

Common Statistical Methods for Clinical Research with SAS® Examples

Third Edition

Glenn A. Walker
Jack Shostak

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**Glenn A. Walker
Jack Shostak**

**THE
POWER
TO KNOW®**

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PREFACE TO THE THIRD EDITION

Aristotle, one of mankind's greatest thinkers, rooted his basis of human understanding in the notion that universal phenomena can be explained through perception and rationalization. As such, Aristotle conjectured that a 10-pound object should fall through the air 10 times faster than a similar 1-pound object. This, and other intuitive suppositions of his based on logic, did not stand up to the scientific method.

We now know that understanding natural phenomena requires more than observation and reasoning. Nearly every behavior that exists is subject to variation and fluctuation due to a vast array of influencing forces, many unknown or unrecognized. This variation is further compounded by less than perfect human perception, measurement error, human bias, or just everyday mistakes. The scientific method, traced back to Francis Bacon in the late 16th century, is designed to maximize one's certainty in the face of the chaos through which one must wade to get to an ultimate truth, and it has become the time-tested gold standard by which new entries are added to the human knowledgebase. Even so, the scientific method has itself evolved with its demands for improved signal detectors that better filter out the noise, and in the past 30 years, the role of mathematics and statistics has become recognized as one of the most important vehicles toward that end. Statistics has become like the hammer in a scientist's toolbox—one of the indispensable tools—especially in the pursuit of new drugs and treatments for human ailments. We present this wholly new updated book in this spirit of scientific investigation and as one additional contribution to complement the many educational resources available to practicing scientists.

Since its first publication in 1996, this book has undergone numerous updates and improvements even as mathematical approaches to statistical analysis have evolved. This new edition reflects some of those evolutionary changes, which include the apparent progression of mathematical statistics toward a unified modeling theory whose applications are made possible by modern high speed computing technology, and are manifested in SAS procedures like PROC MIXED, PROC GENMOD, and PROC GLIMMIX. Today, the applied statistician has the resources to analyze complex statistical experiments—those from mixed or non-linear models, those whose observations have complex correlation structures, are plagued with missing values, or come from non-traditional normal distributions, those requiring models with various parameterizations or differing link functions—all with just a few lines of SAS code, a feat that may have seemed like science fiction back in 1996.

Still, it's difficult for fledgling practitioners to pick up the new techniques without some overview of the traditional methods that led to them. We continue to include those here, and even though theoretical details, for the most part, are necessarily omitted in favor of the practical aspects of everyday applications, you will find some of the basics that motivate the methodology and more detailed discussions of how to apply them. The powerful ODS Graphics features built into most SAS procedures beginning with SAS 9.2 enable a wide array of graphics plots with minimal effort, and we now illustrate many of the figures using this new SAS resource. All of the code has been updated to SAS 9.2,

and PROC MIXED is now used as one of the main modeling tools, replacing GLM in many instances. Overall, you should find more complete explanations of some of the most widely used methodology for the analysis of clinical trials. Also, we've tried to build these updates and enhancements into our presentation without appreciably altering the original structure of the book.

That structure is marked by a discussion of each of the statistical methods most commonly used in clinical research, one method per chapter, each subdivided into sections that provide (i) an overview, (ii) a basic description of the method, (iii) examples, and (iv) numerous details and extensions of the methodology. We continue to believe this type of presentation not only makes it easy for a beginning student to follow as progressively more complex statistical methods are introduced, but that it also provides an easy-to-use reference for those seeking guidance in a specific area. The material is targeted toward anyone whose experience includes basic statistics and SAS programming, while much of it can be absorbed by clinical researchers in other disciplines with no previous exposure to either statistics or SAS. Additionally, many details are included that can benefit experienced statisticians as well.

This book is designed to have particular appeal to those involved in clinical research, biometrics, epidemiology, and other health- or medical-related research applications. Medical students might find the overview approach to statistical application without the theoretical details particularly helpful. Although SAS has been chosen as the primary tool for data analysis due to its widespread use in the pharmaceutical industry, it is not the main focus of this book, and previous SAS experience is not necessary as the SAS coding is kept at a rudimentary level. However, such a book would be impossible without SAS. The examples, which include complete data sets, design layouts, and the complete SAS code, should help readers at all levels more efficiently grasp the statistical concepts presented. Like the previous editions, hypothesis testing is the focus of inferential testing in this book, and examples, for the most part, are worked through manually as well as using SAS. This unique approach bridges the gap between the analytic approaches learned in introductory statistics courses and the algorithmic nature of applying statistical software. It's always reassuring for the student to see that both approaches result in the same answer!

We'd like to thank the reviewers for their insight and invaluable suggestions, and the SAS editors for their patience in bringing the final manuscript to print. Over the years, SAS Press has assembled a number of excellent books with practical appeal, many focusing on statistical application and some specifically linked to the medical field. We hope this offering will complement that prestigious library.

*Glenn A. Walker
Jack Shostak
February 2010*

PREFACE TO THE SECOND EDITION

This second edition expands the first edition with the inclusion of new sections, examples, and extensions of statistical methods described in the first edition only in their most elementary forms. New methods sections include analysis of *crossover designs* (Chapter 9) and multiple comparison methods (Appendix D). Chapters that present *repeated measures analysis* (Chapter 8), *linear regression* (Chapter 10), *analysis of covariance* (Chapter 11), the *chi-square test* (Chapter 16), and *logistic regression* (Chapter 20) have been notably expanded, and 50% more examples have been added throughout the book. A new chapter of exercises has also been added to give the reader practice in applying the various methods presented. Also new in this edition is an introduction to α -adjustments for interim analyses (Chapter 2).

Although many of the new features will have wide appeal, some are targeted to the more experienced data analyst. These include discussion of the proportional odds model, the clustered binomial problem, collinearity in multiple regression, the use of time-dependent covariates with *Cox regression*, and the use of generalized estimating equations in *repeated measures analysis*. These methods, which are based on more advanced concepts than those found in most of the book, are routinely encountered in data analysis applications of clinical investigations and, as such, fit the description of ‘common statistical methods for clinical research’. However, so as not to overwhelm the less experienced reader, these concepts are presented only briefly, usually by example, along with references for further reading.

First and foremost, this is a statistical methods book. It is designed to have particular appeal to those involved in clinical research, biometrics, epidemiology, and other health or medical related research applications. Unlike other books in the SAS Books by Users (BBU) library, SAS is not the primary focus of this book. Rather, SAS is presented as an indispensable tool that greatly simplifies the analyst’s task. While consulting for dozens of companies over 25 years of statistical application to clinical investigation, I have never seen a successful clinical program that did not use SAS. Because of its widespread use within the pharmaceutical industry, I include SAS here as the ‘tool’ of choice to illustrate the statistical methods.

The examples have been updated to Version 8 of SAS, however, the programming statements used have been kept ‘portable’, meaning that most can be used in earlier versions of SAS as they appear in the examples, unless otherwise noted. This includes the use of portable variable and data set names, despite accommodation for use of long names beginning with Version 8. Because SAS is

not the main focus of this book but is key to efficient data analysis, programming details are not included here, but they can be found in numerous references cited throughout the book. Many of these references are other books in the Books by Users program at SAS, which provide the details, including procedure options, use of ODS, and the naming standards that are new in Version 8. For statistical programming, my favorites include **Categorical Data Analysis Using the SAS System**, Second Edition, by Stokes, Davis, and Koch (2000) and **Survival Analysis Using the SAS System, A Practical Guide**, by Paul Allison (1995).

I welcome and appreciate reader comments and feedback through the SAS Publications Web site.

*Glenn A. Walker
July 2002*

PREFACE TO THE FIRST EDITION

This book was written for those involved in clinical research and who may, from time to time, need a guide to help demystify some of the most commonly used statistical methods encountered in our profession.

All too often, I have heard medical directors of clinical research departments express frustration at seemingly cryptic statistical methods sections of protocols which they are responsible for approving. Other nonstatisticians, including medical monitors, investigators, clinical project managers, medical writers and regulatory personnel, often voice similar sentiment when it comes to statistics, despite the profound reliance upon statistical methods in the success of the clinical program. For these people, I offer this book (sans technical details) as a reference guide to better understand statistical methods as applied to clinical investigation and the conditions and assumptions under which they are applied.

For the clinical data analyst and statistician new to clinical applications, the examples from a clinical trials setting may help in making the transition from other statistical fields to that of clinical trials. The discussions of 'Least-Squares' means, distinguishing features of the various SAS® types of sums-of-squares, and relationships among various tests (such as the *Chi-Square Test*, the *Cochran-Mantel-Haenszel Test* and the *Log-Rank Test*) may help crystallize the analyst's understanding of these methods. Analysts with no prior SAS experience should benefit by the simplified SAS programming statements provided with each example as an introduction to SAS analyses.

This book may also aid the SAS programmer with limited statistical knowledge in better grasping an overall picture of the clinical trials process. Many times knowledge of the hypotheses being tested and appropriate interpretation of the SAS output relative to those hypotheses will help the programmer become more efficient in responding to the requests of other clinical project team members.

Finally, the medical student will find the focused presentation on the specific methods presented to be of value while proceeding through a first course in biostatistics.

For all readers, my goal was to provide a unique approach to the description of commonly used statistical methods by integrating both manual and computerized solutions to a wide variety of examples taken from clinical research. Those who learn best by example should find this approach rewarding. I have found no other book which demonstrates that the SAS output actually *does* have the same results as the manual solution of a problem using the calculating formulas. So ever reassuring this is for the student of clinical data analysis!

Each statistical test is presented in a separate chapter, and includes a brief, non-technical introduction, a synopsis of the test, one or two examples worked

manually followed by an appropriate solution using the SAS statistical package, and finally, a discussion with details and relevant notes.

Chapters 1 and 2 are introductory in nature, and should be carefully read by all with no prior formal exposure to statistics. Chapter 1 provides an introduction to statistics and some of the basic concepts involved in inference-making. Chapter 2 goes into more detail with regard to the main aspects of hypothesis testing, including significance levels, power and sample size determination. For those who use analysis-of-variance, Appendix C provides a non-technical introduction to ANOVA methods. The remainder of the book may be used as a text or reference. As a reference, the reader should keep in mind that many of the tests discussed in later chapters rely on concepts presented earlier in the book, strongly suggesting prerequisite review.

This book focuses on statistical hypothesis testing as opposed to other inferential techniques. For each statistical method, the test summary is clearly provided, including the null hypothesis tested, the test statistic and the decision rule. Each statistical test is presented in one of its most elementary forms to provide the reader with a basic framework. Many of the tests discussed have extensions or variations which can be used with more complex data sets. The 18 statistical methods presented here (Chapters 3-20) represent a composite of those which, in my experience, are most commonly used in the analysis of clinical research data. I can't think of a single study I've analyzed in nearly 20 years which did not use at least one of these tests. Furthermore, many of the studies I've encountered have used exclusively the methods presented here, or variations or extensions thereof. Thus, the word 'common' in the title.

Understanding of many parts of this book requires some degree of statistical knowledge. The clinician without such a background may skip over many of the technical details and still come away with an overview of the test's applications, assumptions and limitations. Basic algebra is the only prerequisite, as derivations of test procedures are omitted, and matrix algebra is mentioned only in an appendix. My hope is that the statistical and SAS analysis aspects of the examples would provide a springboard for the motivated reader, both to go back to more elementary texts for additional background and to go forward to more advanced texts for further reading.

Many of the examples are based on actual clinical trials which I have analyzed. In all cases, the data are contrived, and in many cases fictitious names are used for different treatments or research facilities. Any resemblance of the data or the tests' results to actual cases is purely coincidental.

*Glenn A. Walker
May 1996*

CHAPTER 1

Introduction & Basics

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1.1 Statistics—the Field

In some ways, we are all born statisticians. Inferring general patterns from limited knowledge is nearly as automatic to the human consciousness as breathing. Yet, when inference is formalized through the science of mathematics to the field called **Statistics**, it often becomes clouded by preconceptions of abstruse theory. Let's see if we can provide some formalization to this natural process of rational inference without getting bogged down in theoretical details.

The purpose of the field of **Statistics** is to characterize a *population* based on the information contained in a *sample* taken from that population. The sample information is conveyed by functions of the observed data, which are called *statistics*. The field of **Statistics** is a discipline that endeavors to determine which functions are the most relevant in the characterization of various populations. (The concepts of ‘populations’, ‘samples’, and ‘characterization’ are discussed in this chapter.)

For example, the arithmetic mean might be the most appropriate statistic to help characterize certain populations, while the median might be more appropriate for others. Statisticians use statistical and probability theory to develop new methodology and apply the methods best suited for different types of data sets.

Applied Statistics can be viewed as a set of methodologies used to help carry out scientific experiments. In keeping with the *scientific method*, applied statistics consists of developing a hypothesis, determining the best experiment to test the hypothesis, conducting the experiment, observing the results, and making conclusions. The statistician’s responsibilities include: study design, data collection, statistical analysis, and making appropriate inferences from the data. In doing so, the statistician seeks to limit bias, maximize objectivity, and obtain results that are scientifically valid.

■ ***Populations***

A *population* is a universe of entities to be characterized but is too vast to study in its entirety. The population in a clinical trial would be defined by its limiting conditions, usually specified via study inclusion and exclusion criteria.

Examples of populations include:

- patients with mild-to-moderate hypertension
- obese teenagers
- adult, insulin-dependent, diabetic patients.

The first example has only one limiting factor defining the population, that is, mild-to-moderate hypertension. This population could be defined more precisely as patients with diastolic blood pressure within a specific range of values as an inclusion criterion for the clinical protocol. Additional criteria would further limit the population to be studied.

The second example uses both age and weight as limiting conditions, and the third example uses age, diagnosis, and treatment as criteria for defining the population.

It is important to identify the population of interest in a clinical study at the time of protocol development, because the population is the ‘universe’ to which statistical inferences might apply. Severely restricting the population by using many specific criteria for admission might ultimately limit the clinical indication to a restricted subset of the intended market.

■ ***Samples***

You can describe a population by describing some representative entities in it. Measurements obtained from sample entities tend to characterize the entire population through inference.

The degree of representation of the entities in a sample that is taken from the population of interest depends on the sampling plan used. The simplest type of sampling plan is called a ‘simple random sample’. It describes any method of selecting a sample of population entities such that each entity has the same chance of being selected as any other entity in the population. It’s easy to see how random samples should represent the population, and the larger the sample, the greater the representation.

The method of obtaining a simple random sample from the population-of-interest is not always clear-cut. Simple random samples are rarely, if ever, used in clinical trials. Imagine the patients who comprise the populations in the three examples cited earlier, living all over the world. This would make the collection of a simple random sample an overwhelming task.

Although inferences can be biased if the sample is not random, adjustments can sometimes be used to control bias introduced by non-random sampling. An entire branch of **Statistics**, known as *Sampling Theory*, has been developed to provide alternative approaches to simple random sampling. Many of these approaches have the goal of minimizing bias. The techniques can become quite complex and are beyond the scope of this overview.

For logistical reasons, clinical studies are conducted at a convenient study center with the assumption that the patients enrolled at that center are typical of those that might be enrolled elsewhere. Multi-center studies are often used to reduce bias that could arise due to patient characteristics or procedural anomalies that might be unique to a specific center.

Stratified sampling is another technique that is often used to obtain a better representation of patients. Stratified sampling uses random samples from each of several subgroups of a population, which are called ‘strata’. Enrollment in a study is sometimes stratified by disease severity, age group, or some other characteristic of the patient.

Because inferences from non-random samples might not be as reliable as those made from random samples, the clinical statistician must specifically address the issue of selection bias in the analysis. Statistical methods can be applied to determine whether the treatment group assignment ‘appears’ random for certain response variables. For example, baseline values might be lower for Group A than Group B in a comparative clinical study. If Group A shows a greater response, part of that perceived response might be a regression-toward-the-mean effect, that is, a tendency to return to normal from an artificially low baseline level. Such effects should be investigated thoroughly to avoid making faulty conclusions due to selection bias.

Additional confirmatory studies in separate, independent samples from the same population can also be important in allaying concerns regarding possible sampling biases.

■ **Characterization**

So how is the population characterized from a sample? Statistical methods used to characterize populations can be classified as descriptive or inferential.

Descriptive statistics are used to describe the distribution of population measurements by providing estimates of central tendency and measures of variability, or by using graphical techniques such as histograms. *Inferential* methods use probability to express the level of certainty about estimates and to test specific hypotheses.

Exploratory analyses represent a third type of statistical procedure used to characterize populations. Although exploratory methods use both descriptive and inferential techniques, conclusions cannot be drawn with the same level of certainty because hypotheses are not pre-planned. Given a large data set, it is very

likely that at least one statistically significant result can be found by using exploratory analyses. Such results are ‘hypothesis-generating’ and often lead to new studies prospectively designed to test these new hypotheses.

Two main inferential methods are confidence interval estimation and hypothesis testing, which are discussed in detail later in this chapter.

1.2 Probability Distributions

An understanding of basic probability concepts is essential to grasp the fundamentals of statistical inference. Most introductory statistics texts discuss these basics, therefore, only some brief concepts of probability distributions are reviewed here.

Each outcome of a statistical experiment can be mapped to a numeric-valued function called a ‘random variable’. Some values of the random variable might be more likely to occur than others. The probability distribution associated with the random variable X describes the likelihood of obtaining certain values or ranges of values of the random variable.

For example, consider two cancer patients, each having a 50-50 chance of surviving at least 3 months. Three months later, there are 4 possible outcomes, which are shown in Table 1.1.

TABLE 1.1 Probability Distribution of Number of Survivors (n=2)

Outcome	Patient 1	Patient 2	X	Probability
1	Died	Died	0	0.25
2	Died	Survived	1	0.25
3	Survived	Died	1	0.25
4	Survived	Survived	2	0.25

Each outcome can be mapped to the random variable X , which is defined as the number of patients surviving at least 3 months. X can take the values 0, 1, or 2 with probabilities 0.25, 0.50, and 0.25, respectively, because each outcome is equally likely.

The probability distribution for X is given by P_x as follows:

X	P_x
0	0.25
1	0.50
2	0.25

■ ***Discrete Distributions***

The preceding example is a *discrete probability* distribution because the random variable X can only take discrete values, in this case, integers from 0 to 2.

The *binomial* distribution is, perhaps, the most commonly used discrete distribution in clinical biostatistics. This distribution is used to model experiments involving n independent trials, each with 2 possible outcomes, say, ‘event’ or ‘non-event’, and the probability of ‘event’, p, is the same for all n trials. The preceding example, which involves two cancer patients, is an example of a binomial distribution in which n = 2 (patients), p = 0.5, and ‘event’ is survival of at least 3 months.

Other commonly used discrete distributions include the *poisson* and the *hypergeometric* distributions.

■ ***Continuous Distributions***

If a random variable can take any value within an interval or continuum, it is called a *continuous* random variable. Height, weight, blood pressure, and cholesterol level are usually considered continuous random variables because they can take any value within certain intervals, even though the observed measurement is limited by the accuracy of the measuring device.

The probability distribution for a continuous random variable cannot be specified in a simple form as it is in the discrete example above. To do that would entail an infinite list of probabilities, one for each possible value within the interval. One way to specify the distribution for continuous random variables is to list the probabilities for ranges of X-values. However, such a specification can also be very cumbersome.

Continuous distributions are most conveniently approximated by functions of the random variable X, such as P_x . Examples of such functions are

$$P_x = 2x \quad \text{for } 0 < x < 1$$

or

$$P_x = ae^{-ax} \quad \text{for } 0 < x < \infty$$

The *normal* distribution is the most commonly used continuous distribution in clinical research statistics. Many naturally occurring phenomena follow the normal distribution, which can be explained by a powerful result from probability theory known as the *Central Limit Theorem*, discussed in the next section.

The normal probability distribution is given by the function

$$P_x = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \quad \text{for } -\infty < x < \infty$$

where μ and σ are called ‘parameters’ of the distribution. For any values of μ and σ (>0), a plot of P_x versus x has a ‘bell’ shape (illustrated in Appendix B).

Other common continuous distributions are the *exponential* distribution, the *chi-square* distribution, the *F*-distribution, and the Student *t*-distribution. Appendix B lists some analytic properties of common continuous distributions used in statistical inference (mentioned throughout this book). The *normal*, *chi-square*, *F*- and *t*-distributions are all interrelated, and some of these relationships are shown in Appendix B.

Whether discrete or continuous, every probability distribution has the property that the sum of the probabilities over all X-values equals 1.

■ ***The Central Limit Theorem***

The *Central Limit Theorem* states that, regardless of the distribution of measurements, sums and averages of a large number of like measurements tend to follow the normal distribution. Because many measurements related to growth, healing, or disease progression might be represented by a sum or an accumulation of incremental measurements over time, the normal distribution is often applicable to clinical data for large samples.

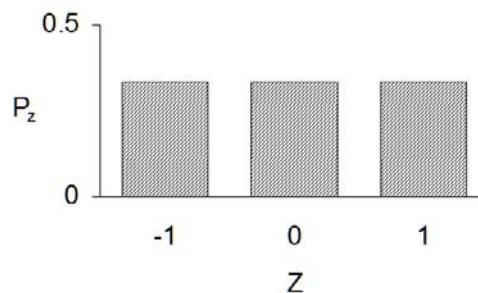
To illustrate the *Central Limit Theorem*, consider the following experiment. A placebo (inactive pill) is given to n patients, followed by an evaluation one hour later. Suppose that each patient's evaluation can result in ‘improvement,’ coded as +1, ‘no change’ (0), or ‘deterioration’ (-1), with each result equally probable. Let X_1, X_2, \dots, X_n represent the measurements for the n patients, and define Z to be a random variable that represents the sum of these evaluation scores for all n patients,

$$Z = X_1 + X_2 + \dots + X_n$$

For $n = 1$, the probability distribution of Z is the same as X , which is constant for all possible values of X . This is called a ‘uniform’ distribution. See Figure 1.1.

FIGURE 1.1 Probability

Z	P _Z
-1	1/3
0	1/3
+1	1/3



For $n = 2$, there are 9 equally probable outcomes resulting in 5 possible, distinct values for Z , as shown in Table 1.2.

