SciaNews

Covariates in 2x2 Cross-Over Trials

M. Gao, J. Wang and M. Schulz, SciAn Clinical

Summary

This article illustrates the use of analysis-of-covariance to explore the association between a covariate and the treatment effect in a 22 cross-over trial. The type I sum of squares is used in the analysis and the results are identical to the 2 separate analyses of covariance presented by Jones and Kenward (1989). An illustration of the analyses based on the example used by Jones and Kenward is presented. Based on the expected mean squares, the appropriateness of the hypothesis tests on the main and interaction effects is discussed.

1 Introduction

The cross-over design has been widely used in clinical trials. The simplest cross-over design is the 22 design. In this design each subject receives two different treatments, say A and B, in two different treatment periods. The order in which the treatments are received in a subject depends on his/her sequence group assignment. Each subject is randomly assigned to one of two sequence groups. Patients randomized to one sequence group receive treatment A first and then cross over to treatment B, while patients assigned to the other sequence group receive the treatments in the reverse order. The design may include a wash-out period between the treatment periods to eliminate any carry-over effects.

In a 22 cross-over trial, the typical effects that are estimated are the treatment, period and sequence group effects. However, the last effect is aliased with the carry-over effect and treatment-by-period interaction (i.e. these 3 effects cannot be estimated independently). The aliasing is due to the fact that the design yields only four means (a mean response for each period and sequence group) which allow the estimation of three parameters, in addition to the overall grand mean.

Since some effects are already aliased in a 22 design, one might question if the design allows us to detect a relationship between the treatment effect and a covariate (either continuous, such as age, or categorical, such as gender) in the event that such an association is suspected at the analysis stage. In fact, if one incorporates additionally a covariate term and a covariate-by-treatment interaction term in the 22 model and perform an ANOVA using SAS (based on type III sum of squares), the resulting ANOVA table would give zero degree of freedom for the covariate term. This suggests that the model is over-parameterized.

In their book "*Design and Analysis of Cross-Over Trials*", Jones and Kenward (1989) presented an analysis approach to test if a treatment effect is related to a covariate in a 22 design. The approach consists of two separate analyses-of-covariance. The objectives of this article are to present an alternative *single* analysis which is equivalent to the separate analyses-of-covariance and to discuss the interpretation of the results. SAS codes will be provided for the analyses and an illustration based on the example used by Jones and Kenward will be given in section 3.

2 Methods

Suppose that a linear association between the treatment effect and a continuous covariate is suspected after the collection of data from a 22 cross-over trial. Generally, a test of this association would involve testing if a covariate-by-treatment interaction term in the model is equal to zero (i.e. testing if the slope of the linear relationship between response and covariate under treatment A is different from that under treatment B).

Jones and Kenward presented the following analysis which incorporates covariate-related terms in the models. The approach consists of two separate analyses-of-covariance, with one performed on the subject totals (i.e. summing the responses over 2 periods in each subject) and the other on the within-subject differences (i.e. period 1 response minus period 2 response in each subject). For illustration, the SAS codes for the analyses using the procedure PROC GLM are as follows:

```
PROC GLM;
CLASS GROUP;
MODEL TOTAL = COVARIAT|GROUP;
RUN;
PROC GLM;
CLASS GROUP;
MODEL DIFF = COVARIAT|GROUP;
RUN;
```

where TOTAL, DIFF, COVARIAT and GROUP denote the subject totals, within-subject differences, covariate and sequence group, respectively.

The analysis with the subject totals as the responses provides a partition of the between-subject sum-of-squares contributed from the covariate, the sequence group and the covariate-by-sequence group interaction. The model terms in the SAS codes with the within-subject differences as the responses are identical to those in the analysis of the subject totals. However, the meanings of the terms are quite different. In the analysis of the differences, the covariate, group and covariate-by-group interaction terms actually correspond to the covariate-by-period interaction, treatment effect, and covariate-by-treatment interaction, respectively. Hence, for testing an association between the treatment effect and a covariate, the appropriate term is the covariate-by-group interaction (corresponding to the covariate-by-treatment interaction) in the analysis of the differences. In the above analyses of covariance, the type I sum of squares of the SAS output are used.

As an alternative to the two separate analyses-of-covariance, we introduce a single analysis-of-covariance performed on the observed responses. The approach is equivalent to the separate analyses for testing the hypotheses of interest, and it can be implemented using the following SAS codes:

```
PROC GLM;
CLASS SUBJECT PERIOD TREAT;
MODEL RESPONSE=COVARIAT GROUP COVARIATGROUP SUBJECT(GROUP)
PERIOD COVARIATPERIOD TREAT COVARIATTREAT/E1;
RANDOM SUBJECT(GROUP)/TEST;
RUN;
```

where RESPONSE is the treatment outcome, COVARIAT, GROUP, SUBJECT, PERIOD and TREAT denote, respectively, the covariate, sequence group, study subject, period and treatment. The term of interest for the testing of a covariate-dependent treatment effect is the covariate-by-treatment interaction. The results of the above analysis correspond to those of the separate analyses of covariance. The correspondence between the single analysis and the separate analyses are shown in detail in section 3. The above MODEL can be simplified by taking out the

COVARIAT*GROUP and the COVARIATPERIOD terms if the covariate-by-sequence group and covariate-by-period interactions are assumed to be absent.

