive assumption that missing values are missing completely at random (MCAR). MAR and MCAR suggest that the missing data represent a random sample from all data that would have been available, and therefore, the results are not biased by their exclusion. Unfortunately, there is no way to test the assumption of MAR or MCAR.

With only about 5% of the data missing and no apparent patterns related to treatment or discontinuation, the missing value structure of Example 2 appears consistent with the MAR assumption. However, this is not usually the case in clinical studies. Because there is frequently missing data in clinical trials from early discontinuation of patients due to ineffectiveness or side effects, assumptions of MAR or MCAR are often violated in such data sets.

However, GEE analysis is well-suited for use with covariates, but only with large data sets. The results of GEE analysis might not be reliable for smaller data sets. Generally, if there are no more than 3 or 4 explanatory variables (including 'Treatment Group'), a minimum sample size of 50 to 100 patient id desirable.

The GEE approach does not depend so heavily on the correct specification of the correlation structure. To illustrate this robustness, the significance of the interaction term of Example 2 is seen to be consistent for different correlation structures, as shown below for the exchangeable (or compound symmetric) (CS) working correlation structure and for a user-defined structure (USER()). The user-defined type is patterned after the estimated correlations shown in the SAS output for PROC MIXED where the correlations are high (e.g. 0.9) for adjacent time points then decrease and level off to 0.7 for time points farther apart. The interaction p-values of 0.0192 for the exchangeable and 0.0182 for the user-defined correlation structure are about the same as that obtained using the autoregressive structure (p=0.0164),

REFERENCES

SAS Institute Inc. 2011. SAS/STAT® 9.2 User's Guide. Cary, NC: SAS Institute Inc.

"Common Statistical Methods for Clinical Research with SAS Examples, Second Edition"- Glenn A. Walker

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DISCLAIMER

All views and opinions documented here are those of the author and do not necessarily represent the opinion, recommendation and practices of Syneos Health.

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