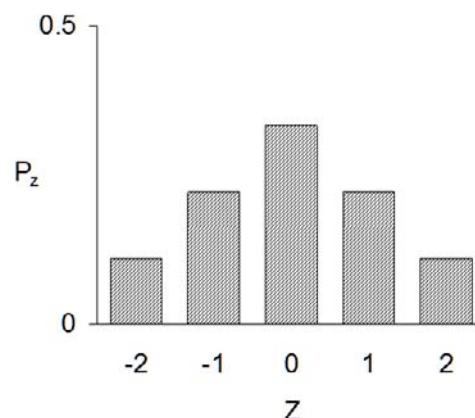
TABLE 1.2 All Possible Equally Probable Outcomes (n=2)

Patient 1	Patient 2	Z	Prob.
-1	-1	-2	1/9
-1	0	-1	1/9
0	-1	-1	1/9
-1	+1	0	1/9
0	0	0	1/9
+1	-1	0	1/9
0	+1	+1	1/9
+1	0	+1	1/9
+1	+1	+2	1/9

The resulting probability distribution for Z is shown in Figure 1.2.

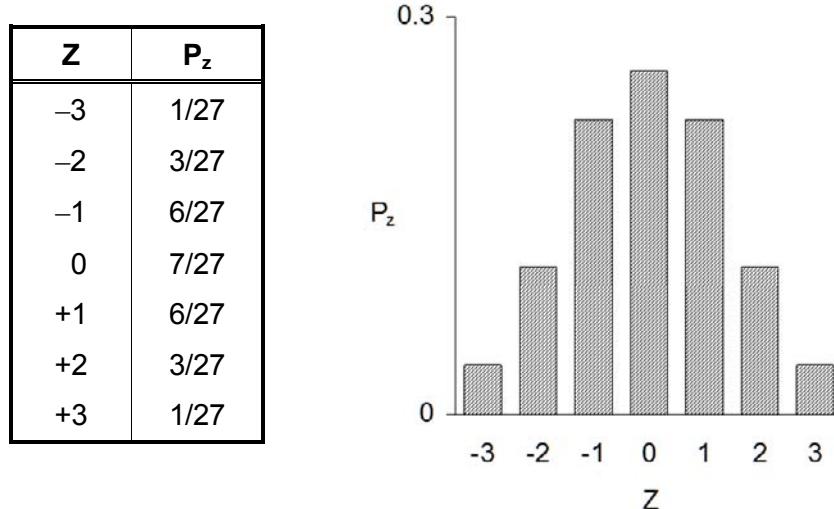
FIGURE 1.2 Probability Distribution for $Z = X_1 + X_2$

Z	P _Z
-2	1/9
-1	2/9
0	3/9
+1	2/9
+2	1/9



For $n = 3$, Z can take values from -3 to $+3$. See Figure 1.3 for the distribution.

FIGURE 1.3 Probability Distribution for $Z = X_1 + X_2 + X_3$

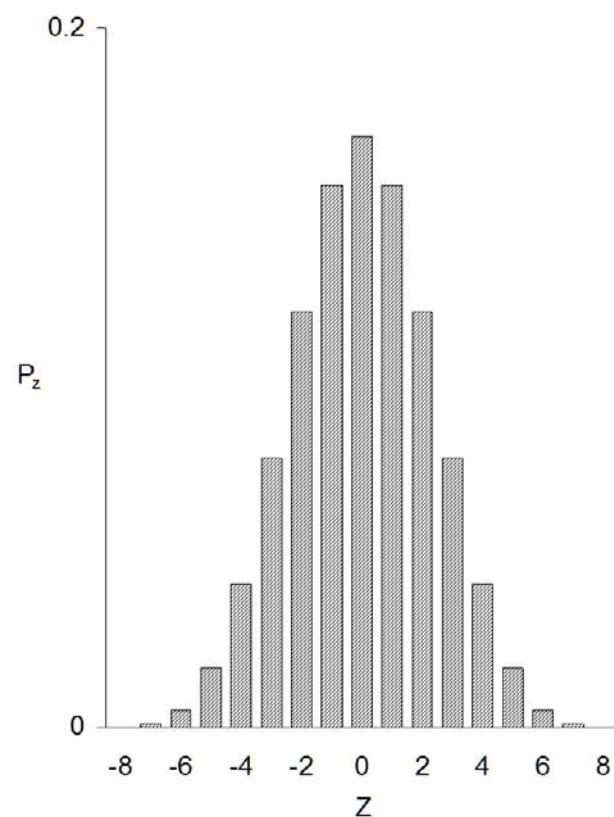


You can see from the histograms that, as n becomes larger, the distribution of Z takes on the bell-shaped characteristic of the normal distribution. The distribution of Z for 8 patients ($n = 8$) is shown in Figure 1.4.

While the probability distribution of the measurements (X) is ‘uniform’, the sum of these measurements (Z) is a random variable that tends toward a normal distribution as n increases. The *Central Limit Theorem* states that this will be the case regardless of the distribution of the X measurements. Because the sample mean, \bar{x} , is the sum of measurements (multiplied by a constant, $1/n$), the *Central Limit Theorem* implies that \bar{x} has an approximate normal distribution for large values of n regardless of the probability distribution of the measurements that comprise \bar{x} .

FIGURE 1.4 Probability Distribution for
 $Z = X_1 + X_2 + X_3 + X_4 + X_5 + X_6 + X_7 + X_8$

Z	P _z
-8	0.000
-7	0.001
-6	0.005
-5	0.017
-4	0.041
-3	0.077
-2	0.119
-1	0.155
0	0.169
+1	0.155
+2	0.119
+3	0.077
+4	0.041
+5	0.017
+6	0.005
+7	0.001
+8	0.000



1.3 Study Design Features

Sound statistical results can be valid only if the study plan is well thought out and accompanied by appropriate data collection techniques. Even the most sophisticated statistical tests might not lead to valid inferences or appropriate characterizations of the population if the study itself is flawed. Therefore, it is imperative that statistical design considerations be addressed in clinical studies during protocol development.

There are many statistical design considerations that go into the planning stage of a new study. The probability distribution of the primary response variables will help predict how the measurements will vary. Because greater variability of the measurements requires a larger sample size, distributional assumptions enable the computation of sample-size requirements to distinguish a real trend from statistical variation. Determining the sample size is discussed in Chapter 2.

Methods to help reduce response variability can also be incorporated into the study design. Features of controlled clinical trials such as *randomization* and *blinding*, and statistical ‘noise-reducing’ techniques (such as the use of covariates, stratification or blocking factors, and the use of within-patient controls) are ways to help control extraneous variability and focus on the primary response measurements.

■ **Controlled Studies**

A controlled study uses a known treatment, which is called a ‘control’, along with the test treatments. A control may be inactive, such as a placebo or sham, or it may be another active treatment, perhaps a currently marketed product.

A study that uses a separate, independent group of patients in a control group is called a *parallel-group* study. A study that gives both the test treatment and the control to the same patients is called a *within-patient control* study.

A controlled study has the advantage of being able to estimate the pure therapeutic effect of the test treatment by comparing its perceived benefit relative to the benefit of the control. Because the perceived benefit might be due to numerous study factors other than the treatment itself, a conclusion of therapeutic benefit cannot be made without first removing those other factors from consideration. Because the controls are subject to the same study factors, treatment effect *relative to* control, instead of absolute perceived benefit, is more relevant in estimating actual therapeutic effect.

■ **Randomization**

Randomization is a means of objectively assigning experimental units or patients to treatment groups. In clinical trials, this is done by means of a randomization schedule generated prior to starting the enrollment of patients.

The randomization scheme should have the property that any randomly selected patient has the same chance as any other patient of being included in any treatment group. Randomization is used in controlled clinical trials to eliminate systematic treatment group assignment, which might lead to bias. In a non-randomized setting, patients with the most severe condition might be assigned to a group based on the treatment's anticipated benefit. Whether this assignment is intentional or not, this creates bias because the treatment groups would represent samples from different populations, some of whom might have more severe conditions than others. Randomization filters out such selection bias and helps establish baseline comparability among the treatment groups.

Randomization provides a basis for unbiased comparisons of the treatment groups. Omitting specific responses from the analysis is a form of tampering with this randomization and will probably bias the results if the exclusions are made in a non-randomized fashion. For this reason, the primary analysis of a clinical trial is often based on the ‘intent-to-treat’ principle, which includes all randomized patients in the analysis even though some might not comply with protocol requirements.

Blinded Randomization

Blinded (or masked) randomization is one of the most important features of a controlled study. Single-blind, double-blind, and even triple-blind studies are common among clinical trials.

A *single-blind* study is one in which the patients are not aware of which treatment they receive. Many patients actually show a clinical response with medical care even if they are not treated. Some patients might respond when treated with a placebo but are unaware that their medication is inactive. These are examples of the well-known *placebo effect*, which might have a psychological component dependent on the patient's belief that he is receiving appropriate care. A 20% or greater placebo response is not uncommon in many clinical indications.

Suppose that a response, Y , can be represented by a true therapeutic response component, TR , and a placebo effect, PE . Letting subscripts A and P denote 'active' and 'placebo' treatments, respectively, the estimated therapeutic benefit of the active compound might be measured by the difference

$$Y_A - Y_P = (TR_A + PE_A) - (TR_P + PE_P)$$

Because a placebo has no therapeutic benefit, $TR_P = 0$. With $PE_\Delta = PE_A - PE_P$, you obtain

$$Y_A - Y_P = TR_A + PE_\Delta$$

When patients are unaware of their treatment, the placebo effect (PE) should be the same for both groups, making $PE_\Delta = 0$. Therefore, the difference in response values estimates the true therapeutic benefit of the active compound.

However, if patients know which treatment they have been assigned, the placebo effect in the active group might differ from that of the control group, perhaps due to better compliance or expectation of benefit. In this case, the estimate of therapeutic benefit is contaminated by a non-zero PE_Δ .

In addition, bias, whether conscious or not, might arise if the investigator does not evaluate all patients uniformly. Evaluation of study measurements (such as global assessments and decisions regarding dosing changes, visit timing, use of concomitant medications, and degree of follow-up relating to adverse events or abnormal labs) might be affected by the investigator's knowledge of the patient's treatment. Such bias can be controlled by *double-blinding* the study, which means that information regarding treatment group assignment is withheld from the investigator as well as the patient.

Double-blinding is a common and important feature of a controlled clinical trial, especially when evaluations are open to some degree of subjectivity. However, double-blinding is not always possible or practical. For example, test and control treatments might not be available in the same formulation. In such cases, treatment can sometimes be administered by one investigator and the evaluations performed

by a co-investigator at the same center in an attempt to maintain some sort of masking of the investigator.

Studies can also be *triple-blind*, wherein the patient, investigator, and clinical project team (including the statistician) are unaware of the treatment administered until the statistical analysis is complete. This reduces a third level of potential bias—that of the interpretation of the results.

Selection of appropriate statistical methods for data analysis in confirmatory studies should be done in a blinded manner whenever possible. Usually, this is accomplished through the development of a statistical analysis plan prior to completing data collection. Such a plan helps remove the potential for biases associated with data-driven methodology. It also eliminates the ability to select a method for the purpose of producing a result closest to the outcome that is being sought.

■ ***Selection of Statistical Methods***

Features of controlled clinical trials, such as randomization and blinding, help to limit bias when making statistical inferences. The statistical methods themselves might also introduce bias if they are ‘data-driven’, that is the method is selected based on the study outcomes. In most cases, the study design and objectives will point to the most appropriate statistical methods for the primary analysis. These methods are usually detailed in a formal analysis plan prepared prior to data collection and, therefore, represent the best ‘theoretical’ methodology not influenced by the data.

Often, sufficient knowledge of the variability and distribution of the response in Phase 3 or in pivotal trials is obtained from previous studies. If necessary, there are ways to confirm distributional assumptions based on preliminary blinded data in order to fully pre-specify the methodology. Because different statistical methods might lead to different conclusions, failure to pre-specify the methods might lead to the appearance of selecting a method that results in the most desirable conclusion.

Methodology bias is one concern addressed by an analysis plan. More importantly, pre-specifying methodology helps to ensure that the study objectives are appropriately addressed. The statistical method selected will depend very strongly on the actual objective of the study. Consider a trial that includes three doses of an active compound and an inactive placebo. Possible study objectives include determining if

- there is any difference among the four groups being studied.
- any of the active doses is better than the placebo.
- the highest dose is superior to the lower doses.
- there is a dose-response.

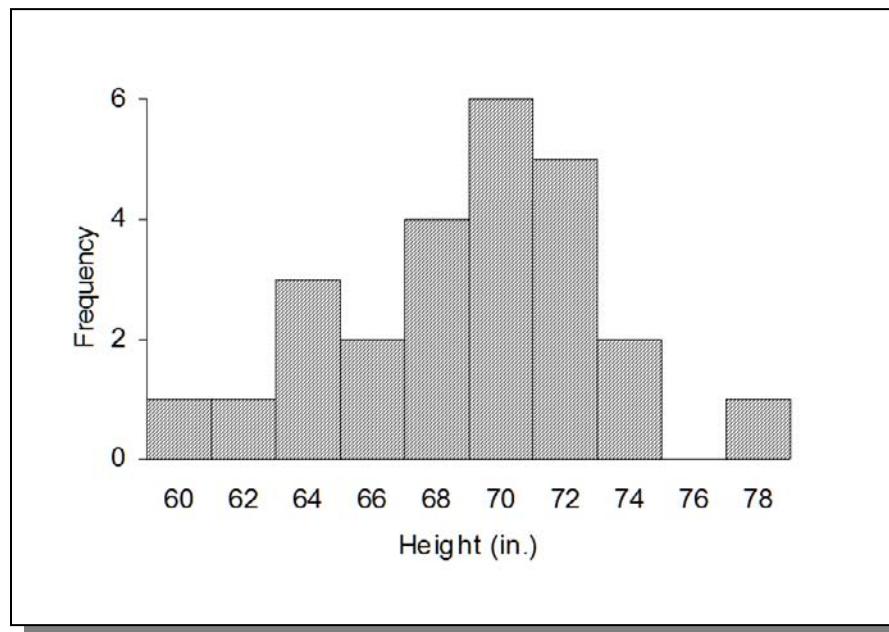
A different statistical method might be required for each of these objectives. The study objective must be clear before the statistical method can be selected.

1.4 Descriptive Statistics

Descriptive statistics describe the probability distribution of the population. This is done by using histograms to depict the shape of the distribution, by estimating distributional parameters, and by computing various measures of central tendency and dispersion.

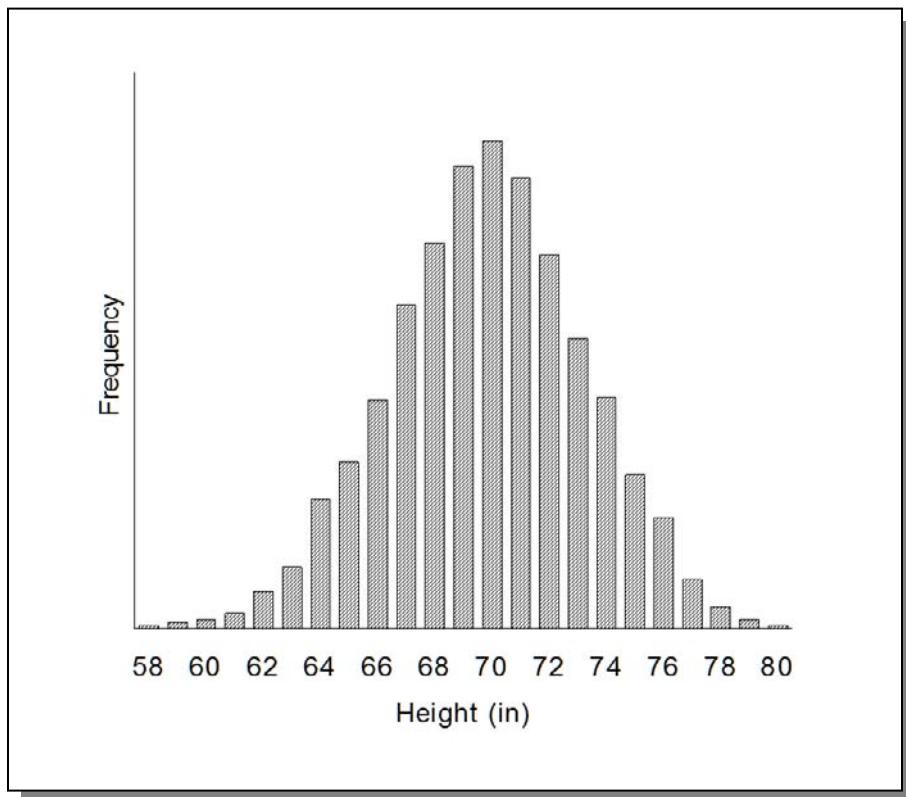
A *histogram* is a plot of the measured values of a random variable by their frequency. For example, height measurements for 16-year-old male students can be described by a sample histogram based on 25 students. See Figure 1.5.

FIGURE 1.5 Histogram of Height Measurements (n=25)



If more-and-more measurements are taken, the histogram might begin looking like a ‘bell-shaped’ curve, which is characteristic of a normal distribution. See Figure 1.6.

FIGURE 1.6 Histogram of Height Measurements (n=300)



If you assume the population distribution can be modeled with a known distribution (such as the normal), you need only estimate the parameters associated with that distribution in order to fully describe it. The binomial distribution has only one parameter, p , which can be directly estimated from the observed data. The normal distribution has two parameters, μ and σ^2 , representing the mean and variance, respectively.

Suppose a sample of n measurements, denoted by x_1, x_2, \dots, x_n is obtained. Various descriptive statistics can be computed from these measurements to help describe the population. These include measures of *central tendency*, which describe the center of the distribution, and measures of *dispersion*, which describe the variation of the data. Common examples of each are shown in Table 1.3.

In addition to distributional parameters, you sometimes want to estimate parameters associated with a statistical model. If an unknown response can be modeled as a function of known or controlled variables, you can often obtain valuable information regarding the response by estimating the weights or coefficients of each of these known variables. These coefficients are called *model parameters*. They are estimated in a way that results in the greatest consistency between the model and the observed data.

TABLE 1.3 Common Descriptive Statistics

Measures of 'Central Tendency'	
<i>Arithmetic Mean</i>	$\bar{x} = (\sum x_i) / n = (x_1 + x_2 + \dots + x_n) / n$
<i>Median</i>	the middle value, if n is odd; the average of the two middle values if n is even (50^{th} percentile)
<i>Mode</i>	the most frequently occurring value
<i>Geometric Mean</i>	$(\prod x_i)^{1/n} = (x_1 \cdot x_2 \cdot \dots \cdot x_n)^{1/n}$
<i>Harmonic Mean</i>	$n / \sum(x_i)^{-1} = n \{ (1/x_1) + (1/x_2) + \dots + (1/x_n) \}^{-1}$
<i>Weighted Mean</i>	$\bar{x}_w = (\sum w_i x_i) / W$, where $W = \sum w_i$
<i>Trimmed Mean</i>	Arithmetic mean omitting the largest and smallest observations
<i>Winsorized Mean</i>	Arithmetic mean after replacing outliers with the closest non-outlier values

Measures of 'Dispersion'	
<i>Variance</i>	$s^2 = \sum(x_i - \bar{x})^2 / (n - 1)$
<i>Standard Deviation</i>	$s = \text{square root of the variance}$
<i>Standard Error (of the mean)</i>	$(s^2 / n)^{1/2} = \text{Standard deviation of } \bar{x}$
<i>Range</i>	Largest value - Smallest value
<i>Mean Absolute Deviation</i>	$(\sum x_i - \bar{x}) / n$
<i>Inter-Quartile Range</i>	$75^{\text{th}} \text{ percentile} - 25^{\text{th}} \text{ percentile}$
<i>Coefficient of Variation</i>	s / \bar{x}

Descriptive statistical methods are often the only approach that can be used for analyzing the results of pilot studies or Phase I clinical trials. Due to small sample sizes, the lack of blinding, or the omission of other features of a controlled trial, statistical inference might not be possible. However, trends or patterns observed in the data by using descriptive or exploratory methods will often help in building hypotheses and identifying important cofactors. These new hypotheses can then be tested in a more controlled manner in subsequent studies, wherein inferential statistical methods would be more appropriate.

1.5 Inferential Statistics

The two primary statistical methods for making inferences are confidence interval estimation and hypothesis testing.

■ *Confidence Intervals*

Population parameters, such as the mean (μ) or the standard deviation (σ), can be estimated by using a point estimate, such as the sample mean (\bar{x}) or the sample standard deviation (s). A *confidence interval* is an interval around the point estimate that contains the parameter with a specific high probability or confidence level. A 95% confidence interval for the mean (μ) can be constructed from the sample data with the following interpretation: If the same experiment were conducted a large number of times and confidence intervals were constructed for each, approximately 95% of those intervals would contain the population mean (μ).

The general form of a confidence interval is $[\theta_L - \theta_U]$, where θ_L represents the lower limit and θ_U is the upper limit of the interval. If the probability distribution of the point estimate is symmetric (such as the normal distribution), the interval can be found by

$$\hat{\theta} \pm C \cdot \sigma_{\hat{\theta}}$$

where $\hat{\theta}$ is the point estimate of the population parameter θ , $\sigma_{\hat{\theta}}$ is the standard error of the estimate, and C represents a value determined by the probability distribution of the estimate and the significance level that you want. When $\sigma_{\hat{\theta}}$ is unknown, the estimate $\hat{\sigma}_{\hat{\theta}}$ may be used.

For example, for α between 0 and 1, a $100(1-\alpha)\%$ confidence interval for a normal population mean (μ) is

$$\bar{x} \pm Z_{\alpha/2} \cdot \sigma / \sqrt{n}$$

where the point estimate of μ is \bar{x} , the standard error of \bar{x} is σ/\sqrt{n} , and the value of $Z_{\alpha/2}$ is found in the normal probability tables (See Appendix A.1). Some commonly used values of α and the corresponding critical Z -values are

α	$Z_{\alpha/2}$
0.10	1.645
0.05	1.96
0.02	2.33
0.01	2.575

In most cases, the standard deviation (σ) will not be known. If it can be estimated using the sample standard deviation (s), a $100(1-\alpha)\%$ confidence interval for the mean (μ) can be formed as

$$\bar{x} \pm t_{\alpha/2} \cdot s / \sqrt{n}$$

where $t_{\alpha/2}$ is found from the Student-t probability tables (see Appendix A.2) based on the number of degrees of freedom, in this case, $n-1$. For example, a value of $t_{\alpha/2} = 2.093$ would be used for a 95% confidence interval when $n = 20$.

Many SAS procedures will print point estimates of parameters with their standard errors. These point estimates can be used to form confidence intervals using the general form for $\hat{\theta}$ that is given above. Some of the most commonly used confidence intervals are for population means (μ), differences in means between two populations ($\mu_1 - \mu_2$), population proportions (p), and differences in proportions between two populations ($p_1 - p_2$). For each of these, the form for $\hat{\theta}$ and its standard error are shown in Table 1.4.