Note that in the MODEL statement of the above SAS codes, the option E1 is specified to choose the type I sum of squares for the analysis. The type II and type III sum of squares are not estimable for all the effects in the above model. The problems of using the type I sum of squares in the analysis can be shown based on the expected mean squares.

Expected Mean Squares (Type I) in the Single Analysis of Covariance

Source	d.f.	Expected MS (type I)
Between subjects:		
Covariate	1	$w2 + 2b2 + Q(C, S, CS, CP, CT)$
Sequence group	1	$w2 + 2b2 + Q(S, CS)$
Covariate Sequence group	1	$w2 + 2b2 + Q(CS)$
Between-subject residuals	$(n1 + n2 - 4)$	$w2 + 2b2$
Within subjects:		
Period	1	$w2 + Q(P, CP, T, CT)$
Covariate Period	1	$w2 + Q(CP, T, CT)$
Treatment	1	$w2 + Q(T, CT)$
Covariate Treatment	1	$w2 + Q(CT)$
Within-subject residuals	$(n1 + n2 - 4)$	$w2$

In the above table, $w2$ and $b2$ denote the variances of the within-subject residuals and of the between-subject residuals, respectively, and $Q()$ refers to a quadratic form involving the specified effects. The letters C, S, P, and T in the quadratic forms represent the covariate, sequence group, period, and treatment effects, respectively, and n1 and n2 denote the sample sizes in the 2 sequence groups.

The expected mean squares serve as a guide for the construction of appropriate significance tests. The quadratic forms for the covariate sequence group and covariate treatment terms contain the single interaction effects. Hence, the tests on these interaction terms based on the type I sum of squares are uncontaminated by other effects. On the other hand, all other effects in the model cannot be tested independently based on the type I sum of squares due to the entangled effects in their quadratic forms. For instance, the expected mean square for the treatment term contains a quadratic form involving both the treatment effect and covariate-by-treatment interaction. Thus a test on the treatment term based on the type I sum of squares is not a test on the treatment effect alone. For an appropriate interpretation of the analysis results, it is very important to recognize any entangled effects in the quadratic forms, and consequently, to assess the appropriateness of a test of interest.

In the situation where the analysis of covariance reveals a significant covariate-by-treatment interaction, a display of the treatment effects at different values of the covariate would be helpful to enhance understanding of this interaction. Recall that a test of the covariate-by-treatment interaction is a test if the difference in the slopes (response versus covariate) between the two treatments is equal to zero. In the single analysis of covariance, it can be shown that the estimated slope difference is equal to the averaged slope under one treatment minus that under the other treatment (the estimated slope difference can be obtained from the SAS output by specifying the option, SOLUTION, in the MODEL statement). To create the plot, one obtains the slope and intercept from the regression of the response versus covariate for each treatment and period combination (i.e. Treatment A and Period 1, Treatment A and period 2, Treatment B and Period 1, and Treatment B and period 2). The estimated slopes and intercepts are then averaged

for each treatment. The averaged slopes and intercepts are used to plot two straight lines, one for each treatment.

When a significant covariate-by-treatment interaction exists, the graph is expected to show two non-parallel lines. The treatment effects (difference between the two lines) can be examined at different values of the covariate. If the two lines are not crossed, one may choose to report an overall treatment effect (which can be estimated via the SOLUTION option in the MODEL statement and is equivalent to the difference between the averaged intercepts). However, if the two lines are crossed, an overall treatment effect would not be meaningful.

3 Example

A 22 cross-over trial for investigating the relationship between plasma oestradiol levels and visuo-spatial ability in women undergoing in-vitro fertilization (IVF) was used as an example by Jones and Kenward (1989). The response, a measure of visuo-spatial ability, is the time taken to complete a task assessed via the embedded figures test (EFT). For each subject, visuo-spatial ability was assessed in both the proliferative phase and at the end of ovarian hyperstimulation. These two conditions, corresponding to low and high oestradiol concentrations respectively, define the two 'treatments' in the trial. Subjects were randomly assigned to two sequence groups, with one group undergoing the first-period test in the proliferative phase and the other group undergoing the first-period test at the end of ovarian hyperstimulation. Due to the skewed distribution, the log transformed EFT score was used as the response in the analysis. As there is a known strong association between IQ and the EFT score, the IQ score obtained at the time of the first-period test was introduced as a covariate. The inclusion of IQ as a covariate allows the investigation of a possible association between IQ and the effect of condition (treatment) on EFT score.

