

## 1 Introduction

In analysis of covariance we wish to test equality of group means, after first adjusting for the effect of a continuous covariate. In our first (exercise) example, we see that the raw  $y = \text{change}$  values look quite different between groups. However,  $y$  is linearly related to  $x = \text{the age of the subject}$ , so that  $y_{ij} = \beta_0 + \beta_1 x_{ij} + \varepsilon_{ij}$ . In the example the group means  $\bar{x}_1$ , and  $\bar{x}_2$  were not equal, and this inequality of the  $x$  values falsely led to a larger group difference for  $y$ . Even if the age means were equal for the two groups, variability among the  $x$  values can also obscure the true group difference for  $y$  by creating large within-group variation. Thus the  $x$  variable is a nuisance variable, and we wish to adjust for it as we test hypotheses about the  $y$  variable. This approach is quite similar to using a blocking factor in a randomized block design to remove nuisance variation.

## 2 Two assumptions

To perform a standard ANCOVA, we will make two assumptions. The first of these assumptions we can check with a statistical test discussed below. The first assumption is that of parallelism. We assume that the slope  $\beta_1$  is equal for each group. This assumption allows us to make a global conclusion about the treatment that is applicable for all values of  $x$ . If this assumption does not hold we then have to qualify any statements about the treatment, because it can change as  $x$  changes. A test for the parallelism assumption is discussed below. The second assumption is that the covariate  $x$  is unaffected by the treatment. As an example if we are comparing the weight of two species of mice at 1 year of age, we may consider using their weights at 6 months as a covariate. The problem is that if weight differs at 1 year by species, it will also likely differ at 6 months as well. If we adjust for weight at 6 months, we will probably remove the true difference due to species. This assumption must be considered each time ANCOVA is used.

## 3 ANCOVA assuming parallelism

In the most common ANCOVA application, we assume a linear relationship between  $x$  and  $y$ . If the slope ( $\beta$ ) is equal in the groups then the regression lines are parallel, and the test for equality of group means is equivalent to equality of the intercepts among groups. If we have a completely randomized design for the treatment, using  $\beta$  as the slope the model is:

$$y_{ij} = \mu_i + \beta(x_{ij} - \bar{x}_{..}) + e_{ij}.$$

The test is conducted in Proc GLM or Proc MIXED by adding the covariate to the MODEL statement, but not to the CLASS statement. See our SAS example for an illustration.

## 4 Testing the parallelism assumption

Complications arise if the regression lines for the separate groups are not all parallel. When parallelism fails, then the comparison of  $y$  values depends on the  $x$  values, as the  $y$  comparison will change with  $x$ . We can test this assumption in Proc GLM by introducing a new crossproduct term to the model, as shown in the example SAS code.

## 5 Comparison of means

If we have more than 2 groups, if the parallelism assumption is satisfied and we have rejected the global null hypothesis of equality of means, we may then want to do additional tests such as pairwise tests of means. Multiple comparison tests should be based on the adjusted group means, in which the mean covariate value  $\bar{x}_{..}$  is used to compare groups. Then we have

$$\bar{y}_{i(adj)} = \bar{y}_{i\cdot} - \hat{\beta}(\bar{x}_{i\cdot} - \bar{x}_{..}) ,$$

where  $\bar{x}_{..}$  is the mean of  $x_{ij}$  for the entire data set. The LSMEANS statement automatically calculates adjusted means in a model with covariates.

## 6 Final remarks

- 1) We have discussed ANCOVA for the completely randomized design, but it can be used with other ANOVA designs as well.
- 2) We have required that the relationship between  $y$  and  $x$  be linear, but ANCOVA can be used with nonlinear patterns as well. In these cases it remains important to have parallelism.
- 3) It is preferred that the ranges of the  $x$  values overlap between different treatment groups. If they do not overlap at all, then the ANCOVA test is extrapolating beyond observed  $x$  values, which may not be justified.
- 4) Comparing ANCOVA to blocking: If  $y$  and  $x$  are linearly related then ANCOVA is more efficient than using a randomized block design, if not then a randomized block design may be better.
- 5) ANCOVA versus differencing: Differencing (analyzing  $y - x$ ) is a special case of ANCOVA in which  $\beta = 1$ .
- 6) For heterogeneous slopes, an article by Hendrix *et al.* (*Biometrics*, 1982, pp 641-650) discusses some approaches to data analysis. Also see our example SAS code for an unequal slopes situation.