TABLE 1.4 Confidence Interval Components Associated with Means and Proportions

θ	$\hat{\theta}$	$\sigma_{\hat{\theta}}^2$	$\hat{\sigma}_{\hat{\theta}}^2$	C
μ	\bar{x}	σ^2 / n	s^2 / n	$Z_{\alpha/2}$ if σ is known; $t_{\alpha/2}$ if σ is unknown
$\mu_1 - \mu_2$	$\bar{x}_1 - \bar{x}_2$	$\sigma_1^2/n_1 + \sigma_2^2/n_2$	$s^2 (1/n_1 + 1/n_2)$	$Z_{\alpha/2}$ if σ_1 and σ_2 are known; $t_{\alpha/2}$ if σ_1 or σ_2 is unknown. If unknown, assume equal variances and use $s^2 = [(n_1-1)s_1^2 + (n_2-1)s_2^2]/(n_1 + n_2 - 2)$
p	$\hat{p} = x/n$	$p(1-p)/n$	$\hat{p}(1-\hat{p})/n$	$Z_{\alpha/2}$ (x 'events' in n binomial trials)*
$p_1 - p_2$	$\hat{p}_1 - \hat{p}_2$	$p_1(1-p_1)/n_1 + p_2(1-p_2)/n_2$	$\hat{p}_1(1-\hat{p}_1)/n_1 + \hat{p}_2(1-\hat{p}_2)/n_2$	$Z_{\alpha/2}$ ($\hat{p}_i = x_i/n_i$ for $i = 1, 2$)*

* applies to large samples

■ **Hypothesis Testing**

Hypothesis testing is a means of formalizing the inferential process for decision-making purposes. It is a statistical approach for testing hypothesized statements about population parameters based on logical argument.

To understand the concept behind the hypothesis test, let's examine a form of deductive argument from logic, using the following example:

If you have an apple, you do not have an orange. You have an orange. Therefore, you do not have an apple.

The first two statements of the argument are premises and the third is the conclusion. The conclusion is logically deduced from the two premises, and its truth depends on the truth of the premises.

If **P** represents the first premise and **Q** represents the second premise, the argument may be formulated as

$$\begin{array}{ll} \text{if } \mathbf{P} \text{ then not } \mathbf{Q} & \text{(conditional premise)} \\ \mathbf{Q} & \text{(premise)} \\ \hline \text{therefore, not } \mathbf{P} & \text{(conclusion)} \end{array}$$

This is a deductively valid argument of logic that applies to any two statements, **P** and **Q**, whether true or false. Note that if you have both an apple and an orange, the conditional premise would be false, which makes the conclusion false because the argument is still valid.

Statistical arguments take the same form as this logical argument, but statistical arguments must account for random variations in statements that might not be known to be completely true. A statistical argument might be paraphrased from the logical argument above as

$$\begin{array}{ll} \text{if } \mathbf{P} \text{ then } \textit{probably not } \mathbf{Q} & \text{(conditional premise)} \\ \mathbf{Q} & \text{(premise)} \\ \hline \text{therefore, } \textit{probably not } \mathbf{P} & \text{(conclusion)} \end{array}$$

The following examples illustrate such ‘statistical arguments’.

Example 1

Statements:

P = the coin is fair

Q = you observe 10 tails in a row

Argument:

If the coin is fair, you would probably not observe 10 tails in a row. You observe 10 tails in a row. Therefore, the coin is probably not fair.

Example 2

Statements:

P = Drug A has no effect on arthritis

Q = from a sample of 25 patients, 23 showed improvement in their arthritis after taking Drug A

Argument:

If Drug A has no effect on arthritis, you would probably not see improvement in 23 or more of the sample of 25 arthritic patients treated with Drug A. You observe improvement in 23 of the sample of 25 arthritic patients treated with Drug A. Therefore, Drug A is probably effective for arthritis.

In the first example, you might initially suspect the coin of being biased in favor of tails. To test this hypothesis, assume the null case, which is that the coin is fair. Then, design an experiment that consists of tossing the coin 10 times and recording the outcome of each toss. You decide to reject the hypothesis concluding that the coin is biased in favor of tails if the experiment results in 10 consecutive tails.

Formally, the study is set out by identifying the hypothesis, developing a test criterion, and formulating a decision rule. For Example 1,

Null hypothesis:	the coin is fair
Alternative:	the coin is biased in favor of tails
Test criterion:	the number of tails in 10 consecutive tosses of the coin
Decision rule:	reject the null hypothesis if all 10 tosses result in 'tails'

First, establish the hypothesis **P**. The hypothesis is tested by observing the results of the study outcome **Q**. If you can determine that the probability of observing **Q** is very small when **P** is true and you do observe **Q**, you can conclude that **P** is probably not true. The degree of certainty of the conclusion is related to the probability associated with **Q**, assuming **P** is true.

Hypothesis testing can be set forth in an algorithm with 5 parts:

- the null hypothesis (abbreviated H_0)
- the alternative hypothesis (abbreviated H_A)
- the test criterion
- the decision rule
- the conclusion.

The null hypothesis is the statement **P** translated into terms involving the population parameters. In Example 1, 'the coin is fair' is equivalent to 'the probability of tails on any toss is $\frac{1}{2}$ '. Parametrically, this is stated in terms of the binomial parameter p , which represents the probability of tails.

$$H_0: p \leq 0.5$$

The alternative hypothesis is 'not **P**', or

$$H_A: p > 0.5$$

Usually, you take 'not **P**' as the hypothesis to be demonstrated based on an acceptable risk for defining '*probably*' as used in Examples 1 and 2.

The test criterion or 'test statistic' is some function of the observed data. This is statement **Q** of the statistical argument. Statement **Q** might be the number of tails in 10 tosses of a coin or the number of improved arthritic patients, as used in Examples 1 and 2, or you might use a more complex function of the data. Often the test statistic is a function of the sample mean and variance or some other summary statistics.

The decision rule results in the rejection of the null hypothesis if unlikely values of the test statistic are observed when assuming the test statistic is true. To determine a decision rule, the degree of such ‘unlikeliness’ needs to be specified. This is referred to as the *significance level* of the test (denoted α) and, in clinical trials, is often (but not always) set to 0.05. By knowing the probability distribution of the test statistic when the null hypothesis is true, you can identify the most extreme $100\alpha\%$ of the values as a rejection region. The decision rule is simply to reject H_0 when the test statistic falls in the rejection region.

See Chapter 2 for more information about significance levels.

1.6 Summary

This introductory chapter provides some of the basic concepts of statistics, gives an overview of statistics as a scientific discipline, and shows that the results of a statistical analysis can be no better than the data collected. You’ve seen that the researcher must be vigilant about biases that can enter into a data set from a multitude of sources. With this in mind, it is important to emphasize the correct application of statistical techniques in study design and data collection as well as at the analysis stage.

Statistical methods used to characterize populations from sample data can be classified as descriptive or inferential, most notably, parameter estimates by confidence intervals and hypothesis testing. These techniques are the focus of the methods presented in this book, Chapters 4 through 22.

CHAPTER 2

Topics in Hypothesis Testing

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2.1 Significance Levels

When conducting hypothesis testing, an erroneous conclusion is made if the null hypothesis is rejected when it is really true. This error is called a Type I error, and its probability is denoted by α , which is known as the ‘significance level’ of the test.

When setting up the hypothesis test, the rejection region is selected based on a predetermined value for α , usually a small value such as 0.05. This means that there is only a 5% chance of rejecting a true null hypothesis.

For example, suppose that administration of a drug was suspected to cause increased alkaline phosphatase levels in adult males, a population known to have an alkaline phosphatase mean of 60 U/l in a certain laboratory. To test this, the null and alternative hypotheses are set as

$$H_0: \mu = 60$$

versus

$$H_A: \mu > 60$$

where μ represents the population mean alkaline phosphatase in all men who might qualify to receive the drug and be tested at this testing facility.

A sample of n men treated with the drug is observed, and their alkaline phosphatase levels are measured. The *Z-test* which is based on the standard normal distribution and computed from the sample mean \bar{x} is chosen as the test statistic. According to the *Central Limit Theorem* (Chapter 1), \bar{x} has a normal distribution with mean μ and standard error σ/\sqrt{n} for large n , so that

$$Z = \frac{\bar{x} - \mu}{\sigma / \sqrt{n}}$$

has a ‘standard normal’ distribution (see Appendix B).

The null hypothesis would be contradicted if the sample mean \bar{x} is much greater than the known mean, 60. The decision rule is to reject H_0 in favor of H_A when the test statistic is too large, computed under the assumption that H_0 is true,

$$Z_0 = \frac{\bar{x} - 60}{\sigma / \sqrt{n}}$$

The rejection region is $Z_0 > c$, where c is selected according to the chosen significance level α . That is,

$$\alpha = \Pr(\text{reject } H_0 \text{ when } H_0 \text{ is true}) = \Pr(Z_0 > c)$$

The critical value, c , can be denoted by Z_α , which is found from widely available tables of the probabilities for the standard normal distribution, including Appendix A.1 of this book. For the commonly used value of $\alpha = 0.05$, $Z_\alpha = 1.645$.

Suppose that previous laboratory testing at the study laboratory established a mean alkaline phosphatase level of 60 U/l with a standard deviation of $\sigma = 15$. A current sample of 100 treated men resulted in a sample mean of 62 U/l. The *Z-test* summary is

null hypothesis:	$H_0: \mu = 60$
alt. hypothesis:	$H_A: \mu > 60$
test statistic:	$Z_0 = \frac{\bar{x} - 60}{\sigma / \sqrt{n}} = \frac{62 - 60}{15 / \sqrt{100}} = 1.33$
rejection region:	Reject H_0 if $Z_0 > 1.645$ at significance level $\alpha = 0.05$
conclusion:	Because $1.33 < 1.645$, do not reject H_0 . Insufficient evidence exists to indicate an increase in mean alkaline phosphatase levels.

2.2 Power

Accepting the null hypothesis when it is not true is a second type of error that can occur when testing a hypothesis. This is known as a Type II error and has the probability β .

For a given test, β is partly determined by the choice for α . Ideally, both α and β would be small. However, in general, there is an inverse relationship between α and β for a fixed sample size, n . Decreasing α (the probability of a Type I error) increases β (the probability of a Type II error) and, if taken too far, tends to render the test *powerless* in its ability to detect real deviations from the null hypothesis.

A test's *power* is defined by $1 - \beta$, the probability of rejecting the null hypothesis when it is not true. For the fixed significance level α , the sample size will determine β and, therefore, the power of the test.

In the example discussed in Section 2.1, if you accept H_0 and conclude that there is no increase in mean alkaline phosphatase levels with treatment, you would be guilty of a Type II error if a true increase goes undetected by the statistical test. Until the test's power can be investigated, you must conclude that there is 'insufficient evidence to indicate a change' rather than 'there is no change'.

Note that β is not only a function of the significance level and the sample size, but also of the true mean, μ . The Type II error probability for this alkaline phosphatase example is given by

$$\begin{aligned}\beta &= \Pr(\text{accept } H_0 \text{ when } H_A \text{ is true}) \\ &= \Pr(Z_0 \leq 1.645 \text{ when } \mu > 60)\end{aligned}$$

which will differ for each alternative value of $\mu (> 60)$.

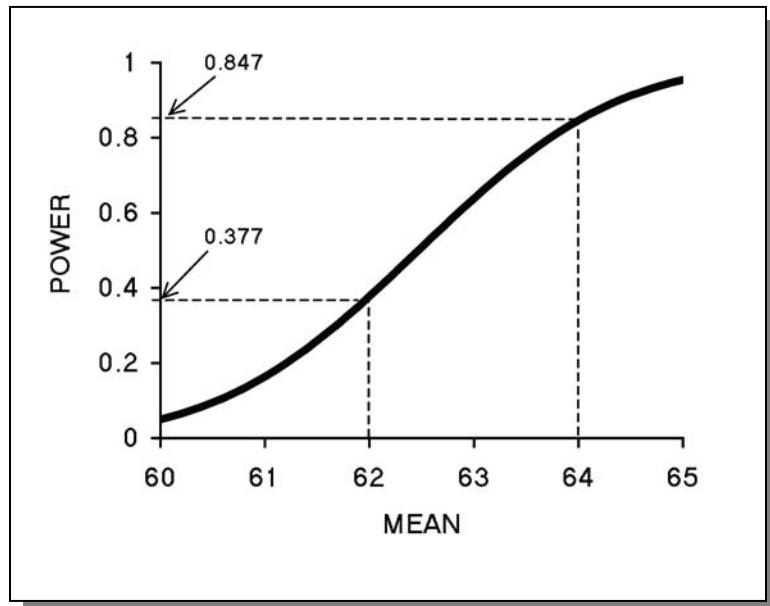
For example, the probability of a Type II error when $\mu = 64$ is

$$\begin{aligned}\beta &= \Pr(Z_0 \leq 1.645 \text{ when } \mu = 64) \\ &= \Pr\left(\frac{\bar{x} - 60}{\sigma/\sqrt{n}} \leq 1.645 \text{ when } \mu = 64\right) \\ &= \Pr\left(\frac{\bar{x} - 64}{\sigma/\sqrt{n}} \leq 1.645 - \frac{4}{\sigma/\sqrt{n}}\right) \\ &= \Pr(Z \leq -1.022)\end{aligned}$$

because $\sigma = 15$ and $n = 100$. From the normal probability tables (Appendix A.1), you obtain $\beta = 0.153$ and a power of $1 - \beta = 0.847$. Similar calculations when $\mu = 62$ result in $\beta = 0.623$, which gives a power of 0.377.

The power function of the test can be described by a plot of alternative values of μ vs. the power, computed as demonstrated in the preceding equations. Figure 2.1 shows the power curve of the *Z-test* for our example.

FIGURE 2.1 Power Curve for the Z-Test



Power curves are important for determining the best test statistic to use. When more than one statistical test is a logical candidate for testing the same hypothesis, the statistician uses the test's power function to determine the more powerful test for a range of likely alternative values. Power curves are also important in sample size determination during study design. Sample size calculations are discussed in Section 2.5.

2.3 One-Tailed and Two-Tailed Tests

The form of the alternative hypothesis determines whether the test is a *one-* or *two-tailed* test. The alkaline phosphatase example is a *one-tailed test* because the alternative hypothesis $H_A: \mu > 60$ is only concerned with alternative mean values in one direction, that is, greater than 60. The *two-tailed* alternative for this example would be specified as $H_A: \mu \neq 60$, which indicates an interest in alternative values of the mean either greater or less than 60.

The rejection region for the *two-tailed Z-test* would include both very large *and* very small values of the test statistic. For a significance level of α , you reject H_0 in favor of the two-tailed alternative if $Z_0 > Z_{\alpha/2}$ or $Z_0 < -Z_{\alpha/2}$. For $\alpha = 0.05$, $\alpha/2 = 0.025$ in each 'tail' of the normal distribution, obtaining $Z_{0.025} = 1.96$ as the critical value for rejection from the normal probability tables (Appendix A.1). The

rejection region for the two-tailed Z-test when $\alpha = 0.05$ is $|Z_0| > 1.96$ (that is, $Z_0 > 1.96$ or $Z_0 < -1.96$) due to the symmetry of the normal distribution.

When the distribution of the test statistic is not symmetric, the relationship between one- and two-tailed tests is more complex and beyond the scope of this book. However, it should be noted that for some underlying distributions, such as the chi-square or F-distributions (discussed in other sections), a ‘two-tailed’ test is more appropriately referred to as a ‘two-sided’ test since the distribution only has one ‘tail’ (see Appendix B.4).

2.4 p-Values

Formally, the conclusion of a statistical hypothesis test is to ‘reject’ or to ‘not reject’ the null hypothesis at a pre-set significance level. Another way to convey a test’s level of significance is with its p-values. The p-value is the actual probability of obtaining the calculated test statistic or a value in a more extreme part of the rejection region, when H_0 is true.

In the alkaline phosphatase example discussed previously, the test statistic of $Z_0 = 1.33$ is obtained. The p-value is computed as

$$p = \Pr(Z_0 \geq 1.33, \text{ assuming } H_0 \text{ is true}) = 0.0918$$

based on the normal probability tables (Appendix A.1). Calculated p-values less than the pre-set significance level, such as 0.05, would be considered statistically significant.

If the probability distribution of the test statistic is symmetric, the p-value that corresponds to a *two-tailed test* can be halved to obtain the *p-value* corresponding to the *one-tailed-test*. This is true for the *Z-test* (Section 2.1), which has the standard normal distribution (Chapter 1), and for the *t-test* (Chapters 4, 5), which has the Student t-distribution, both of which are symmetrically distributed about 0.

The results of a statistical test are often described as ‘highly significant’ when very small p-values (such as $p < 0.001$, $p < 0.0001$, etc.) are obtained.

2.5 Sample Size Determination

Sample size requirements are determined from the desired power of the test, the significance level, the measurement variability, and the stated alternative to the null hypothesis. In designing comparative clinical studies, $\alpha = 0.05$ is often used and a power of at least 80% is sought. Variability is estimated from previous studies or known data sets, and the alternative value of the hypothesis is determined by that which is clinically important.

Many excellent sources are available that show methods for computing sample sizes, including Lachin (1981) and the SAS documentation for the POWER procedure (see *SAS/STAT User’s Guide*). In the initial planning stages of a

clinical study, ballpark approximations of sample size requirements under various conditions are often needed for budgetary and other considerations. We give here three examples commonly used in determining sample sizes for clinical studies, which can be used for ‘quick and dirty’ approximations. These approximate formulas are based on the commonly used significance level of $\alpha = 0.05$.

■ *One-Sample Test of a Mean*

For the *one-sample Z-test* about a population mean (presented earlier in Section 2.1) or the *one sample t-test* (Chapter 4), the sample size needed is given by

$$n = \frac{W}{(\Delta/\sigma)^2}$$

where σ is the standard deviation, Δ represents the difference between the hypothesized value and the alternative value, and W is obtained from Table 2.1 based on the power and type of test. If σ is unknown, the sample standard deviation (s) estimated from previous studies may be substituted.

In the alkaline phosphatase example discussed previously, the sample size required to detect an increase in mean alkaline phosphatase from 60 to 63 U/l, based on a standard deviation of 15, is

$$n = 6.2 / (3/15)^2 = 155 \text{ patients}$$

This sample size results in a power of 80% based on a one-tailed significance level of 0.05.

■ *Two-Sample Comparison of Means*

The comparison of two means using a *two-sample Z-test* or the *two-sample t-test* (Chapter 5) typically tests whether the difference in means between two independent groups is 0. The alternative is that the difference is a non-zero value, such as, Δ . If patients will be equally divided between two groups based on a standard deviation in each group of σ , the sample size for each group is found by

$$n = \frac{2 \cdot W}{(\Delta/\sigma)^2}$$

where W is obtained from Table 2.1. Again, the sample standard deviation, s , may be substituted when σ is unknown.

For example, based on a *two-tailed test* at 90% power and a standard deviation of 10, a difference in means of at least 5 can be detected with a sample size of

$$n = 2(10.5) / (5/10)^2 = 84 \text{ patients per group}$$

■ Two-Sample Comparison of Proportions

The comparison of two, independent, binomial proportions using a normal approximation (*Z-test*) or *chi-square test* (Chapter 16) tests for a 0 difference in proportions

$$H_0: p_1 - p_2 = 0$$

The alternative is that the difference is a non-zero value, say, Δ . The sample size (per group) that is required to detect a difference when the true difference is Δ is given by

$$n = \frac{2 \cdot W \cdot \bar{p} \cdot (1 - \bar{p})}{\Delta^2}$$

where $\bar{p} = (p_1 + p_2) / 2$, and W is obtained from Table 2.1. As in the two-sample means comparison, this formula can be used when the study calls for two groups that have the same number of patients in each.

For example, the sample size needed to detect a difference in true response rates of 25% vs. 35% with at least 80% power, is found as

$$n = 2(7.85)(0.3)(0.7) / (.1^2) = 330 \text{ patients per group}$$

This is based on a *two-tailed test* at a significance level of 0.05.

TABLE 2.1 W Values for $\alpha = 0.05$

POWER	β	W	
		One-Tailed	Two-Tailed
70%	0.30	4.7	6.2
75%	0.25	5.4	6.9
80%	0.20	6.2	7.85
85%	0.15	7.2	9.0
90%	0.10	8.6	10.5
95%	0.05	10.8	13.0

PROC POWER, available in SAS 9.1 and later, makes it possible to compute sample sizes. The SAS code is shown below for producing the sample sizes for the three examples just presented: One-Sample Test of a Mean, Two-Sample Comparison of Means, and Two-Sample Comparison of Proportions. In each case, the `sides` parameter is used to specify a one- or two-tailed hypothesis, the power is specified (as a fraction between 0 and 1), and the sample size is requested by setting the corresponding parameter as missing (`ntotal = .`, `npergroup = .`).

```

proc power;
    onesamplemeans test = t
        mean = 63
        nullmean = 60
        std = 15
        sides = 1
        power = 0.8
        ntotal = . ;
    title 'One-Sample Test of a Mean';
run;

proc power;
    twosamplemeans test = diff
        meandiff = 5
        stddev = 10
        sides = 2
        power = 0.9
        npergroup = . ;
    title 'Two-Sample Comparison of Means';
run;

proc power;
    twosamplefreq test = pchi
        groupproportions = (0.25 0.35)
        nullproportiondiff = 0
        power = 0.8
        sides = 2
        npergroup = . ;
    title 'Two-Sample Comparison of Proportions';
run;

```

The output (not shown here) gives sample sizes of $n = 156$, $n = 86$, and $n = 329$ per group for the three examples, respectively, which is within 1 or 2 of our approximated versions. (Note: The approximating formulas would not be appropriate for small sample sizes.)

PROC POWER can also be used to compute the power for a given sample size and effect size, and to compute the detectable effect size for a given sample size and power. This is a very versatile procedure which can also be used in the analysis-of-variance, multiple regression, and rank comparison of survival curves. A corresponding procedure, GLMPOWER, enables power and sample size calculations for more complex designs using linear statistical modeling under PROC GLM. New power and sample size analyses are available in SAS 9.2 and later for logistic regression and non-parametric procedures as well. For more information, see the *SAS/STAT User's Guide*.