Jones and Kenward analyzed the data from the above example using two separate analyses-of-covariance, with one on the subject totals and the other on the within-subject differences. The results are presented in Tables 1 and 2.

Table 1. Analysis of covariance of the subject totals

Source	d.f.	SS	MS	F
Covariate (IQ)	1	21.030	21.030	14.68
Group	1	2.982	2.982	2.08
Covariate (IQ) Group	1	2.463	2.463	1.72
Residual	19	27.214	1.432	

A large F-ratio for the covariate (IQ) in the analysis of the subject totals suggests a strong association between the covariate (IQ) and the response (EFT score).

Table 2. Analysis of covariance of the within-subject differences (period 1 - period 2)

Source	d.f.	SS	MS	F
Correction factor (Period)	1	6.775	6.775	24.11
Covariate (IQ Period)	1	0.072	0.072	0.26
Group (Treatment)	1	2.434	2.434	8.65
Covariate Group (IQ Treatment)	1	0.654	0.654	2.32

Residual	19	5.344	0.281	

In the above table, the model terms corresponding to the single analysis are given in parentheses. Also note that a 'correction factor' is included, which is the difference between the uncorrected and corrected total sums of squares. The correction factor corresponds to the period main effect.

Based on the analysis of the differences, Jones and Kenward claimed that no effects associated with IQ are found, but there are large period and treatment effects. However, the tests on the treatment and period terms, as shown previously in the table of expected mean squares (section 2), involve not only treatment and period single effects but also other effects. Therefore these tests are not appropriate tests on the treatment and period effects unless the other effects involved in the corresponding quadratic forms are negligible.

The data in this example are subjected to the single analysis-of-covariance proposed in this article. The results are shown in Table 3.

Table 3. Analysis of Covariance of the Full Model

Source	d.f.	SS	MS	F	p
Between subjects:					
Covariate (IQ)	1	10.52	10.52	14.69	0.001
Group	1	1.49	1.49	2.09	0.165
Covariate(IQ) Group	1	1.22	1.22	1.71	0.207
Between-subject residuals	19	13.61	0.72		
Within subjects:					
Period	1	3.39	3.39	24.09	0.000
Covariate (IQ) Period	1	0.04	0.04	0.26	0.616
Treatment	1	1.22	1.22	8.66	0.008
Covariate (IQ) Treatment	1	0.32	0.32	2.31	0.145
Within-subject residuals	19	2.67	0.14		

By comparing the results of Table 3 with those in Tables 1 and 2, it can be seen that the sums of squares in the single analysis are halved while the corresponding degrees of freedom (d.f.) and *F*-ratios are the same as the separate analyses.

As indicated by the expected mean squares (section 2), Table 3 provides the right test for the covariate-by-treatment interaction. The results for the example show no evidence of the presence of the interaction.

4 Discussion

This paper illustrates the use of analysis-of-covariance to explore the association between a covariate and the treatment effect if a relationship is suspected after the collection of data from a 22 cross-over trial. The single analysis-of-covariance uses the type I sum of squares and is analogous to the 2 separate analyses of covariance given by Jones and Kenward (1989). The example in this paper uses a continuous covariate, which may be a baseline measurement of the response variable. In fact, the approach can be applied to a categorical covariate also.

If the analysis of covariance reveals a significant covariate-by-treatment interaction, the interpretation of the treatment main effect requires particular caution. In their analysis of the

example, Jones and Kenward claimed 'large' period and treatment effects based on the F-ratios calculated using type I sums of squares. This interpretation can be misleading. According to the expected mean squares, the test for the treatment effect is appropriate only when the covariate-by-treatment interaction is absent. For a correct interpretation of the analysis results, it is very important to recognize any entangled effects in the quadratic forms, and consequently, to assess the appropriateness of a test of interest.

In the presence of a covariate-by-treatment interaction, a graphical display of the treatment effects at different values of the covariate are proposed . The graph is expected to show two non-parallel lines. A summary of an overall treatment effect may not be precluded if the two lines are not crossed. However, if the two lines are crossed, an overall treatment effect would not be meaningful.

In the case of a non-significant covariate-by-treatment interaction, the model may be reduced to the usual model for a 22 cross-over design (without any covariate terms) and the data analyzed using the type III sum of squares.

References

Jones, B. and Kenward, M.G., *Design and Analysis of Cross-Over Trials.* Chapman and Hall, London, New York, 1989.

SAS Institute Inc., *SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 1 & 2,* Cary, NC: SAS Institute Inc., 1989.