2.6 Multiple Testing

Consider the alkaline phosphatase example discussed at the beginning of this chapter and suppose that two such studies are conducted, one in each of two independent centers. The significance level, α , is the probability of an erroneously significant finding, i.e., the probability of a significant result in Center 1 or in Center 2 when H_0 is true. Using the law of probability that states: For any event A,

$$\Pr(A) = 1 - \Pr(\text{not } A)$$

you have, $\alpha = 1 - \Pr(\text{a non-significant result in Centers 1 and 2, when } H_0 \text{ is true})$. If α_1 and α_2 represent the significance levels of the tests for Centers 1 and 2, respectively, you have,

$$\Pr(\text{a non-significant result in Center } i \text{ when } H_0 \text{ is true}) = 1 - \alpha_i \text{ (for } i = 1, 2)$$

Applying a law of probability which states that for any two independent events A and B,

$$\Pr(A \text{ and } B) = \Pr(A) \cdot \Pr(B)$$

you obtain $\alpha = 1 - (1 - \alpha_1)(1 - \alpha_2)$. If $\alpha_1 = \alpha_2 = 0.05$, you have

$$\alpha = 1 - (1 - 0.05)^2 = 0.0975$$

In general, if there are k centers or k independent tests, each conducted at a significance level of 0.05, the overall significance level, α , is

$$\alpha = 1 - (1 - 0.05)^k$$

which is seen to increase markedly even for small values of k, as shown here.

k	α
1	0.050
2	0.098
3	0.143
4	0.185
5	0.226
10	0.401
15	0.537
20	0.642

This illustrates a problem encountered with simultaneously conducting many hypothesis tests. Although the tests usually will not be independent, the fact is that the overall significance level will differ from the significance level at which the individual tests are performed.

Multiple hypothesis testing arises in a number of ways in the analysis of clinical trials data as discussed below, and the researcher must be aware of any effects on the overall conclusions resulting from these situations.

■ ***Multiple Comparisons of Treatment Response***

One type of multiple testing situation arises in the comparison of a single response variable among more than two randomized treatment groups. With three groups, for example, a low-active-dose group, a high-active-dose group, and a placebo group, you might want to compare the response of each active group to that of the placebo group, and compare the responses of the low-dose and the high-dose groups.

Multiple comparisons can be designed to answer specific questions, such as

- Which, if any, treatments are better than a control group?
- Does response improve with increasing doses of a treatment?
- Which of several doses is the most effective?
- What is the smallest effective dose?
- Which treatments are statistically inferior to the ‘best’?
- Is there a reversal of dose response at a higher dose?

In some cases, a statistical test designed to detect very specific alternative hypotheses can be applied to help answer such questions. The *Jonckheere-Terpstra test* for monotonic dose response and the *Cochran-Armitage test* for linear trend are two examples. (In SAS, you would use the JT and TREND options in the TABLES statement with PROC FREQ—see the *SAS/STAT User’s Guide* for details). Often, contrasts (linear functions) of group means can be used to help answer specific questions about the relationships of responses among groups. Sets of contrasts can be simultaneously tested by using multiple comparison methods to adjust the p-values in order to maintain control of the overall significance levels.

Most commonly, the goals of multiple comparisons of treatment effects are (i) to perform all pairwise comparisons among the treatment groups, and (ii) to test each active treatment group against a single control group.

With K ($K \geq 3$) groups, there are $K \cdot (K-1)/2$ possible pairwise comparisons. However, when comparing each treated group with a control, there are only $K-1$ comparisons, a substantial reduction from the case of all pairwise comparisons, as shown in Table 2.2.

TABLE 2.2 Number of Group Comparisons for K=3 to 8

Number of Groups	Number of Pairwise Comparisons	Number of Comparisons with 'Control'
		K-1
3	3	2
4	6	3
5	10	4
6	15	5
7	21	6
8	28	7

In a study with 5 dose groups, for example, there would be 10 possible pairwise comparisons. If each of these is conducted at a significance level of 0.05, the overall significance level is affected so that the likelihood of obtaining at least one erroneous finding might increase substantially. Fortunately, this problem is easily overcome by using one or more of the many approaches to multiple comparisons that will control overall significance levels.

A vast array of multiple testing methods is available in SAS. Multiple comparison procedures can be carried out by using appropriate options in the MEANS statement in PROC ANOVA or in PROC GLM, or by using the LSMEANS statement with the ADJUST= option in PROC GLM, PROC MIXED or PROC GLIMMIX. Certain multiple testing methods can also be conducted by using PROC MULTTEST or the PROBMC function in a DATA step. See Appendix E for some examples.

■ *Multiple Response Variables*

Another instance in which multiple testing arises is when conducting individual tests on many response variables. For example, testing for significant pre- to post-study changes in laboratory values by conducting individual *Z*- or *t*-tests on a large number of laboratory parameters might result in chance significance. With 20 independent *t*-tests each at a significance level of 0.05, you would expect one test result to be significant due to chance variation when there is no real deviation from the null hypothesis. If 30 or 50 or 100 tests are conducted on the laboratory data, although not independent, one might expect a number of these to be falsely significant.

Multiple testing situations might also arise when a study has more than one primary endpoint associated with establishing treatment efficacy. The overall significance level for efficacy depends on whether at least one, some, or all of the endpoints must individually attain a certain level of significance. Considerations must be given as to which combinations of primary response variables must show significance before treatment efficacy can be declared. Certain combinations might require use of a multivariate statistical method or an adjustment of the individual significance levels used to test each variable. Some of the many p-value adjustment

methods available to address this multiplicity problem are discussed in Appendix E in the context of multiple comparisons.

While very conservative, a simple Bonferroni adjustment is often used in these situations. For example, when efficacy may be claimed if just one of k co-primary response variables is significant, the *Bonferroni* method calls for testing each of the k response variables at a significance level of α/k to maintain an overall significance level of α . A less conservative method can be used by taking into account the correlations among the response variables. Pocock, et al. (1987) have shown that with k normally distributed response variables and a correlation of ρ between any pair, the tests can be conducted at a significance level that is slightly higher than the Bonferroni value. This is illustrated in Table 2.3 for a *two-tailed test* with an overall significance level of 0.05.

TABLE 2.3 Adjusted Significance Levels Needed to Maintain an Overall 0.05 Level When Testing k Co-Primary Endpoints with Correlation ρ

k	Bonferroni	$\rho = 0.3$	$\rho = 0.5$	$\rho = 0.7$	$\rho = 0.9$
2	0.025	0.026	0.027	0.029	0.035
3	0.017	0.018	0.019	0.022	0.029
4	0.012	0.013	0.015	0.017	0.025

■ *Interim Analyses*

Interim analyses of ongoing studies represent another situation involving multiple testing. Use of interim analyses has become highly accepted in large or lengthy clinical research studies. In some situations, it is looked upon as unethical to continue a study when there is overwhelming evidence of the efficacy of a new therapy. By continuing such a study, patients might receive a placebo or another less effective treatment that deprives them of the more effective treatment. Assuming there are no safety issues, it is generally preferable to make the new therapy available to patients as soon as possible.

When a decision is made to stop or to continue the study or to change the study in some fundamental way based on an interim look at the data, the final significance levels will be altered. *Group sequential methods* are special statistical approaches that can be applied to handle such problems, and in most cases, offer adjustments in order to maintain an overall significance level at a pre-determined value. The group sequential methods most commonly used in clinical trials include those described by Pocock (1977), O'Brien and Fleming (1979), and Lan and DeMets (1983). These are discussed in the sections that follow.

Because the issue of interim analyses can affect the overall significance level, careful planning at the design stage is very important in studies with anticipated interim analyses in order to protect the overall α . The study protocol and statistical analysis plans should specifically lay out the details, including

- the number of interim analyses that will be done
- when they will be conducted
- their purpose
- which variables will be analyzed
- how the analyses will be handled
- any adjustments to be made to the significance levels
- who will remain blinded.

When interim analyses occur without pre-planning, careful documentation must be kept to avoid compromising the study integrity.

To maintain an overall significance level, such as $\alpha = 0.05$, the interim analyses must be conducted at significance levels somewhat less than 0.05. One method is to conduct each interim analysis at a very small α -level, such as 0.001 or less, so that the final analysis can be conducted near the 0.05 level. This is a very conservative approach because it is extremely difficult to find a difference between treatment groups at interim testing at such a reduced α -level. However, it might accomplish the purpose of identifying overwhelming treatment differences early in the analysis while permitting almost a full α -level test at the final analysis.

Pocock's Approach

Pocock (1977) proposed a group sequential method whereby the analysis at each stage of testing, including the final analysis, is conducted at the same reduced significance level, α_p , in order to maintain an overall α of 0.05. Values of α_p are shown in Table 2.4 for 1 to 4 planned interim analyses. These ‘adjusted alphas’ can be obtained using PROC SEQDESIGN in SAS, as shown in Appendix H (Section H.2).

TABLE 2.4 Pocock’s α_p for $\alpha = 0.05$

Number of Analyses	Number of Interims	α_p
2	1	0.029
3	2	0.022
4	3	0.018
5	4	0.016

O'Brien-Fleming Approach

A drawback of Pocock's method is that the final analysis is conducted at a level much smaller than the 0.05 level. The O'Brien-Fleming approach (1979), probably the most widely used method in handling interim analyses of clinical trials, overcomes this objection, but at the expense of requiring even greater conservatism at the early interims. This method uses progressively increasing α levels at each interim analysis (say, α_{OF}), so that the final analysis is conducted close to the 0.05 level while maintaining an overall α of 0.05. As shown in Table 2.5, the final analysis is conducted at an α_{OF} level between 0.04 and 0.05 when there are 4 or fewer planned interim analyses.

TABLE 2.5 O'Brien-Fleming's α_{OF} for $\alpha = 0.05$

Number of Analyses	Number of Interims	Interim Analysis				Final
		1	2	3	4	
2	1	0.005				0.048
3	2	0.0005	0.014			0.045
4	3	0.00005	0.0042	0.019		0.043
5	4	0.00001	0.0013	0.008	0.023	0.041

For example, in a two-stage design that uses the O'Brien-Fleming approach with one scheduled interim analysis, hypothesis testing would be conducted at an interim significance level of $\alpha_{OF} = 0.005$. If significance is found in favor of the test treatment, the study may be stopped with sufficient statistical evidence of efficacy. If the 0.005 level of significance is not reached at the interim, the study continues to normal completion, at which time the hypothesis testing is conducted at a significance level of $\alpha_{OF} = 0.048$. This stagewise procedure will have an overall significance of $\alpha = 0.05$. These adjusted alpha levels can be obtained using the SEQDESIGN procedure in SAS, as demonstrated in Appendix H (Section H.3).

When a study is designed to include one or more interim analysis, sample sizes can be pre-determined to achieve the power you want in a way that is similar to that discussed previously in this chapter. Because the final analysis of a group sequential design is conducted at an alpha level smaller than the nominal $\alpha = 0.05$, sample size requirements for studies involving interim analyses are generally larger than for similar fixed sample size studies (i.e., those with no planned interim analyses). One of the features of the O'Brien-Fleming approach is that sample size requirements are very close to those of the fixed sample size study, usually no more than 2% to 3% higher, in order to achieve the same power. Furthermore, these sample sizes are generally smaller than those needed under Pocock's method.

Lan-DeMets α -Spending Function

The O'Brien-Fleming approach was developed with the assumption that a pre-specified number of interim analyses will be performed at approximately, equally spaced intervals during the study, based on patient accrual. Simulation studies have shown that the procedure is not greatly affected under non-extreme deviations from this assumption. In many cases, the number or timing of interim analyses cannot be pre-specified. Lengthy trials, for example, might simply request an interim every 6 months, and patient accrual might not be uniform through each of those 6-month periods.

Lan and DeMets (1983) introduced a method for handling the multiplicity problem when the number of interim analyses is not known at the planning stage. This method is based on an ' α -spending function' that allocates a portion of the overall significance level, α , for testing at each interim analysis, based on the amount of information available at that analysis ('information fraction').

The information fraction is usually based on the ratio of the number of patients available for interim analysis to the total anticipated sample size if the study were to go to completion. In some cases, the information fraction can be the fraction of elapsed time or, in the case of survival analysis, the number of deaths observed relative to the number of deaths expected.

A number of spending functions have been proposed for use with the Lan-DeMets method, including the spending function that is based on the O'Brien-Fleming approach. The rejection boundaries and interim testing levels can be obtained by using a computer program capable of evaluating multivariate normal probabilities. PROC SEQDESIGN and PROC SEQTEST, available in SAS 9.2 and later, can be used for this purpose.

Table 2.6 shows the portion of α available for all interim analyses up-to and including a specified information fraction. This cumulative α uses the O'Brien-Fleming spending function and assumes a *two-tailed symmetric test* with overall $\alpha = 0.05$. In Table 2.6, you see that only 0.00305 of the overall α (0.05) is allocated for all interim analyses conducted by the midpoint of the study (information fraction = 0.5). Appendix H (Section H.4) shows the SAS code to obtain the cumulative alphas shown in Table 2.6.

TABLE 2.6 Cumulative Lan-DeMets α -Spending Function for the O'Brien-Fleming Spending Function, Two-Tailed Test at Overall $\alpha = 0.05$

Information Fraction	Cumulative α
0.1	0.00000
0.2	0.00000
0.25	0.00001
0.3	0.00009
0.333	0.00021
0.4	0.00079
0.5	0.00305
0.6	0.00762
0.667	0.01210
0.7	0.01477
0.75	0.01930
0.8	0.02442
0.9	0.03629
1.0	0.05000

Interim analysis testing levels (α_{LD}) for the O'Brien-Fleming spending function under the Lan-DeMets method are shown in Table 2.7. These assume equally spaced interim analyses during the study and are based on a *two-tailed test* with an overall significance level of 0.05.

For example, a study involving three, equally spaced interim analyses would entail testing at the 0.044 level at the final analysis, in order to maintain an overall two-tailed α of 0.05 (assuming the study goes to planned completion). As seen in Table 2.7, stopping the study at any interim stage would require overwhelming evidence of efficacy, namely, significance at <0.0001 at the first stage, <0.003 at the second stage, or <0.018 at the third stage of testing. See Appendix H (Section H.5) for use of PROC SEQDESIGN to obtain the interim testing alpha levels found in Table 2.7.

TABLE 2.7 Lan-DeMets α_{LD} for Overall $\alpha = 0.05$ Using the O'Brien-Fleming Spending Function, Equally Spaced Interims

Number of Interims	Number of Analyses	Interim Analysis					Final
		1	2	3	4		
1	2	0.003					0.049
2	3	<0.001	0.012				0.046
3	4	<0.0001	0.003	0.018			0.044
4	5	<0.00001	<0.001	0.007	0.022		0.042

■ *Multiple Studies*

You've seen how overall significance levels can be affected by multiple treatment comparisons, multiple response variables, and multiple analyses during an ongoing study. Your overall conclusions about a drug development program can, likewise, be affected by multiple studies.

What if a health food company wanted to promote a new herbal product for some condition, such as weight loss or baldness? Obviously, it would be much easier to sell if the product's efficacy were supported by statistically significant results from a well-controlled, randomized study. Since some studies will result in a significant effect by chance, the company might be able to get such a result simply by conducting many studies. It's tempting to cite only the positive one in their advertising.

A variation of this practice occurs in many professional publications, where publishers of some medical journals are more likely to publish articles that show 'significant' results. This is understandable since the readers prefer to read 'interesting' results, i.e., those which depict a new treatment or procedure as significantly better than a standard. However, the significance is really questionable without information on how many other studies were conducted and how many had a different outcome.

Unfortunately, these non-scientific practices are common. You can easily see the effect on one's conclusion when conducting multiple clinical studies, but only reporting selected results. It is one reason ethical drug development is controlled by agencies that require drug developers to submit protocols for regulatory review before the study is conducted. Results from negative studies are then not so easy to 'hide'. It is also a reason why the FDA requires at least two independent, pivotal studies for most new drug applications.

There are many advocates for a universal clinical trial registration database that would include the results of all studies conducted on any given pharmaceutical product. In fact, great strides toward this objective have been made in recent years, with many drug firms participating. It's always important to put the results of any one study into context.

There are ways of consolidating study outcomes with methods such as meta-analysis. If numerous independent studies have been conducted on the same treatment, perhaps with differing outcomes, meta-analysis is a way of combining them to obtain an overall conclusion. This is a broad subject, and there are many books available showing the various techniques for meta-analysis.

In short, reproducibility is an important aspect of the scientific method, and the researcher must be cognizant of the effect of multiple studies when making statements about overall efficacy of a product. This is another of the multiple-testing issues that are important for clinical development of a new treatment.

2.7 Summary

This chapter presents a discussion of some of the most fundamental elements of hypothesis testing, including significance levels, power, p-values, illustration of the difference between *one-* and *two-tailed tests*, the idea behind sample size computations, and the effect of multiple testing on significance levels, including interim analyses. Many of the statistical terms used in this chapter are frequently used by clinical researchers, both statisticians and non-statisticians, from protocol development through regulatory submission. The concepts presented here form a basis for a general overview of hypothesis testing, the primary inferential tool used in presenting the statistical methods in Chapters 4 through 22.

CHAPTER 3

The Data Set TRIAL

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3.1 Introduction

This chapter presents data from a very simple, hypothetical clinical trial that will be used to illustrate many of the methods discussed throughout this book. First, through the use of very rudimentary case report forms (CRFs), you'll see how response data can be collected in various formats: dichotomous, categorical, or numeric. Then, one of several methods available in SAS for creating a data set from these CRFs is illustrated. The data set TRIAL is used to demonstrate some elementary data summarization methods using SAS. Finally, the data set TRIAL is used in the exercises presented in Chapter 23. The exercises are designed to give the reader practice in applying the statistical techniques discussed in this book.

Beginning with this chapter, this book assumes some elementary knowledge of the SAS DATA step and Base SAS procedures (e.g., PROC PRINT and PROC SORT). For more information about how to read data into SAS and working with variables, you can refer to *The Little SAS Book: A Primer* by Delwiche and Slaughter. Jack Shostak's book, *SAS Programming in the Pharmaceutical Industry*, is also an excellent reference and a companion book to this, as it is geared toward programming in SAS with emphasis on clinical research projects. Both of these books are published by SAS Press.

3.2 Data Collection

Consider a very simple trial used to study a clinical response from each of 100 patients. The design is a multi-center, randomized, double-blind, parallel study conducted at three study centers. Patients are randomized in equal numbers to one of two treatment groups. The objective is to compare the efficacy of experimental drug (A) with that of reference drug (B) in reducing or eliminating the symptoms of an unspecified disease. The primary response variable that measures the severity of the symptoms is obtained by using a patient-rated global assessment at the end of the trial. Three types of responses are considered:

- presence or absence of symptoms (a dichotomous response)
- discrete severity rating (an ordered categorical response)
- percentile rating using a visual analog scale (VAS) (a continuous numeric response).

Sample CRFs, which illustrate how these responses might be obtained, are shown in Figures 3.1 to 3.3. The forms include basic patient information (patient identification, age, and gender), site number, and visit date in addition to the response measure. These forms would typically be filled out upon examination of the patient by the physician, physician's assistant, or the site's clinical research monitor.

FIGURE 3.1 Sample Case Report Form I (Dichotomous Response)

PROTOCOL NO. _____	SITE NO. _____	
PATIENT INFORMATION		
PATIENT NO. _____	PATIENT INITIALS <input type="text"/> <input type="text"/> <input type="text"/>	VISIT DATE <input type="text"/> <input type="text"/> <input type="text"/> mm <input type="text"/> <input type="text"/> dd <input type="text"/> yy
AGE (years) _____	GENDER: <input type="text"/> <input type="text"/> Male Female	
GLOBAL ASSESSMENT [To be completed at study termination]		
Are symptoms present now?		
YES <input type="text"/>	NO <input type="text"/>	

FIGURE 3.2 Sample Case Report Form 2 (Categorical Response)

PROTOCOL NO. _____	SITE NO. _____		
PATIENT INFORMATION			
PATIENT NO. _____	PATIENT INITIALS <input type="text"/> <input type="text"/> <input type="text"/>	VISIT DATE <input type="text"/> <input type="text"/> <input type="text"/> mm dd yy	
AGE (years) _____	GENDER: <input type="text"/> <input type="text"/> Male Female		
GLOBAL ASSESSMENT [To be completed at study termination] Check box corresponding to how your symptoms are now			
No Symptoms <input type="checkbox"/>	Mild <input type="checkbox"/>	Moderate <input type="checkbox"/>	Severe <input type="checkbox"/>

FIGURE 3.3 Sample Case Report Form 3 (Continuous Numeric Response)

PROTOCOL NO. _____	SITE NO. _____	
PATIENT INFORMATION		
PATIENT NO. _____	PATIENT INITIALS <input type="text"/> <input type="text"/> <input type="text"/>	VISIT DATE <input type="text"/> <input type="text"/> <input type="text"/> mm dd yy
AGE (years) _____	GENDER: <input type="text"/> <input type="text"/> Male Female	
GLOBAL ASSESSMENT [To be completed at study termination] Place an 'X' on the line corresponding to how you feel now		
0 (No symptoms)	100 (Most severe)	

3.3 Creating the Data Set TRIAL

Create and store the data set TRIAL in the directory bookfiles\examples\sas on Drive C. The SAS code for creating this data set is shown below.

All three response types are input as follows:

- The dichotomous response variable is named RESP. It has coded values of 0 (if symptoms are absent) and 1 (if symptoms are present);
- The categorical response variable is named SEV. It is coded as 0, 1, 2, or 3 for ‘none’, ‘mild’, ‘moderate’, and ‘severe’;
- The continuous numeric response variable is named SCORE. Its values can range between 0 and 100. These values represent the percentage of the greatest possible severity perceived by the patient.

Because these data are used for illustrative purposes only, the values of the SCORE variable ❶ are entered and, for ease in constructing the data set, the other response variables (RESP and SEV) are computed rather than entered manually. RESP is set to 0 or 1, based on whether SCORE is 0 or >0, respectively ❷. The severity category (SEV) field is created as follows: SCORE values of 1 to 30 are defined as ‘Mild’; 31 to 69, ‘Moderate’; and 70 to 100, ‘Severe’ ❸.

SAS Code for Creating the Data Set TRIAL

```
libname examp 'c:\bookfiles\examples\sas';

data examp.trial;
  input trt $ center pat sex $ age score @@; ❶
  resp = (score gt 0);
  /* resp=0 (symptoms are absent), =1 (symptoms are present) */ ❷
  if (score = 0) then sev = 0;          /* "No Symptoms" */
  if ( 1 le score le 30) then sev = 1; /* "Mild Symptoms" */
  if (31 le score le 69) then sev = 2; /* "Moderate Symptoms" */
  if (score ge 70) then sev = 3;       /* "Severe Symptoms" */
  datalines;
A 1 101 M 55 5    A 1 104 F 27 0    A 1 106 M 31 35
A 1 107 F 44 21   A 1 109 M 47 15   A 1 111 F 69 70
A 1 112 F 31 10   A 1 114 F 50 0    A 1 116 M 32 20
A 1 118 F 39 25   A 1 119 F 54 0    A 1 121 M 70 38
A 1 123 F 57 55   A 1 124 M 37 18   A 1 126 F 41 0
A 1 128 F 48 8    A 1 131 F 35 0    A 1 134 F 28 0
A 1 135 M 27 40   A 1 138 F 42 12   A 2 202 M 58 68
A 2 203 M 42 22   A 2 206 M 26 30   A 2 207 F 36 0
A 2 210 F 35 25   A 2 211 M 51 0    A 2 214 M 51 60
A 2 216 F 42 15   A 2 217 F 50 50   A 2 219 F 41 35
A 2 222 F 59 0    A 2 223 F 38 10   A 2 225 F 32 0
A 2 226 F 28 16   A 2 229 M 42 48   A 2 231 F 51 45
A 2 234 F 26 90   A 2 235 M 42 0    A 3 301 M 38 28
A 3 302 M 41 20   A 3 304 M 65 75   A 3 306 F 64 0
A 3 307 F 30 30   A 3 309 F 64 5    A 3 311 M 39 80
```

```

A 3 314 F 57 85    A 3 315 M 61 12    A 3 318 F 45 95
A 3 319 F 34 26    A 3 321 M 39 10    A 3 324 M 27  0
A 3 325 F 56 35    B 1 102 M 19 68    B 1 103 F 51 10
B 1 105 M 45 20    B 1 108 F 44 65    B 1 110 M 32 25
B 1 113 M 61 75    B 1 115 M 45 83    B 1 117 F 21  0
B 1 120 F 19 55    B 1 122 F 38  0    B 1 125 M 37 72
B 1 127 F 53 40    B 1 129 M 48  0    B 1 130 F 36 80
B 1 132 M 49 20    B 1 133 F 28  0    B 1 136 F 34 45
B 1 137 F 57 95    B 1 139 F 47 40    B 1 140 M 29  0
B 2 201 F 63 10    B 2 204 M 36 49    B 2 205 M 36 16
B 2 208 F 48 12    B 2 209 F 42 40    B 2 212 F 32  0
B 2 213 M 24 88    B 2 215 M 40 59    B 2 218 M 31 24
B 2 220 F 45 72    B 2 221 F 27 55    B 2 224 M 56 70
B 2 227 F 41  0    B 2 228 F 24 65    B 2 230 M 44 30
B 2 232 M 37 32    B 2 233 F 33  0    B 3 303 M 40 26
B 3 305 M 46 15    B 3 308 M 59 82    B 3 310 F 62 38
B 3 312 M 52 40    B 3 313 F 33 40    B 3 316 M 62 87
B 3 317 M 52 60    B 3 320 F 32  2    B 3 322 F 43  0
B 3 323 F 51 35
;

```

First, sort the data set by patient number, and then obtain a printout (see **OUTPUT 3.1**) by using the following SAS statements:

```

proc sort data = examp.trial;
   by pat;
run;
proc print data = examp.trial;
   var pat trt center sex age resp sev score;
   title 'Printout of Data Set TRIAL, Sorted by PAT';
run;

```

OUTPUT 3.1 Results from PROC PRINT: The Data Set TRIAL

Printout of Data Set TRIAL, Sorted by PAT									
Obs	pat	trt	center	sex	age	resp	sev	score	
1	101	A	1	M	55	1	1	5	
2	102	B	1	M	19	1	2	68	
3	103	B	1	F	51	1	1	10	
4	104	A	1	F	27	0	0	0	
5	105	B	1	M	45	1	1	20	
6	106	A	1	M	31	1	2	35	
7	107	A	1	F	44	1	1	21	
8	108	B	1	F	44	1	2	65	
9	109	A	1	M	47	1	1	15	
10	110	B	1	M	32	1	1	25	
11	111	A	1	F	69	1	3	70	
12	112	A	1	F	31	1	1	10	
13	113	B	1	M	61	1	3	75	
14	114	A	1	F	50	0	0	0	
15	115	B	1	M	45	1	3	83	
16	116	A	1	M	32	1	1	20	
17	117	B	1	F	21	0	0	0	
18	118	A	1	F	39	1	1	25	
19	119	A	1	F	54	0	0	0	
20	120	B	1	F	19	1	2	55	
21	121	A	1	M	70	1	2	38	

OUTPUT 3.1 Results from PROC PRINT: The Data Set TRIAL (continued)

22	122	B	1	F	38	0	0	0
23	123	A	1	F	57	1	2	55
24	124	A	1	M	37	1	1	18
25	125	B	1	M	37	1	3	72
26	126	A	1	F	41	0	0	0
27	127	B	1	F	53	1	2	40
28	128	A	1	F	48	1	1	8
29	129	B	1	M	48	0	0	0
30	130	B	1	F	36	1	3	80
31	131	A	1	F	35	0	0	0
32	132	B	1	M	49	1	1	20
33	133	B	1	F	28	0	0	0
34	134	A	1	F	28	0	0	0
35	135	A	1	M	27	1	2	40
36	136	B	1	F	34	1	2	45
37	137	B	1	F	57	1	3	95
38	138	A	1	F	42	1	1	12
39	139	B	1	F	47	1	2	40
40	140	B	1	M	29	0	0	0
41	201	B	2	F	63	1	1	10
42	202	A	2	M	58	1	2	68
43	203	A	2	M	42	1	1	22
44	204	B	2	M	36	1	2	49
45	205	B	2	M	36	1	1	16
46	206	A	2	M	26	1	1	30
47	207	A	2	F	36	0	0	0
48	208	B	2	F	48	1	1	12
49	209	B	2	F	42	1	2	40
50	210	A	2	F	35	1	1	25
51	211	A	2	M	51	0	0	0
52	212	B	2	F	32	0	0	0
53	213	B	2	M	24	1	3	88
54	214	A	2	M	51	1	2	60
55	215	B	2	M	40	1	2	59
56	216	A	2	F	42	1	1	15
57	217	A	2	F	50	1	2	50
58	218	B	2	M	31	1	1	24
59	219	A	2	F	41	1	2	35
60	220	B	2	F	45	1	3	72
61	221	B	2	F	27	1	2	55
62	222	A	2	F	59	0	0	0
63	223	A	2	F	38	1	1	10
64	224	B	2	M	56	1	3	70
65	225	A	2	F	32	0	0	0
66	226	A	2	F	28	1	1	16
67	227	B	2	F	41	0	0	0
68	228	B	2	F	24	1	2	65
69	229	A	2	M	42	1	2	48
70	230	B	2	M	44	1	1	30
71	231	A	2	F	51	1	2	45
72	232	B	2	M	37	1	2	32
73	233	B	2	F	33	0	0	0
74	234	A	2	F	26	1	3	90
75	235	A	2	M	42	0	0	0
76	301	A	3	M	38	1	1	28
77	302	A	3	M	41	1	1	20
78	303	B	3	M	40	1	1	26
79	304	A	3	M	65	1	3	75
80	305	B	3	M	46	1	1	15

OUTPUT 3.1 Results from PROC PRINT: The Data Set TRIAL (continued)

81	306	A	3	F	64	0	0	0
82	307	A	3	F	30	1	1	30
83	308	B	3	M	59	1	3	82
84	309	A	3	F	64	1	1	5
86	311	A	3	M	39	1	3	80
87	312	B	3	M	52	1	2	40
88	313	B	3	F	33	1	2	40
89	314	A	3	F	57	1	3	85
90	315	A	3	M	61	1	1	12
91	316	B	3	M	62	1	3	87
92	317	B	3	M	52	1	2	60
93	318	A	3	F	45	1	3	95
94	319	A	3	F	34	1	1	26
95	320	B	3	F	32	1	1	2
96	321	A	3	M	39	1	1	10
97	322	B	3	F	43	0	0	0
98	323	B	3	F	51	1	2	35
99	324	A	3	M	27	0	0	0
100	325	A	3	F	56	1	2	35

3.4 Statistical Summarization

- Data summarization would naturally begin by using simple descriptive summaries, starting with the summary statistics for the variable SCORE for each treatment group.

First use PROC MEANS to summarize the numeric variables, SCORE, and AGE for each treatment group (see **OUTPUT 3.2**).

```
proc sort data = examp.trial;
   by trt;
run;

proc means mean std n min max data = examp.trial;
   by trt;
   var score age;
   title "Summary Statistics for 'SCORE' and 'AGE' Variables";
run;
```

Now, use PROC UNIVARIATE (see **OUTPUT 3.3**).

```
proc univariate data = examp.trial;
   by trt;
   var score;
   title "Expanded Summary Statistics for 'SCORE' ";
run;
```

- Next, write the mean and median values for SCORE for each treatment group and study center combination to an output data set, and then print the results (see **OUTPUT 3.4**).

```

proc sort data = examp.trial;
  by trt center;
run;
proc univariate noint data = examp.trial;
  by trt center;
  var score;
  output out = summry
    n      = num
    mean   = avescore
    median = medscore;
run;
proc print data = summry;
  title "Summary Statistics for 'SCORE' by Treatment
        Group & Study Center";
run;

```

- Visualize the distribution of scores using a histogram (see **OUTPUT 3.5**).

```

proc format;
  value $trtfmt 'A' = 'trt = A'  'B' = 'trt = B';
run;
proc univariate data=examp.trial noint;
  class trt;
  histogram score / midpoints = 10 30 50 70 90 nrows = 2
    cfill=ltgray;
  format trt $trtfmt.;
  title h=1 "Histogram of 'SCORE' by Treatment Group";
run;

```

- Obtain response rates by treatment group using PROC FREQ (see **OUTPUT 3.6**).

```

proc format;
  value rspfmt  0 = '0=Abs.'  1 = '1=Pres';
run;

proc freq data = examp.trial;
  tables trt*resp / nocol nopct;
  format resp rspfmt.;
  title 'Summary of Response Rates by Treatment Group';
run;

```

- Obtain the frequency tables for the severity categories using PROC FREQ (see **OUTPUT 3.7**).

```

proc format;
  value sevfmt  0 = '0=None'
                1 = '1=Mild'
                2 = '2=Mod.'
                3 = '3=Sev.'  ;
run;

```

```

proc freq data = examp.trial;
  tables trt*sev / nocol nopct;
  format sev sevfmt.;
  title 'Severity Distribution by Treatment Group';
run;

```

- Obtain the frequency distribution of severity category by treatment group stratified by SEX (see **OUTPUT 3.8**).

```

proc freq data = examp.trial;
  tables sex*trt*sev / nocol nopct;
  format sev sevfmt.;
  title 'Severity Distribution by Treatment Group and Sex';
run;

```

- Obtain a histogram of severity for each treatment group (see **OUTPUT 3.9**).

```

proc format;
  value $trtfmt 'A' = 'trt = A' 'B' = 'trt = B';
run;
proc univariate data=examp.trial noprint;
  class trt;
  histogram sev / midpoints = 0 1 2 3 nrows = 2 cfill=ltgray;
  format trt $trtfmt.;
  title h=1 "Histogram of 'SEV' by Treatment Group";
run;

```

The SORT, PRINT, FORMAT, MEANS, UNIVARIATE, and FREQ procedures represent some of the most basic SAS procedures. The SAS analyses used throughout this book require familiarity of these basic procedures. More information about these and other SAS procedures, including syntax and available options, can be found in numerous SAS books (e.g., Delwiche and Slaughter (2008), Elliott (2000), the *Base SAS Procedures Guide*, or SAS Help and Documentation). The output generated from the preceding code provides a small sampling of the types of summaries available from the vast wealth of summarization possibilities using the various SAS procedures and options.

OUTPUT 3.2 Results from PROC MEANS

Summary Statistics for 'SCORE' and 'AGE' Variables					
----- trt=A -----					
The MEANS Procedure					
Variable	Mean	Std Dev	N	Minimum	Maximum
score	26.6730769	26.9507329	52	0	95.0000000
age	43.7307692	12.2187824	52	26.0000000	70.0000000
----- trt=B -----					
Variable	Mean	Std Dev	N	Minimum	Maximum
score	38.3333333	29.6937083	48	0	95.0000000
age	41.3333333	11.7949382	48	19.0000000	63.0000000

OUTPUT 3.3 Results from PROC UNIVARIATE

Expanded Summary Statistics for 'SCORE'			
----- trt=A -----			
The UNIVARIATE Procedure			
Variable: score			
Moments			
N	52	Sum Weights	52
Mean	26.6730769	Sum Observations	1387
Std Deviation	26.9507329	Variance	726.342006
Skewness	1.02297616	Kurtosis	0.13964333
Uncorrected SS	74039	Corrected SS	37043.4423
Coeff Variation	101.04096	Std Error Mean	3.73739421
Basic Statistical Measures			
Location		Variability	
Mean	26.67308	Std Deviation	26.95073
Median	20.00000	Variance	726.34201
Mode	0.00000	Range	95.00000
		Interquartile Range	36.50000
Tests for Location: Mu0=0			
Test	-Statistic-	-----p Value-----	
Student's t	t 7.136811	Pr > t	<.0001
Sign	M 19.5	Pr >= M	<.0001
Signed Rank	S 390	Pr >= S	<.0001
Quantiles (Definition 5)			
Quantile Estimate			
100% Max	95.0		
99%	95.0		
95%	85.0		
90%	70.0		
75% Q3	39.0		
50% Median	20.0		
25% Q1	2.5		
10%	0.0		
5%	0.0		
1%	0.0		
0% Min	0.0		
Extreme Observations			
-----Lowest----		----Highest---	
Value	Obs	Value	Obs
0	51	75	41
0	42	80	45
0	38	85	46
0	33	90	37
0	31	95	48

OUTPUT 3.3 Results from PROC UNIVARIATE (continued)

Expanded Summary Statistics for 'SCORE'			
----- trt=B -----			
Moments			
N 48 Sum Weights 48 Mean 38.3333333 Sum Observations 1840 Std Deviation 29.6937083 Variance 881.716312 Skewness 0.21433323 Kurtosis -1.2051435 Uncorrected SS 111974 Corrected SS 41440.6667 Coeff Variation 77.4618477 Std Error Mean 4.28591762			
Basic Statistical Measures			
Location Variability			
Mean 38.33333 Std Deviation 29.69371 Median 39.00000 Variance 881.71631 Mode 0.00000 Range 95.00000 Interquartile Range 54.00000			
Tests for Location: Mu0=0			
Test -Statistic- -----p Value-----			
Student's t t 8.94402 Pr > t <.0001 Sign M 19.5 Pr >= M <.0001 Signed Rank S 390 Pr >= S <.0001			
Quantiles (Definition 5)			
Quantile Estimate			
100% Max 95 99% 95 95% 87 90% 82 75% Q3 65 50% Median 39 25% Q1 11 10% 0 5% 0 1% 0 0% Min 0			
Extreme Observations			
----Lowest---- -----Highest---			
Value Obs Value Obs			
0 99 82 92 0 89 83 59 0 85 87 96 0 78 88 79 0 72 95 70			

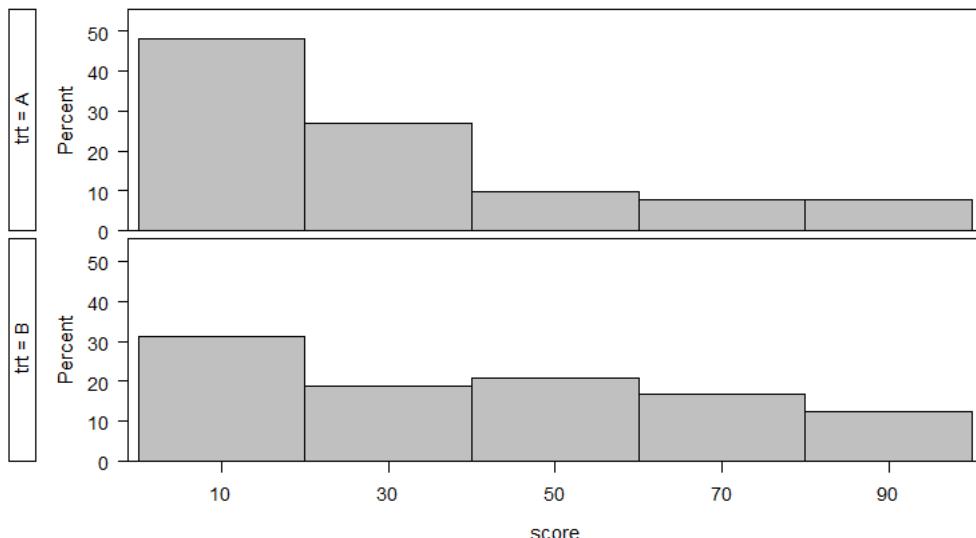
OUTPUT 3.4 Summary Statistics in an Output Data Set

Summary Statistics for 'SCORE' by Treatment Group & Study Center

Obs	trt	center	num	avescore	medscore
1	A	1	20	18.6000	13.5
2	A	2	18	28.5556	23.5
3	A	3	14	35.7857	27.0
4	B	1	20	39.6500	40.0
5	B	2	17	36.5882	32.0
6	B	3	11	38.6364	38.0

OUTPUT 3.5 Histogram from PROC UNIVARIATE

Histogram of 'SCORE' by Treatment Group



OUTPUT 3.6 Results from PROC FREQ: Dichotomous Response Frequencies

Summary of Response Rates by Treatment Group

The FREQ Procedure
Table of trt by resp

trt	resp	Frequency	0=Abs.	1=Pres	Total
A					
		13		39	52
		25.00		75.00	
B					
		9		39	48
		18.75		81.25	
Total		22		78	100

OUTPUT 3.7 Results from PROC FREQ: Severity Frequencies by Treatment Group

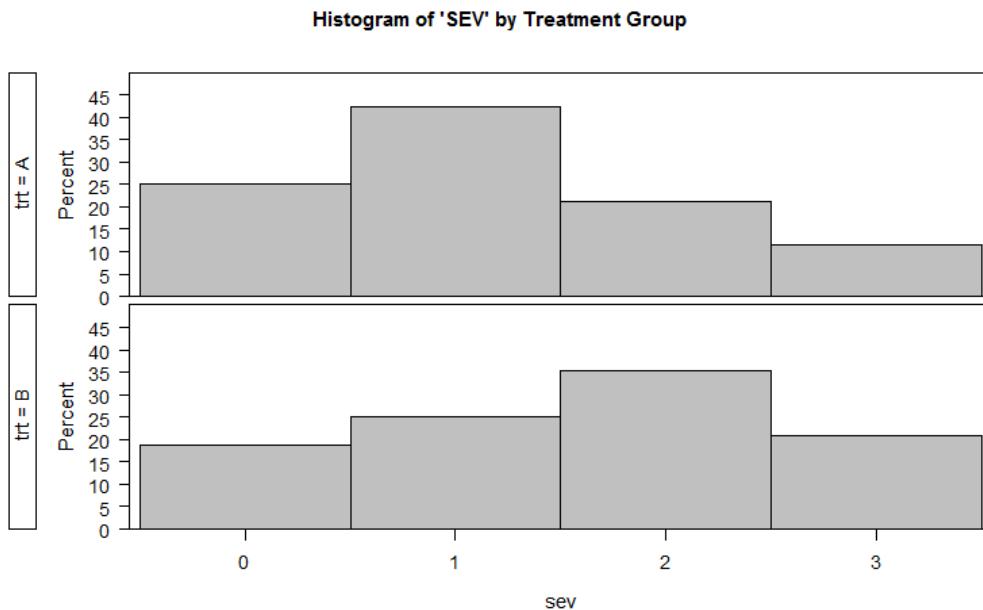
Severity Distribution by Treatment Group					
The FREQ Procedure					
Table of trt by sev					
trt	sev				
Frequency	Row Pct	0=None	1=Mild	2=Mod.	3=Sev. Total
A		13	22	11	6 52
		25.00	42.31	21.15	11.54
B		9	12	17	10 48
		18.75	25.00	35.42	20.83
Total		22	34	28	16 100

OUTPUT 3.8 Results from PROC FREQ: Severity Frequencies by Gender and Treatment Group

Severity Distribution by Treatment Group and Sex					
The FREQ Procedure					
Table 1 of trt by sev Controlling for sex=F					
trt	sev				
Frequency	Row Pct	0=None	1=Mild	2=Mod.	3=Sev. Total
A		10	12	5	4 31
		32.26	38.71	16.13	12.90
B		7	4	11	3 25
		28.00	16.00	44.00	12.00
Total		17	16	16	7 56

Table 2 of trt by sev Controlling for sex=M					
trt	sev				
Frequency	Row Pct	0=None	1=Mild	2=Mod.	3=Sev. Total
A		3	10	6	2 21
		14.29	47.62	28.57	9.52
B		2	8	6	7 23
		8.70	34.78	26.09	30.43
Total		5	18	12	9 44

OUTPUT 3.9 Results from PROC UNIVARIATE: SEV by Treatment Group



3.5 Summary

This chapter illustrates how data from a simplified hypothetical clinical trial can be collected and entered into a SAS data set, and how simple statistical summaries and graphics can be obtained for the key data. Three types of response variables are presented: a dichotomous binary variable (**resp**), an ordered categorical variable (**sev**), and a continuous numeric variable (**score**). These types of responses are representative of those most often encountered in clinical studies. The data set **trial** is used in the exercises in Chapter 23.

CHAPTER 4

The One-Sample *t*-Test

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4.1 Introduction

The *one-sample t-test* is used to infer whether an unknown population mean differs from a hypothesized value. The test is based on a single sample of ‘n’ measurements from the population.

A special case of the *one-sample t-test* is often used to determine if a mean response changes under different experimental conditions by using paired observations. Paired measurements most often arise from repeated observations for the same patient, either at two different time points (e.g., pre- and post-study) or from two different treatments given to the same patient. Because the analyst uses the changes (differences) in the paired measurements, this test is sometimes referred to as the ‘paired-difference’ *t*-test. A hypothesized mean difference of 0 might be interpreted as ‘no clinical response’.

The *one-sample t-test*, along with its two-sample counterpart presented in the next chapter, have undoubtedly been the most frequently cited inferential tests used in statistics.

4.2 Synopsis

A sample of n data points, y_1, y_2, \dots, y_n , is randomly selected from a normally distributed population with unknown mean, μ . This mean is estimated by the sample mean, \bar{y} . You hypothesize the mean to be some value, μ_0 . The greater the deviation between \bar{y} and μ_0 , the greater the evidence that the hypothesis is untrue.

The test statistic t is a function of this deviation, standardized by the standard error of \bar{y} , namely, s/\sqrt{n} . Large values of t will lead to rejection of the null hypothesis,

H_0 . When H_0 is true, t has the Student-t probability distribution with $n-1$ degrees of freedom.

The *one-sample t-test* is summarized as follows:

null hypothesis:	$H_0: \mu = \mu_0$
alt. hypothesis:	$H_A: \mu \neq \mu_0$
test statistic:	$t = \frac{\bar{y} - \mu_0}{s / \sqrt{n}}$
decision rule:	reject H_0 if $ t > t_{\alpha/2, n-1}$

The value $t_{\alpha/2, n-1}$ represents the ‘critical t-value’ of the Student t-distribution at a two-tailed significance level, α , and $n-1$ degrees of freedom. This value can be obtained from Appendix A.2 or from SAS, as described later (see Section 4.4.4).

The *one-sample t-test* is often used in matched-pairs situations, such as testing for pre- to post-study changes. The goal is to determine whether a difference exists between two time points or between two treatments based on data values from the same patients for both measures. The ‘paired-difference’ t-test is conducted using the procedures outlined for the *one-sample t-test* with μ =the mean difference (sometimes denoted μ_d), $\mu_0= 0$, and the y_i ’s = the paired-differences. Examples 4.1 and 4.2 demonstrate application of the *one-sample t-test* in a non-matched and a matched situation, respectively.

4.3 Examples

¶ Example 4.1—Body-Mass Index

In a number of previous Phase I and II studies of male, non-insulin-dependent diabetic (NIDDM) patients conducted by Mylitech Biosystems, Inc., the mean body mass index (BMI) was found to be 28.4. An investigator has 17 male NIDDM patients enrolled in a new study and wants to know if the BMI from this sample is consistent with previous findings. BMI is computed as the ratio of weight in kilograms to the square of the height in meters. What conclusion can the investigator make based on the following height and weight data from his 17 patients?

TABLE 4.1 Raw Data for Example 4.1

Patient Number	Height (cm)	Weight (kg)	Patient Number	Height (cm)	Weight (Kg)
1	178	101.7	10	183	97.8
2	170	97.1	11	n/a	78.7
3	191	114.2	12	172	77.5
4	179	101.9	13	183	102.8
5	182	93.1	14	169	81.1
6	177	108.1	15	177	102.1
7	184	85.0	16	180	112.1
8	182	89.1	17	184	89.7
9	179	95.8			

Solution

First, calculate BMI as follows: convert height in centimeters to meters by dividing by 100, square this quantity, and then divide it into the weight. BMI data (y_i) are shown below, along with the squares and summations. Because no height measurement is available for Patient No. 11, BMI cannot be computed and is considered a missing value.

TABLE 4.2 Computation of Sum of Squares / Example 4.1

Patient Number	BMI (y)	BMI^2 (y^2)	Patient Number	BMI (y)	BMI^2 (y^2)
1	32.1	1030.30	10	29.2	852.85
2	33.6	1128.87	11	.	.
3	31.3	979.94	12	26.2	686.26
4	31.8	1011.43	13	30.7	942.28
5	28.1	789.98	14	28.4	806.30
6	34.5	1190.58	15	32.6	1062.08
7	25.1	630.33	16	34.6	1197.07
8	26.9	723.55	17	26.5	701.96
9	29.9	893.96			

TOTALS $\Sigma y_i = 481.5$

$\Sigma (y_i)^2 = 14627.74$

The sample mean BMI, \bar{y} , and standard deviation, s , are computed from the $n=16$ patients with non-missing data as follows. The formula shown for s is equivalent to that given in Chapter 1, but it's in a more convenient format for manual computations.

$$\bar{y} = \frac{\sum y_i}{n} = \frac{481.5}{16} = 30.093$$

and

$$s = \sqrt{\frac{\sum y_i^2 - n \cdot (\bar{y})^2}{(n - 1)}} = \sqrt{\frac{14627.74 - 16 \cdot (30.093)^2}{15}} = 3.03$$

With a 0.05 significance level, the test summary becomes

null hypothesis:	$H_0: \mu = 28.4$
alt. hypothesis:	$H_A: \mu \neq 28.4$
test statistic:	$t = \frac{\bar{y} - \mu_0}{s/\sqrt{n}} = \frac{30.093 - 28.4}{3.03/\sqrt{16}} = 2.23$
decision rule:	reject H_0 if $ t > t_{0.025, 15} = 2.131$
conclusion:	Because $2.23 > 2.131$, you reject H_0 and conclude that the BMI of the new patients differs from that of the patients studied previously.

SAS Analysis of Example 4.1

The SAS code and output for analyzing this data set are shown on the next two pages. The data are read into the data set `diab` ① using the INPUT statement ②. (The INFILE statement can be used if the data are already in an external file.) The response variable `BMI` (body mass index) is computed ③ as each record is read in.

The program prints a listing of the data by using PROC PRINT ④. The *t-test* is conducted with the TTEST procedure, specifying the null value of 28.4 for H_0 ⑤. The output shows a t-statistic of 2.23 ⑥, which corroborates the manual calculations performed. The test is significant if the p-value ⑦ is less than the significance level of the test. In this case, you reject the null hypothesis at a two-tailed significance level of 0.05 because the p-value of 0.041 is less than 0.05.

The output automatically shows the BMI summary statistics, namely, the mean **❸** and the standard deviation **❹**. The standard error and the upper and lower confidence limits associated with a 95% confidence interval are also shown for both the mean and standard deviation.

SAS Code for Example 4.I

```
data diab;
    input patno wt_kg ht_cm @@;
    bmi = wt_kg / ((ht_cm/100)**2); ❶
    datalines; ❷
    1 101.7 178      2  97.1 170
    3 114.2 191      4 101.9 179
    5  93.1 182      6 108.1 177
    7  85.0 184      8  89.1 182
    9  95.8 179      10 97.8 183
   11  78.7   .      12 77.5 172
   13 102.8 183      14 81.1 169
   15 102.1 177      16 112.1 180
   17  89.7 184
;

proc print data = diab; ❸
    var patno ht_cm wt_kg bmi;
    format bmi 5.1;
    title1 'One-Sample t-Test';
    title2 'EXAMPLE 4.1: Body-Mass Index Data';
run;

proc ttest h0=28.4 data=diab; ❹
    var bmi;
run;
```

Output 4.1 SAS Output for Example 4.1

One-Sample t-Test EXAMPLE 4.1: Body-Mass Index Data					
Obs	patno	ht_cm	wt_kg	bmi	
1	1	178	101.7	32.1	
2	2	170	97.1	33.6	
3	3	191	114.2	31.3	
4	4	179	101.9	31.8	
5	5	182	93.1	28.1	
6	6	177	108.1	34.5	
7	7	184	85.0	25.1	④
8	8	182	89.1	26.9	
9	9	179	95.8	29.9	
10	10	183	97.8	29.2	
11	11	.	78.7	.	
12	12	172	77.5	26.2	
13	13	183	102.8	30.7	
14	14	169	81.1	28.4	
15	15	177	102.1	32.6	
16	16	180	112.1	34.6	
17	17	184	89.7	26.5	

The TTEST Procedure					
Variable: bmi					
N	Mean	Std Dev	Std Err	Minimum	Maximum
16	30.0934 ⑧	3.0323 ⑨	0.7581	25.1063	34.5988
Mean	95% CL Mean	Std Dev	95% CL Std Dev		
30.0394	28.4776 31.7092	3.0323	2.2400 4.6930		
DF	t Value	Pr > t			
15	2.23 ⑥	0.0411 ⑦			

Example 4.2—Paired-Difference in Weight Loss

Mylitech is developing a new appetite suppressing compound for use in weight reduction. A preliminary study of 35 obese patients provided the following data on patients' body weights (in pounds) before and after 10 weeks of treatment with the new compound. Does the new treatment look at all promising?

TABLE 4.3. Raw Data for Example 4.2

Subject Number	Weight In Pounds		Subject Number	Weight In Pounds	
	Pre-	Post-		Pre-	Post-
1	165	160	19	177	171
2	202	200	20	181	170
3	256	259	21	148	154
4	155	156	22	167	170
5	135	134	23	190	180
6	175	162	24	165	154
7	180	187	25	155	150
8	174	172	26	153	145
9	136	138	27	205	206
10	168	162	28	186	184
11	207	197	29	178	166
12	155	155	30	129	132
13	220	205	31	125	127
14	163	153	32	165	169
15	159	150	33	156	158
16	253	255	34	170	161
17	138	128	35	145	152
18	287	280			

Solution

First, calculate the weight loss for each patient (y_i) by subtracting the ‘post’- weight from the ‘pre’- weight. The mean and standard deviation of these differences are $\bar{y}_d = 3.457$ and $s = 6.340$ based on $n = 35$ patients. A test for significant mean weight loss is equivalent to testing whether the mean loss, μ_d , is greater than 0. You use a one-tailed test because the interest is in weight loss (not weight ‘change’). The hypothesis test is summarized as follows:

- | | |
|-------------------------|---|
| null hypothesis: | $H_0: \mu_d = 0$ |
| alt. hypothesis: | $H_A: \mu_d > 0$ |
| test statistic: | $t = \frac{\bar{y}_d}{s_d / \sqrt{n}} = \frac{3.457}{6.430 / \sqrt{35}} = 3.23$ |
| decision rule: | reject H_0 if $t > t_{0.05,34} = 1.691$ |
| conclusion: | Because $3.23 > 1.691$, you reject H_0 at a significance level of 0.05, concluding that a significant weight loss occurs with treatment. |

SAS Analysis of Example 4.2

The SAS code and output for analyzing this data set are shown below. The program computes the paired differences (variable=wtloss) ⑩ as the data are read into the data set obese. The program prints a listing of the data by using PROC PRINT. A part of the listing is shown in Output 4.2 ⑪.

As in Example 4.1, PROC TTEST without a CLASS statement can be used to conduct the paired t-test. In this case, you tell SAS to compute the differences, wtloss, so you can simply include the var wtloss statement with PROC TTEST. Alternatively, you may omit the VAR statement and include the PAIRED statement followed by the pair of variables being compared, separated by an asterisk (*). This eliminates the need to compute the differences in a separate DATA step.

This example illustrates the PAIRED statement by including the two variables, wtpre and wtpst ⑫. SAS will automatically compute the paired differences (wtpre-wtpst), and then conduct the t-test. The output shows a t-statistic of 3.23 ⑬, which corroborates the manual computations. Because SAS prints the two-tailed p-value ⑭, this value must be halved to obtain the one-tailed result, $p = 0.0028/2 = 0.0014$. You reject the null hypothesis and conclude that a significant weight loss occurs because this p-value is less than the nominal significance level of $\alpha = 0.05$.

SAS Code for Example 4.2

```
data obese;
  input subj wtpre wtpst @@;
  wtloss = wtpre - wtpst;                                ⑩
  datalines;
  1 165 160    2 202 200    3 256 259    4 155 156
  5 135 134    6 175 162    7 180 187    8 174 172
  9 136 138    10 168 162   11 207 197   12 155 155
  13 220 205   14 163 153   15 159 150   16 253 255
  17 138 128   18 287 280   19 177 171   20 181 170
  21 148 154   22 167 170   23 190 180   24 165 154
  25 155 150   26 153 145   27 205 206   28 186 184
  29 178 166   30 129 132   31 125 127   32 165 169
  33 156 158   34 170 161   35 145 152
;

proc print data = obese;                                ⑪
  var subj wtpre wtpst wtloss;
  title1 'One-Sample t-Test';
  title2 'EXAMPLE 4.2: Paired-Difference in Weight
Loss';
run;
proc ttest data = obese;
  paired wtpre*wtpst;                                  ⑫
run;
```

Output 4.2 SAS Output for Example 4.2

One-Sample t-Test EXAMPLE 4.2: Paired-Difference in Weight Loss					
OBS	subj	wtpre	wtpst	wtloss	
1	1	165	160	5	
2	2	202	200	2	
3	3	256	259	-3	
4	4	155	156	-1	
5	5	135	134	1	
6	6	175	162	13	
7	7	180	187	-7	
8	8	174	172	2	⑪
.	
.	
.	
33	33	156	158	-2	
34	34	170	161	9	
35	35	145	152	-7	

The TTEST Procedure					
Difference: wtpre - wtpst					
N	Mean	Std Dev	Std Err	Minimum	Maximum
35	3.4571	6.3401	1.0717	-7.0000	15.0000
Mean	95% CL Mean	Std Dev	95% CL Std Dev		
3.4571	1.2792	5.6350	6.3401	5.1283	8.3068
DF	t Value	Pr > t			
34	3.23 ⑬	0.0028 ⑭			

4.4 Details & Notes

- **4.4.1** The main difference between the *Z-test* (Chapter 2) and the *t-test* is that the Z-statistic is based on a known standard deviation σ , while the t-statistic uses the sample standard deviation, s , as an estimate of σ . With the assumption of normally distributed data, the variance σ^2 is more closely estimated by the sample variance s^2 as n gets large. It can be shown that the *t-test* is equivalent to the *Z-test* for infinite degrees of freedom. In practice, a ‘large’ sample is usually considered $n \geq 30$. The distributional relationship between the Z- and t-statistics is shown in Appendix B.

- **4.4.2** If the assumption of normally distributed data cannot be made, the mean might not represent the best measure of central tendency. In such cases, a non-parametric rank test, such as the *Wilcoxon signed-rank test* (Chapter 12), might be more appropriate. A test for normality can be carried out in SAS using the NORMAL option in the UNIVARIATE procedure, as illustrated below for Example 4.1:

```
proc univariate normal data = diab;
  var bmi;
run;
```

- **4.4.3** Because Example 4.1 tests for *any* difference from a hypothesized value, a *two-tailed test* is used. A *one-tailed test* would be used when you want to test whether the population mean is strictly greater than *or* strictly less than the hypothesized threshold level (μ_0), such as in Example 4.2. We use the rejection region according to the alternative hypothesis as follows:

Type of Test	Alternative Hypothesis	Corresponding Rejection Region
two-tailed	$H_A: \mu \neq \mu_0$	reject H_0 if $t > t_{\alpha/2,n-1}$ or $t < -t_{\alpha/2,n-1}$
one-tailed (right)	$H_A: \mu > \mu_0$	reject H_0 if $t > t_{\alpha,n-1}$
one-tailed (left)	$H_A: \mu < \mu_0$	reject H_0 if $t < -t_{\alpha,n-1}$

- **4.4.4** Most statistics text books have tables of the t-distribution in an Appendix. These tables often provide the critical t-values for levels of $\alpha = 0.10, 0.05, 0.025, 0.01$, and 0.005 , as in Appendix A.2. Critical t-values can also be found from many statistical programs. In SAS, you may use the function QUANTILE('T',1-a,n-1), where $a=\alpha$ for a *one-tailed test* and $a=\alpha/2$ for a *two-tailed test*. In Example 4.1, the critical t-value of 2.131 with $n=16$ (15 degrees of freedom) can be found with the SAS function QUANTILE ('T', 0.975, 15).

Alternatively, the p-value associated with the test statistic, t , based on v degrees of freedom, can be found by using the SAS function PROBT(t,v), which gives the cumulative probability of the Student-t distribution. Because you want the tail probabilities, the associated two-tailed p-value is $2*(1-PROBT(t, v))$. In Example 4.1, the p-value 0.041 can be confirmed by using the SAS expression $2*(1-PROBT(2.23,15))$. In Example 4.2, the one-tailed p-value 0.0014 can be confirmed by using the SAS expression $1-PROBT(3.226,34)$.

- **4.4.5** A non-significant result does not necessarily imply that the null hypothesis is true, only that insufficient evidence exists to contradict it. Larger sample sizes are often needed and an investigation of the power curve (see Chapter 2) is necessary for ‘equivalency studies’.

- **4.4.6** Statistical significance does not imply causality. In Example 4.2, concluding that the treatment *caused* the significant weight loss would be presumptuous. Causality can better be investigated using a concurrent untreated or control group in the study, and strictly controlling other experimental conditions. In controlled studies, comparison of responses among groups is carried out with tests such as the *two-sample t-test* (Chapter 5) or *analysis of variance* methods (Chapters 6–8).
- **4.4.7** Known values of n measurements uniquely determine the sample mean \bar{y} . But given \bar{y} , the n measurements cannot uniquely be determined. In fact, $n-1$ of the measurements can be freely selected, with the n^{th} determined by \bar{y} . Thus the term $n-1$ degrees of freedom.

Note: The number of degrees of freedom, often denoted by the Greek letter ν (nu), is a parameter of the Student-t probability distribution.

CHAPTER 5

The Two-Sample t-Test

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5.1 Introduction

The *two-sample t-test* is used to compare the means of two independent populations, denoted μ_1 and μ_2 . This test has ubiquitous application in the analysis of controlled clinical trials. Examples might include the comparison of mean decreases in diastolic blood pressure between two groups of patients receiving different antihypertensive agents, or estimating pain relief from a new treatment relative to that of a placebo based on subjective assessment of percent-improvement in two parallel groups.

Assume that the two populations are normally distributed. If the variances are known, you can use a Z-test. However, in most cases, you will need to estimate the variances from the data in which case you use the *two-sample t-test* under the assumption that the two populations have the same variance (σ^2).

5.2 Synopsis

Samples of n_1 and n_2 observations are randomly selected from the two populations, with the measurements from Population i denoted by $y_{i1}, y_{i2}, \dots, y_{in_i}$ (for $i = 1, 2$). The unknown means, μ_1 and μ_2 , are estimated by the sample means, \bar{y}_1 and \bar{y}_2 , respectively. The greater the difference between the sample means, the greater the evidence that the hypothesis of equality of population means (H_0) is untrue.

The test statistic, t , is a function of this difference standardized by its standard error, namely $s(1/n_1 + 1/n_2)^{1/2}$. Large values of t will lead to rejection of the null hypothesis. When H_0 is true, t has the Student-t distribution with $N-2$ degrees of freedom, where $N = n_1+n_2$.

The best estimate of the common unknown population variance (σ^2), is the ‘pooled’ variance (s_p^2), computed as the weighted average of the sample variances using the formula:

$$s_p^2 = \frac{(n_1 - 1) \cdot s_1^2 + (n_2 - 1) \cdot s_2^2}{(n_1 + n_2 - 2)}$$

The *two-sample t-test* is summarized as follows:

null hypothesis: $H_0: \mu_1 = \mu_2$

alt. hypothesis: $H_A: \mu_1 \neq \mu_2$

test statistic:

$$t = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{s_p^2 \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}}$$

decision rule:

reject H_0 if $|t| > t_{\alpha/2, N-1}$

5.3 Examples

Example 5.1—FEV₁ Changes

A new compound, ABC-123, is being developed for long-term treatment of patients with chronic asthma. Asthmatic patients were enrolled in a double-blind study and randomized to receive daily oral doses of ABC-123 or a placebo for 6 weeks. The primary measurement of interest is the resting FEV₁ (forced expiratory volume during the first second of expiration), which is measured before and at the end of the 6-week treatment period. Data (in liters) are shown in the table which follows. Does administration of ABC-123 appear to have any effect on FEV₁?

TABLE 5.1 Raw Data for Example 5.1

ABC-123 Group			Placebo Group		
Patient Number	Baseline	Week 6	Patient Number	Baseline	Week 6
101	1.35	n/a	102	3.01	3.90
103	3.22	3.55	104	2.24	3.01
106	2.78	3.15	105	2.25	2.47
108	2.45	2.30	107	1.65	1.99
109	1.84	2.37	111	1.95	n/a
110	2.81	3.20	112	3.05	3.26
113	1.90	2.65	114	2.75	2.55
116	3.00	3.96	115	1.60	2.20
118	2.25	2.97	117	2.77	2.56
120	2.86	2.28	119	2.06	2.90
121	1.56	2.67	122	1.71	n/a
124	2.66	3.76	123	3.54	2.92

Solution

Let μ_1 and μ_2 represent the mean increases in FEV_1 for the ABC-123 and the placebo groups, respectively. The first step is to calculate each patient's increase in FEV_1 from baseline to Week 6 (shown in Output 5.1). Patients 101, 111, and 122 are excluded from this analysis because no Week-6 measurements are available. The FEV_1 increases (in liters) are summarized by treatment group as follows:

TABLE 5.2 Treatment Group Summary Statistics for Example 5.1

	ABC-123	Placebo
Mean (\bar{y}_i)	0.503	0.284
SD (s_i)	0.520	0.508
Sample Size (n_i)	11	10

The pooled variance is

$$s_p^2 = \frac{(11-1) \cdot 0.520^2 + (10-1) \cdot 0.508^2}{21-2} = 0.265$$

Because you are looking for any effect, use a *two-tailed test* as follows:

null hypothesis:	$H_0: \mu_1 = \mu_2$
alt. hypothesis:	$H_A: \mu_1 \neq \mu_2$
test statistic:	$t = \frac{0.503 - 0.284}{\sqrt{0.265 \left(\frac{1}{11} + \frac{1}{10} \right)}} = 0.974$
decision rule:	reject H_0 if $ t > t_{0.025,19} = 2.093$
conclusion:	Because 0.974 is not > 2.093 , you cannot reject H_0 . You conclude that the samples fail to provide significant evidence of any effect of ABC-123 on FEV ₁ . This test is based on a significance level of $\alpha = 0.05$.

SAS Analysis of Example 5.1

The SAS code for analyzing this data set and the resulting output are shown on the next three pages. The program computes the pre- to post-study changes in FEV₁ ('chg') ①, and then prints a listing of the data by using PROC PRINT ②.

PROC MEANS is used in this example to obtain the summary statistics for the baseline and Week-6 response values for each group ③. The T and PRT options are specified in the PROC MEANS statement to illustrate the *one-sample t-tests* for the significance of the within-group changes (variable=chg) ④. PROC TTEST can also be used for this, as illustrated in Chapter 4. Note that the ABC-123 Group shows a significant increase in mean FEV₁ ($p=0.0094$), while the Placebo Group does not ($p=0.1107$).

The *two-sample t-test* is carried out by using PROC TTEST with the class variable, TRTGRP, specified in a CLASS statement. Assuming equal variances, you use the t-value and p-value ($Pr>|t|$) corresponding to 'Equal' under the 'Variances' column ⑤. The t-statistic 0.97 confirms the result from the manual calculation above. The p-value 0.3425 (>0.05) indicates no significant difference in the FEV₁ increases between groups.

SAS Code for Example 5.1

```
data fev;
    input patno trtgrp $ fev0 fev6 @@;
    chg = fev6 - fev0; ❶
    if chg = . then delete;
    datalines;
101 A 1.35 .      103 A 3.22 3.55      106 A 2.78 3.15
108 A 2.45 2.30   109 A 1.84 2.37      110 A 2.81 3.20
113 A 1.90 2.65   116 A 3.00 3.96      118 A 2.25 2.97
120 A 2.86 2.28   121 A 1.56 2.67      124 A 2.66 3.76
102 P 3.01 3.90   104 P 2.24 3.01      105 P 2.25 2.47
107 P 1.65 1.99   111 P 1.95 .         112 P 3.05 3.26
114 P 2.75 2.55   115 P 1.60 2.20      117 P 2.77 2.56
119 P 2.06 2.90   122 P 1.71 .         123 P 3.54 2.92
;

proc format;
    value $trt 'A' = 'ABC-123'
                  'P' = 'PLACEBO';
run;

proc print data = fev; ❷
    var patno trtgrp fev0 fev6 chg;
    format trtgrp $trt. fev0 fev6 chg 5.2;
    title1 'Two-Sample t-Test';
    title2 'EXAMPLE 5.1: FEV1 Changes';
run;

proc means mean std n t prt data = fev; ❸
    by trtgrp;
    var fev0 fev6 chg;
    format trtgrp $trt. ;
run;

proc ttest data = fev;
    class trtgrp;
    var chg;
    format trtgrp $trt. ;
run;
```

OUTPUT 5.1 SAS Output for Example 5.1

Two-Sample t-Test EXAMPLE 5.1: FEV1 Changes					
Obs	patno	trtgrp	fev0	fev6	chg
1	103	ABC-123	3.22	3.55	0.33
2	106	ABC-123	2.78	3.15	0.37
3	108	ABC-123	2.45	2.30	-0.15
4	109	ABC-123	1.84	2.37	0.53
5	110	ABC-123	2.81	3.20	0.39
6	113	ABC-123	1.90	2.65	0.75
7	116	ABC-123	3.00	3.96	0.96
8	118	ABC-123	2.25	2.97	0.72
9	120	ABC-123	2.86	2.28	-0.58
10	121	ABC-123	1.56	2.67	1.11
11	124	ABC-123	2.66	3.76	1.10
12	102	PLACEBO	3.01	3.90	0.89
13	104	PLACEBO	2.24	3.01	0.77
14	105	PLACEBO	2.25	2.47	0.22
15	107	PLACEBO	1.65	1.99	0.34
16	112	PLACEBO	3.05	3.26	0.21
17	114	PLACEBO	2.75	2.55	-0.20
18	115	PLACEBO	1.60	2.20	0.60
19	117	PLACEBO	2.77	2.56	-0.21
20	119	PLACEBO	2.06	2.90	0.84
21	123	PLACEBO	3.54	2.92	-0.62

Two-Sample t-Test EXAMPLE 5.1: FEV1 Changes					
----- trtgrp=ABC-123 -----					
The MEANS Procedure					
Variable	Mean	Std Dev	N	t Value	Pr > t
fev0	2.4845455	0.5328858	11	15.46	<.0001
fev6	2.9872727	0.5916095	11	16.75	<.0001
chg	0.5027273	0.5198286	11	3.21	0.0094
----- trtgrp=PLACEBO -----					
Variable	Mean	Std Dev	N	t Value	Pr > t
fev0	2.4920000	0.6355365	10	12.40	<.0001
fev6	2.7760000	0.5507006	10	15.94	<.0001
chg	0.2840000	0.5077882	10	1.77	0.1107

OUTPUT 5.1 SAS Output for Example 5.1 (continued)

Two-Sample t-Test EXAMPLE 5.1: FEV1 Changes								
The TTEST Procedure								
Variable: chg								
trtgrp	N	Mean	Std Dev	Std Err	Minimum	Maximum		
ABC 123	11	0.5027	0.5198	0.1567	-0.5800	1.1100		
PLACEBO	10	0.2840	0.5078	0.1606	-0.6200	0.8900		
Diff (1-2)		0.2187	0.5142	0.2247				
trtgrp	Method	Mean	95% CL Mean	Std Dev				
ABC 123		0.5027	0.1535	0.8520	0.5198			
PLACEBO		0.2840	-0.0792	0.6472	0.5078			
Diff (1-2)	Pooled	0.2187	-0.2515	0.6889	0.5142			
Diff (1-2)	Satterthwaite	0.2187	-0.2511	0.6886				
trtgrp	Method	95% CL Std Dev						
ABC 123		0.3632	0.9123					
PLACEBO		0.3493	0.9270					
Diff (1-2)	Pooled	0.3910	0.7510					
Diff (1-2)	Satterthwaite							
Method	Variances	DF	t Value	Pr > t				
Pooled	Equal	19	0.97	0.3425				
Satterthwaite	Unequal	18.888	0.97	0.3420				
Equality of Variances								
Method	Num DF	Den DF	F Value	Pr > F				
Folded F	10	9	1.05	0.9532				

5.4 Details & Notes

- **5.4.1** The assumption of equal variances can be tested using the *F-test*. An *F-test* generally arises as a ratio of variances. When the hypothesis of equal variances is true, the ratio of sample variances should be about 1. The probability distribution of this ratio is known as the *F-distribution* (which is widely used in the *analysis of variance*).

The test for comparing two variances can be made as follows:

null hypothesis:	$H_0: \sigma_1^2 = \sigma_2^2$
alt. hypothesis:	$H_A: \sigma_1^2 \neq \sigma_2^2$
test statistic:	$F = s_U^2 / s_L^2$
decision rule:	reject H_0 if $F > F_c(\alpha/2)$

The subscripts U and L denote ‘upper’ and ‘lower’. The sample with the larger sample variance (s_U^2) is considered the ‘upper’ sample, and the sample with the smaller sample variance (s_L^2) is the ‘lower’. Large values of F indicate a large disparity in sample variances and would lead to rejection of H_0 . The critical F value, F_c , can be obtained from the F-tables in most elementary statistics books or many computer packages, based on n_{U-1} upper and n_{L-1} lower degrees of freedom. In SAS, the critical F value can be found by using the QUANTILE function for the F-distribution:

$$F_c(\alpha/2) = \text{QUANTILE}('F', 1 - \alpha/2, n_{U-1}, n_{L-1})$$

In Example 5.1, compute $F = 0.520^2 / 0.508^2 = 1.048$, which leads to non-rejection of the null hypothesis of equal variances at $\alpha=0.05$ based on $F_c(0.025)=3.96$. As noted in Output 5.1, the p-value associated with this preliminary test is 0.9532 **6**, which can also be obtained using the PROBF function in SAS: $2*(1-\text{PROBF}(1.048,10,9))$. You can proceed with the *t-test* assuming equal variances because the evidence fails to contradict that assumption.

As described in Chapter 6, you can also conduct a t-test using PROC GLM in SAS in which you use *analysis-of-variance* to compare two groups. SAS allows you to test for equal variances using the HOVTEST option in the MEANS statement of PROC GLM. See Sections 6.4.3 and 6.4.9 for more details. In addition, you can turn on ODS Graphics in the TTEST or GLM procedures to provide a graphical summary display, which is helpful in visually comparing the data distribution or variability between groups. This is illustrated for Example 6.1 in Chapter 6.

- **5.4.2** If the hypothesis of equal variances is rejected, the *t-test* might give erroneous results. In such cases, a modified version of the *t-test* proposed by Satterthwaite is often used. The ‘Satterthwaite adjustment’ consists of using the statistic similar to the *t-test* but with approximate degrees of freedom, carried out as follows:

test statistic:	$t' = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$
decision rule:	reject H_0 if $ t' > t_{\alpha/2,q}$

where q represents the approximate degrees of freedom computed as follows (with $w_i = s_i^2 / n_i$):

$$q = \frac{(w_1 + w_2)^2}{\frac{w_1^2}{(n_1 - 1)} + \frac{w_2^2}{(n_2 - 1)}}$$

Although Satterthwaite’s adjustment is not needed for Example 5.1, it can be used to illustrate the computational methods as follows. Compute

$$w_1 = 0.520^2 / 11 = 0.0246$$

and

$$w_2 = 0.508^2 / 10 = 0.0258$$

so that

$$q = \frac{(0.0246 + 0.0258)^2}{\frac{0.0246^2}{10} + \frac{0.0258^2}{9}} = 18.9$$

and

$$t' = \frac{0.503 - 0.284}{\sqrt{\frac{0.520^2}{11} + \frac{0.508^2}{10}}} = 0.975$$

SAS computes these quantities automatically in the output of PROC TTEST (see Output 5.1). Satterthwaite’s *t*-value and approximate degrees of freedom are given for ‘Unequal’ under Variances in the SAS output. (See 7 in Output 5.1.) These results should be used when it is believed that the population variances might differ.

- **5.4.3** The significance level of a statistical test will be altered if it is conditioned on the results of a preliminary test. Therefore, if a *t-test* depends on the results of a preliminary *F-test* for variance homogeneity, the actual significance level might be slightly different than what is reported. This difference gets smaller as the significance level of the preliminary *F-test* increases. With this in mind, you might want to conduct the preliminary *F-test* for variance homogeneity at a significance level greater than 0.05, usually 0.10, 0.15, or even 0.20. For example, if 0.15 were used, Satterthwaite's adjustment would be used if the *F-test* were significant at $p < 0.15$.
- **5.4.4** The assumption of normality can be tested by using the *Shapiro-Wilk test* or the *Kolmogorov-Smirnov test* executed with the `normal` option in PROC UNIVARIATE in SAS. See Section 4.4.2 for the sample SAS code. Rejection of the assumption of normality in the small sample case precludes the use of the *t-test*. As n_1 and n_2 become large (generally ≥ 30), you don't need to rely so heavily on the assumption that the data have an underlying normal distribution in order to apply the *two-sample t-test*. However, with non-normal data, the mean might not represent the most appropriate measure of central tendency. With a skewed distribution, for example, the median might be more representative of the distributional center than the mean. In such cases, a rank test, such as the *Wilcoxon rank-sum test* (Chapter 13) or the *log-rank test* (Chapter 21), should be considered.
- **5.4.5** Note that, if within-group tests are used for the analysis *instead* of the *two-sample t-test*, the researcher might reach a different, erroneous conclusion. In Example 5.1, you might hastily conclude that a significant treatment-related increase in mean FEV_1 exists just by looking at the within-group results. The *two-sample t-test*, however, is the more appropriate test for the comparison of between-group changes because the control group response must be factored out of the response from the active group.

In interpreting the changes, you might argue that the mean change in FEV_1 for the active group is comprised of two additive effects, namely the placebo effect plus a therapeutic benefit. If it can be established that randomization to the treatment groups provides effectively homogeneous groups, you might conclude that the FEV_1 mean change for the active group in Example 5.1 can be broken down as follows:

$$0.503 = 0.284 + 0.219 \\ (\text{Total Effect}) \quad (\text{Placebo Effect}) \quad (\text{Therapeutic Effect})$$

To validate such interpretations of the data, you would first establish baseline comparability of the two groups. The *two-sample t-test* can also be applied for this purpose by analyzing the baseline values in the same way the changes were analyzed in Example 5.1.

- **5.4.6** While larger p-values (> 0.10) give greater credence to the null hypothesis of equality, such a conclusion should not be made without considering the test's power function, especially with small sample sizes. Statistical power is the probability of rejecting the null hypothesis when it is *not* true. This concept is discussed briefly in Chapter 2.
- **5.4.7** In checking for differences between means in either direction, Example 5.1 uses a *two-tailed or two-sided test*. If interest is restricted to differences in only one direction, a *one-tailed test* can be applied in the same manner as described for the *one-sample t-test* (Chapter 4). The probability given in the SAS output, $\text{Pr}>|t|$, can be halved to obtain the corresponding one-tailed p-value. In SAS, you can use the SIDES option with PROC TTEST (SAS 9.2 and later) to indicate the type of test. Specifying SIDES=2 indicates a two-sided or two-tailed test (default), while the options SIDES=U and SIDES=L request a one-sided test to the right (to test values larger than the null value) and to the left (to test values smaller than the null value), respectively.

CHAPTER 6

One-Way ANOVA

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6.1 Introduction

One-way ANOVA (analysis of variance) is used to simultaneously compare two or more group means based on independent samples from each group. The bigger the variation among sample group means relative to the variation of individual measurements within the groups, the greater the evidence that the hypothesis of equal group means is untrue. This concept is illustrated in Appendix C (Section C.1), which discusses some basic concepts of *ANOVA*.

In clinical trials, this *ANOVA* method might be appropriate for comparing mean responses among a number of parallel-dose groups or among various strata based on patients' background information, such as race, age group, or disease severity.

In this chapter, assume the samples are from normally distributed populations, all with the same variance, σ^2 .

6.2 Synopsis

In general, there are k ($k \geq 2$) levels of the factor GROUP. From each, independently sample a number of observations, letting y_{ij} represent the j^{th} measurement from the i^{th} group and n_i represent the number of measurements within Group i ($i = 1, 2, \dots, k$). Data are collected as shown in Table 6.1.

TABLE 6.1 Typical Layout for the One-Way ANOVA

GROUP			
Group 1	Group 2	...	Group k
y_{11}	y_{21}	...	y_{k1}
y_{12}	y_{22}	...	y_{k2}
...
y_{1n_1}	y_{2n_2}	...	y_{kn_k}

The null hypothesis is that of “no Group effect” (i.e., no difference in mean responses among groups). The alternative hypothesis is that “the Group effect is important” (i.e., at least one pair of Group means differs). When H_0 is true, the variation among groups and the variation within groups are independent estimates of the same measurement variation, σ^2 , and their ratio should be close to 1. This ratio is used as the test statistic F, which has the F-distribution with $k-1$ upper and $N-k$ lower degrees of freedom ($N = n_1 + n_2 + \dots + n_k$).

The test summary is given as

null hypothesis:	$H_0: \mu_1 = \mu_2 = \dots = \mu_k$
alt. hypothesis:	$H_A: \text{not } H_0$
test statistic:	$F = \frac{\text{MSG}}{\text{MSE}}$
decision rule:	reject H_0 if $F > F_{N-k}^{k-1}(\alpha)$

MSG is an estimate of the variability among groups, and MSE is an estimate of the variability within groups.

In *ANOVA*, you assume ‘variance homogeneity’, which means that the within-group variance is constant across groups. This can be expressed as

$$\sigma_1^2 = \sigma_2^2 = \dots = \sigma_k^2 = \sigma^2$$

where σ_i^2 denotes the unknown variance of the i^{th} population. The common variance σ^2 is estimated by s^2 , which is a weighted average of the k sample variances:

$$s^2 = \frac{(n_1 - 1) \cdot s_1^2 + (n_2 - 1) \cdot s_2^2 + \dots + (n_k - 1) \cdot s_k^2}{(n_1 + n_2 + \dots + n_k) - k}$$

Recall the formula for the sample variance for Group i (Table 1.3) to be

$$s_i^2 = \sum_{j=1}^{n_i} \frac{(y_{ij} - \bar{y}_i)^2}{n_i - 1}$$

Because s_i^2 is the estimated variance within Group i, s^2 represents an average within-group variation over all groups. In *ANOVA*, s^2 is called the mean square error (MSE), and its numerator is the sum of squares for error (SSE). The ‘error’ is the deviation of each observation from its group mean. If SSE is expressed as the sum of squared errors,

$$SSE = \sum_{i=1}^k \sum_{j=1}^{n_i} (y_{ij} - \bar{y}_i)^2$$

then the pooled variance s^2 is just $SSE / (N-k)$. The denominator $N-k$, where $N = n_1 + n_2 + \dots + n_k$, is the total sample size over all samples and is known as the degrees of freedom associated with the error.

The variability among groups can be measured by the deviation of the average observation in each group from the overall average, \bar{y} . That is, the overall variance obtained by replacing each observation with its group mean (\bar{y}_i), represents the between-group variability MSG. Its numerator is the sum of squares for groups (SSG), computed as

$$SSG = \sum_{i=1}^k n_i (\bar{y}_i - \bar{y})^2$$

where \bar{y} is the mean of all N observations. Each group mean is treated as a single observation, so there are $k-1$ degrees of freedom associated with the SSG. The mean square for the GROUP effect is the sum of squares divided by its degrees of freedom

$$MSG = SSG / (k-1)$$

When the null hypothesis is true, the variation between groups should be the same as the variation within groups. Therefore, under H_0 , the test statistic F should be close to 1 and has an F-distribution with $k-1$ upper degrees of freedom and $N-k$ lower degrees of freedom. Critical F-values based on the F-distribution are used to determine the rejection region (see Section 6.4.1).

6.3 Examples

Example 6.1—HAM-A Scores in GAD

A new serotonin-uptake inhibiting agent, SN-X95, is being studied in subjects with general anxiety disorder (GAD). Fifty-two subjects diagnosed with GAD of moderate or greater severity consistent with the “Diagnostic and Statistical Manual, 4th Edition” (DSM-IV) were enrolled and randomly assigned to one of three treatment groups: 25 mg SN-X95, 100 mg SN-X95, or placebo. After 10 weeks of once-daily oral dosing in a double-blind fashion, a test based on the Hamilton Rating Scale for Anxiety (HAM-A) was administered. This test consists of 14 anxiety-related items (e.g., ‘anxious mood’, ‘tension’, ‘insomnia’, ‘fears’, etc.), each rated by the subject as ‘not present’, ‘mild’, ‘moderate’, ‘severe’, or ‘very severe’. HAM-A test scores were found by summing the coded values of all 14 items using the numeric coding scheme of 0 for ‘not present’, 1 for ‘mild’, 2 for ‘moderate’, 3 for ‘severe’, and 4 for ‘very severe’. The data are presented in Table 6.2. Are there any differences in mean HAM-A test scores among the three groups?

TABLE 6.2 Raw Data for Example 6.1

Lo-Dose (25mg)		Hi-Dose (100mg)		Placebo	
Patient Number	HAM-A	Patient Number	HAM-A	Patient Number	HAM-A
101	21	103	16	102	22
104	18	105	21	107	26
106	19	109	31	108	29
110	n/a	111	25	114	19
112	28	113	23	115	n/a
116	22	119	25	117	33
120	30	123	18	118	37
121	27	127	20	122	25
124	28	128	18	126	28
125	19	131	16	129	26
130	23	135	24	132	n/a
136	22	138	22	133	31
137	20	140	21	134	27
141	19	142	16	139	30
143	26	146	33	144	25
148	35	150	21	145	22
152	n/a	151	17	147	36
				149	32

Solution

Patients who dropped out with no data (Nos. 110, 115, 132, 152) are excluded from the analysis. Arbitrarily assigning subscripts 1 for Lo-Dose, 2 for Hi-Dose, and 3 for Placebo, the group summary statistics for the HAM-A scores are shown in Table 6.3.

TABLE 6.3 Summary Statistics by Treatment Group for Example 6.1

	----- GROUP -----			Overall
	Lo-Dose (i=1)	Hi-Dose (i=2)	Placebo (i=3)	
\bar{y}_i	23.800	21.588	28.000	24.417
s_i	4.974	4.963	5.033	5.588
n_i	15	17	16	48

Compute

$$\begin{aligned} SSG &= 15(23.800 - 24.417)^2 + \\ &\quad 17(21.588 - 24.417)^2 + \\ &\quad 16(28.000 - 24.417)^2 = 347.1 \end{aligned}$$

with $k = 3$ groups, so that

$$MSG = SSG/(k-1) = 347.1 / 2 = 173.6$$

Also,

$$\begin{aligned} SSE &= (21-23.800)^2 + (18-23.800)^2 + \dots + (35-23.800)^2 + \\ &\quad (16-21.588)^2 + (21-21.588)^2 + \dots + (17-21.588)^2 + \\ &\quad (22-28.000)^2 + (26-28.000)^2 + \dots + (32-28.000)^2 = 1120.5 \end{aligned}$$

with $N-k = 48 - 3 = 45$ degrees of freedom, so that $MSE = 1120.5 / 45 = 24.9$.

To check the calculations, you can also compute the MSE, alternatively, as the weighted average of the group variances:

$$MSE = \frac{14 \cdot (4.974^2) + 16 \cdot (4.963^2) + 15 \cdot (5.033^2)}{45} = 24.9$$

The test summary, conducted at a significance level of $\alpha = 0.05$ is shown as

null hypothesis:	$H_0: \mu_1 = \mu_2 = \mu_3$
alt. hypothesis:	$H_A: \text{not } H_0$
test statistic:	$F = 173.6 / 24.9 = 6.97$
decision rule:	reject H_0 if $F > F_{45}^2(0.05) = 3.2$
conclusion:	because $6.97 > 3.2$, you reject H_0 and conclude that there is a significant difference in mean HAM-A scores among the 3 dose groups.

SAS Analysis of Example 6.1

The next four pages provide the code and output for the analysis of Example 6.1 using the GLM procedure in SAS.

The summary statistics are first obtained for each dose group by using PROC MEANS ❶. These statistics are also summarized in Table 6.3.

The key to using PROC GLM is in correctly specifying the MODEL statement, which lists the response variable on the left side of the equal sign and the model factors on the right side of the equal sign. In the *one-way ANOVA*, there is only one model factor. In this example, the model factor is Dose Group (variable=dosegrp). The model factor must also be specified in the CLASS statement to indicate it is a classification factor rather than a numeric covariate. The CLASS statement must precede the MODEL statement.

The output shows the MSE ❷, the MSG ❸, and the *F-test* for the Dose Group effect of 6.97 ❹, which corroborate the manual calculations. The p-value ❺ of 0.0023 (<0.05) indicates that the mean HAM-A scores differ significantly among Dose Groups.

With a significant Dose Group effect, the next question is which group or groups contribute to that difference. The MEANS statement ❻ is used to obtain multiple comparison results using both the pairwise *t-test* ❼ and *Dunnett's test* ➋. Both of these methods reveal significant differences between each active group and placebo, as discussed further in Section 6.4.4. A CONTRAST statement is also included ⬁ to illustrate a customized test (see Section 6.4.5). The ODS Graphics feature is used to depict the box plots for each dose group. These are discussed further in Section 6.4.3.

SAS Code for Example 6.1

```
data gad;
    input patno dosegrp $ hama @@;
    datalines;
101 LO 21 104 LO 18
106 LO 19 110 LO .
112 LO 28 116 LO 22
120 LO 30 121 LO 27
124 LO 28 125 LO 19
130 LO 23 136 LO 22
137 LO 20 141 LO 19
143 LO 26 148 LO 35
152 LO . 103 HI 16
105 HI 21 109 HI 31
111 HI 25 113 HI 23
119 HI 25 123 HI 18
127 HI 20 128 HI 18
131 HI 16 135 HI 24
138 HI 22 140 HI 21
142 HI 16 146 HI 33
150 HI 21 151 HI 17
102 PB 22 107 PB 26
108 PB 29 114 PB 19
115 PB . 117 PB 33
118 PB 37 122 PB 25
126 PB 28 129 PB 26
132 PB . 133 PB 31
134 PB 27 139 PB 30
144 PB 25 145 PB 22
147 PB 36 149 PB 32
;

proc sort data = gad; by dosegrp;
proc means mean std n data = gad;
    by dosegrp; ①
    var hama;
    title1 'One-Way ANOVA';
    title2 'EXAMPLE 6.1: HAM-A Scores in GAD';
run;

ods graphics on;
proc glm data = gad plots = boxplot;
    class dosegrp;
    model hama = dosegrp;
    means dosegrp / hovtest t dunnett('PB'); ⑥
        contrast 'ACTIVE vs. PLACEBO' dosegrp 0.5 0.5 -1; ⑨
run;
ods graphics off;
```

OUTPUT 6.1 SAS Output for Example 6.1

```

One-Way ANOVA
EXAMPLE 6.1: HAM-A Scores in GAD
----- dosegrp=HI -----
The MEANS Procedure ①
Analysis Variable : hama
      Mean      Std Dev      N
----- 21.5882353    4.9630991    17
----- dosegrp=LO -----
Analysis Variable : hama
      Mean      Std Dev      N
----- 23.8000000    4.9742192    15
----- dosegrp=PB -----
Analysis Variable : hama
      Mean      Std Dev      N
----- 28.0000000    5.0332230    16

```

```

The GLM Procedure

Class Level Information
   Class      Levels      Values
dosegrp          3      HI LO PB

Number of Observations Read      52
Number of Observations Used     48

Dependent Variable: hama

      Sum of
Source      DF      Squares      Mean Square      F Value      Pr > F
Model          2      347.149020    173.574510      6.97      0.0023
Error         45      1120.517647    24.900392 ②
Corrected Total 47      1467.666667

      R-Square      Coeff Var      Root MSE      hama Mean
0.236531      20.43698      4.990029      24.41667

      Source      DF      Type I SS      Mean Square      F Value      Pr > F
dosegrp        2      347.1490196    173.5745098      6.97      0.0023

      Source      DF      Type III SS      Mean Square      F Value      Pr > F
dosegrp        2      347.1490196    173.5745098      6.97      0.0023 ③ ④ ⑤

Levene's Test for Homogeneity of hama Variance
ANOVA of Squared Deviations from Group Means

      Source      DF      Sum of
                     Squares      Mean
                     Square      F Value      Pr > F
dosegrp        2      4.0184      2.0092      0.00      0.9980 ⑪
Error         45      44623.5      991.6

```

OUTPUT 6.1 SAS Output for Example 6.1 (continued)

One-Way ANOVA
EXAMPLE 6.1: HAMA Scores in GAD

The GLM Procedure

t Tests (LSD) for hama 7

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	45
Error Mean Square	24.90039
Critical Value of t	2.01410

Comparisons significant at the 0.05 level are indicated by ***.

		Difference	95% Confidence		
		Between	Limits		
		Means			
dosegrp	Comparison				
⑩	PB - LO	4.200	0.588	7.812	***
	PB - HI	6.412	2.911	9.912	***
	LO - PB	-4.200	-7.812	-0.588	***
	LO - HI	2.212	-1.349	5.772	
	HI - PB	-6.412	-9.912	-2.911	***
	HI - LO	-2.212	-5.772	1.349	

Dunnett's t Tests for hama 8

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	45
Error Mean Square	24.90039
Critical Value of Dunnett's t	2.28361

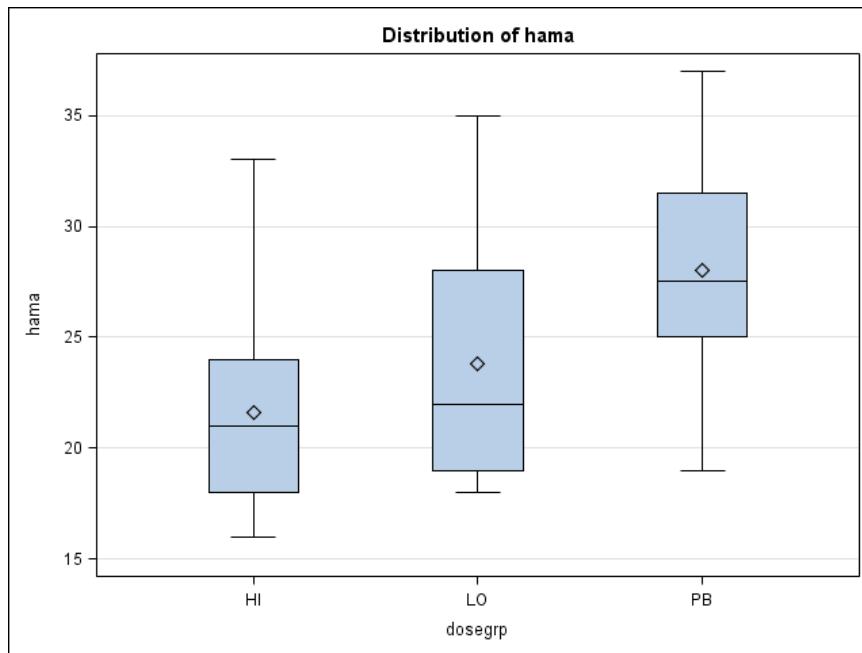
Comparisons significant at the 0.05 level are indicated by ***.

		Difference	Simultaneous		
		Between	95% Confidence		
		Means			
dosegrp	Comparison				
LO	- PB	-4.200	-8.295	-0.105	***
	HI - PB	-6.412	-10.381	-2.443	***

Dependent Variable: hama

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
ACTIVE vs. PLACEBO	1	299.9001075	299.9001075	12.04	0.0012 9

OUTPUT 6.1 SAS Output for Example 6.1 (continued)



6.4 Details & Notes

- **6.4.1** The parameters associated with the F-distribution are the upper and lower degrees of freedom. Many elementary statistical texts provide tables of critical F-values associated with a fixed significance level (usually 0.10, 0.05, 0.01) for various combinations of values for the upper and lower degrees of freedom. F-values or associated probabilities for known degrees of freedom can also be found by using most statistical programs. In SAS, the function QUANTILE('F', $1-\alpha$, udf, ldf), where udf and ldf represent the upper and lower degrees of freedom, respectively, is used to obtain the critical F-values for a significance level of α . The PROBF function can be used to obtain the p-value for a given F-value. For Example 6.1, the SAS statements

```
fcrit = quantile('F', 0.95, 2, 45);  
pval = 1-probf(6.97, 2, 45);
```

could be used to obtain the values of 3.2 for fcrit and 0.0023 for pval.

- **6.4.2** When conducting *analysis of variance*, an ‘ANOVA table’ is a traditional and widely used method of summarizing the results. The ANOVA summary identifies each source of variation, the degrees of freedom, the sum of squares, the mean square, and the F-statistic. An ANOVA table is a conventional way of organizing results for analyses that use many factors. In Example 6.1, there is only one model factor (Dose Group), but it is still convenient to use the ANOVA table format to summarize the results. The ANOVA table for this example is

ANOVA

Source	df	SS	MS	F
Dose Group	2	347.15	173.57	6.97 *
Error	45	1120.52	24.90	
Total	47	1467.67		

* Significant ($p < 0.05$)

■ 6.4.3 A one-way ANOVA requires the assumptions of

- normally distributed data
- independent samples from each group
- variance homogeneity among groups.

You may use ODS Graphics in SAS with PROC GLM to quickly obtain some descriptive data summaries, which can help you visualize whether the data deviate from these assumptions. As shown in Example 6.1, ODS Graphics is used by simply turning it on before the procedure and off at the end of the procedure. The PLOTS option is used to select which of the graphical summaries available in SAS will be used. Plots that may be helpful in checking distributional assumptions include diagnostic charts (PLOTS = DIAGNOSTICS) and box plots (PLOTS = BOXPLOT). The box plot, for example, can help check homogeneity of variance among groups. As shown at the end of Output 6.1, the box plot depicts the range of values for each group shown by the vertical lines, the interquartile range (25–75 percentile) shown by the shaded box, the group means shown by the diamond symbol, and the group medians shown by the horizontal lines within each box.

The *Shapiro-Wilk test* or *Kolmogorov-Smirnov test* for normality are some of the many formal tests available for determining whether the sample data are consistent with the assumption of normality. These tests can be conducted in SAS using options available in PROC UNIVARIATE and PROC GLM. If you are not able to make the assumption of normality, a data transformation might be appropriate. Logarithmic and rank transformations are popular transformations used in clinical data analysis (see Appendix F), and the *Kruskal-Wallis test* (Chapter 14) may be used as an alternative to the *one-way ANOVA* in the absence of normal data.

A number of formal tests, such as *Levene's test* or *Bartlett's test*, can be used to test for homogeneity of variances. In SAS, you simply include the HOVTEST option in the MEANS statement (as in Example 6.1) to run *Levene's test* (default in SAS), one of the most widely used tests for checking the equality of variances among groups. The p-value for equal variances in Example 6.1 is 0.998 ⑩. This, along with the box plot, indicate no differences among the groups in variability.

In the event that equal variances among groups is not a plausible assumption, you can conduct an approximate test, known as *Welch's test*, for comparing the group means. To do this in SAS, you simply add the WELCH option to the MEANS statement in PROC GLM. *Welch's test* is analogous to the *Satterthwaite's* adjustment used in the *two-sample t-test* (Chapter 5) when the variances differ between groups.

- **6.4.4** When comparing more than two means ($k > 2$), a significant *F-test* indicates that at least one pair of means differs, but which pair (or pairs) is not identified by *ANOVA*. If the null hypothesis of equal means is rejected, further analysis must be undertaken to investigate where the differences lie. Numerous multiple comparison procedures are available for conducting such investigations, and many of these are discussed in Appendix E.

For now, attention is confined to two commonly-used multiple comparison methods: one method is a very simple approach to all-pairwise tests, and one method is a very useful way to compare numerous groups with a control. The simplest approach to multiple comparisons is to conduct pairwise *t-tests* for each pair of treatments. This method uses the approach of the *two-sample t-test* discussed in Chapter 5, but rather than using the pooled standard deviation of only the two groups being compared, the pooled standard deviation of all k groups is used (i.e., the square root of the MSE from the *ANOVA*). This method can be carried out in SAS using the T option in the MEANS statement in PROC GLM.

Another approach to multiple comparisons, which compares treatment means with a control group, is called *Dunnett's test*. Special tables are needed to conduct this test manually. The results are printed by SAS if you use the DUNNETT option in the MEANS statement in PROC GLM. You can also use the PROBMC function in the DATA step as illustrated in Appendix E.

The T and DUNNETT options are shown in Example 6.1. The output for the pairwise *t-tests* (LSD) ⑦ shows significant differences in mean HAM-A scores between the placebo group and each of the active-dose groups (indicated by ‘***’), but no difference between the low- and high-dose groups. The results using DUNNETT ⑧ also show significant comparisons of each active treatment group versus the control group (placebo). Note that the SAS code must include the designation ‘PB’ in parentheses after the DUNNETT option to indicate that each dose group is to be compared with the placebo group.

The biggest drawback of using pairwise *t-tests* is the effect on the overall significance level (as described in Chapter 2). If each pairwise test is conducted at a significance level of $\alpha=0.05$, the chance of having at least one erroneous conclusion (among all the comparisons performed) increases with the number of groups, k . Approaches to multiple comparisons that control the overall error rate have been developed, and it is important to consider the use of these methods when the study uses a larger number of groups. *Dunnett's test* controls the overall significance level because it uses an ‘experiment-wise error rate’, as noted in Output 6.1.

Note: For a comprehensive discussion of the numerous other approaches to multiple comparisons, an excellent reference is *Multiple Comparisons and Multiple Tests Using SAS* by Westfall, Tobias, Rom, and Wolfinger, which is part of the SAS Press library.

- **6.4.5** When conducting an *ANOVA*, customized comparisons among combinations of the group means can be made. This is done by testing whether specific linear combinations of the group means (called ‘linear contrasts’) differ from 0.

In Example 6.1, a CONTRAST statement is included in the SAS analysis to compare mean scores between ‘Active’ and ‘Placebo’ ⑨. ‘Active’ represents the pooled results from both the active (‘HI’ and ‘LO’) dose groups, and this overall mean is compared to the results from the placebo group. The CONTRAST statement in PROC GLM is followed by a descriptive label (in this case, ACTIVE vs. PLACEBO), then the name of the effect (dosegrp) and, finally, the contrast specification. The contrast is simply a vector whose elements are coefficients of the parameters, which represent the levels of the effect being tested. Because the effect dosegrp has three levels, you include the 3 coefficients 0.5, 0.5, and -1.0, the order of which must correspond to the order that SAS uses, either alphabetical (e.g., HI, LO, PB) or as specified in an ORDER statement. This specification tells SAS to test

$$H_0: (\text{Hi-Dose mean} + \text{Lo-Dose mean}) / 2 = \text{Placebo mean}$$

The output shows a significant contrast with an F-value of 12.04 (p=0.0012) ⑨.

Any number of CONTRAST statements can be used with each MODEL statement in PROC GLM. You may want to compare the mean responses between the HI and LO dose groups, in addition. For this, you could add a new CONTRAST statement as:

```
contrast 'Hi vs. Low Dose' dosegrp 1 -1 0;
```

- **6.4.6** Multiple comparison results can sometimes be contrary to your intuition. For example, let $\bar{y}_A < \bar{y}_B < \bar{y}_C$, and suppose the analysis determines that the Group A mean is not statistically different from that of Group B, which is not different from C. You might infer that there is no difference among any of the 3 groups while, in fact, there might be a significant difference between Groups A and C. Remember that ‘no difference’ means ‘insufficient evidence to detect a difference’.

- **6.4.7** Sometimes, when a significant *F-test* for groups is found, it is preferable to report the confidence intervals for the group mean responses rather than perform multiple comparisons. A 95% confidence interval for the mean of Group i is found by

$$\bar{y}_i \pm t_{0.025, N-k} \cdot \sqrt{\frac{MSE}{n_i}}$$

In Example 6.1, a 95% confidence interval for the Hi-Dose group is

$$21.588 \pm 2.014 \cdot \sqrt{\frac{24.9}{17}} = (19.2 - 24.0)$$

Similarly, 95% confidence intervals can be found for the Lo-Dose group (21.2 – 26.4) and for the Placebo group (25.5 – 30.5). Such intervals are often depicted graphically in medical journal articles.

By using the ESTIMATE statement in SAS following the MODEL statement, you can obtain the mean and standard error for these calculations. The ESTIMATE statement is used very much like the CONTRAST statement. In this case, you must include an ‘intercept’ term corresponding to the overall mean as follows:

```
estimate 'Hi Dose Group' intercept 1 dosegrp 1 0 0;
estimate 'Lo Dose Group' intercept 1 dosegrp 0 1 0;
estimate 'Placebo Group' intercept 1 dosegrp 0 0 1;
```

- **6.4.8** 95% confidence intervals can also be obtained for the mean difference between any pair of groups (e.g., Group i vs. Group j) by using the formula

$$(\bar{y}_i - \bar{y}_j) \pm t_{0.025, N-k} \sqrt{MSE \left(\frac{1}{n_i} + \frac{1}{n_j} \right)}$$

In Example 6.1, a 95% confidence interval for the difference in mean HAM-A scores between the Placebo and Lo-Dose groups is

$$(28.00 - 23.80) \pm 2.014 \cdot \sqrt{24.9 \left(\frac{1}{16} + \frac{1}{15} \right)} = \\ 4.20 \pm 2.014 \cdot 1.793 = \\ (0.59 \text{ to } 7.81)$$

You can obtain these confidence intervals in SAS by using the T option in the MEANS statement in PROC GLM (see results for the LSD t tests in Output 6.1 ⑩). You can also have SAS compute the estimates of the mean differences and standard errors using the ESTIMATE statements. For the example above for the Lo Dose-Placebo difference, simply include the SAS statement:

```
estimate 'Lo Dose v. Placebo' intercept 1 dosegrp 0 1 -1;
```

following the MODEL statement.

A confidence interval for the difference in means that does not contain 0 is indicative of significantly different means.

Making inferences based on a series of confidence intervals can lead to the same type of inflation of the overall significance level as was discussed with hypothesis testing. Adjustments to the interval widths can be made when using simultaneous confidence intervals by resorting to the same methodology developed for multiple comparison inferences.

- **6.4.9** If there are only two groups ($k = 2$), the p-value for Group effect using an *ANOVA* is the same as that of a *two-sample t-test*. The F- and t-distributions enjoy the relationship that, with 1 upper degree of freedom, the F-statistic is the square of the t-statistic. When $k = 2$, the MSE (computed as a pooled combination of the group sample means) is identical to the pooled variance s_p^2 , which is used in the *two-sample t-test* (Chapter 5). Thus, you can analyze Example 5.1 using PROC GLM instead of PROC TTEST. In that case, the test for equal variances may simply be carried out using the HOVTEST option in a MEANS statement in PROC GLM, and the WELCH option will give the same results as the Satterthwaite's adjustment.
- **6.4.10** Manual computations (with a hand calculator) can be facilitated with standard computing formulas as follows:

$$\text{Total for Group } i \quad G_i = \sum_{j=1}^{n_i} y_{ij}$$

$$\text{Overall Total} \quad G = \sum_{i=1}^k G_i$$

$$\text{Correction Factor} \quad C = \frac{G^2}{N}$$

$$\text{Total Sum of Squares} \quad \text{TOT(SS)} = \sum_i \sum_j y_{ij}^2 - C$$

$$\text{Sum of Squares for Groups} \quad \text{SSG} = \sum_i \frac{G_i^2}{n_i} - C$$

$$\text{Sum of Squares for Error} \quad \text{SSE} = \text{TOT(SS)} - \text{SSG}$$

- **6.4.11** SAS computes the sum of squares in four ways. These are called the Type I, II, III, and IV sums of squares. Computational differences among these types are based on the model used and the missing value structure. For the *one-way ANOVA*, all four types of sums of squares are identical, so it does not matter which type is selected. SAS prints the Type I and III results by default. These different types of sums of squares are discussed in Chapter 7 and Appendix D.

CHAPTER 7

Two-Way ANOVA

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7.1 Introduction

The *two-way ANOVA* is a method for simultaneously analyzing two factors that affect a response. As in the *one-way ANOVA*, there is a group effect, such as treatment group or dose level. The *two-way ANOVA* also includes another identifiable source of variation called a blocking factor, whose variation can be separated from the error variation to give more precise group comparisons. For this reason, the *two-way ANOVA* layout is sometimes called a ‘randomized block design’.

Because clinical studies often use factors such as study center, gender, diagnostic group, or disease severity as a stratification or blocking factor, the *two-way ANOVA* is one of the most common ANOVA methods used in clinical data analysis.

The basic ideas underlying the *two-way ANOVA* are given in Appendix C. A review of the concepts in Section C.2 might be helpful before proceeding with this chapter.

7.2 Synopsis

In general, the randomized block design has g ($g \geq 2$) levels of a ‘group’ factor and b ($b \geq 2$) levels of a ‘block’ factor. An independent sample of measurements is taken from each of the $g \times b$ cells formed by the group-block combinations. Let n_{ij} represent the number of measurements taken in Group i and Block j (Cell $i-j$), and let N represent the number of measurements over all $g \times b$ cells. Letting y_{ijk} denote the k^{th} response in Cell $i-j$ ($k = 1, 2, \dots, n_{ij}$), the general layout of the randomized block design is shown in Table 7.1.

TABLE 7.1 Randomized Block Layout

	Group 1	Group 2	...	Group g
Block 1	$y_{111}, y_{112}, \dots, y_{11n_{11}}$	$y_{211}, y_{212}, \dots, y_{21n_{21}}$...	$y_{g11}, y_{g12}, \dots, y_{g1n_{g1}}$
Block 2	$y_{121}, y_{122}, \dots, y_{12n_{12}}$	$y_{221}, y_{222}, \dots, y_{22n_{22}}$...	$y_{g21}, y_{g22}, \dots, y_{g2n_{g2}}$
...
Block b	$y_{1b1}, y_{1b2}, \dots, y_{1bn_{1b}}$	$y_{2b1}, y_{2b2}, \dots, y_{2bn_{2b}}$...	$y_{gb1}, y_{gb2}, \dots, y_{gbn_{gb}}$

The general entries in a *two-way ANOVA* summary table are represented as shown in Table 7.2.

TABLE 7.2 ANOVA Summary Table for the Two-Way ANOVA

Source	df	SS	MS	F
Group (G)	$g-1$	SSG	MSG	$F_G = MSG/MSE$
Block (B)	$b-1$	SSB	MSB	$F_B = MSB/MSE$
G x B (interaction)	$(g-1)(b-1)$	SSGB	MSGB	$F_{GB} = MSGB/MSE$
Error	$N-gb$	SSE	MSE	
Total	$N-1$	TOT(SS)		

SS represents the sum of squared deviations associated with the factor listed under ‘Source’. These are computed in a way similar to that shown in Chapter 6 for the *one-way ANOVA*.

The mean square (MS) is found by dividing the SS by the degrees of freedom. The MS represents a measure of variability associated with the factor listed under ‘Source’. When there is no effect due to the specified factor, this variability reflects measurement error variability, σ^2 , which is also estimated by MSE.

The F-values are ratios of the effect mean squares to the mean square error (MSE). Under the null hypothesis of no effect, the F-ratio should be close to 1. These F-values are used as the test statistics for testing the null hypothesis of no mean differences among the levels of the factor.

The F-test for group (F_G) tests the primary hypothesis of no group effect. Denoting the mean for the i^{th} group by μ_i , the test summary is summarized as follows:

null hypothesis:	$H_0: \mu_1 = \mu_2 = \dots = \mu_g$
alt. hypothesis:	$H_A: \text{NOT } H_0$
test statistic:	$F_G = \frac{\text{MSG}}{\text{MSE}}$
decision rule:	reject H_0 if $F_G > F_{N-gb}^{g-1}(\alpha)$

The F-test for the block effect (F_B) provides a secondary test, which is used in a similar way to determine if the mean responses differ among blocking levels. A significant block effect often results in a smaller error variance (MSE) and greater precision for testing the primary hypothesis of “no group effect” than if the block effect were ignored. However, there are exceptions to this (see Section 7.4.5).

The Group-by-Block factor ($G \times B$) represents the statistical interaction between the two main effects. If the F-test for interaction is significant, this result indicates that trends across groups differ among the levels of the blocking factor. This is usually the first test of interest in a *two-way ANOVA* because the test for group effects might not be meaningful in the presence of a significant interaction. If the interaction is significant, further analysis might be required, such as application of a one-way ANOVA to compare groups within each level of the blocking factor.

In a two-way ANOVA, you assume that the samples within each cell are normally distributed with the same variance. You can estimate this common variance, σ^2 , by the mean square error (MSE), which is a pooled combination of the cell sample variances, s_{ij}^2 ,

$$s^2 = \frac{\sum_{i=1}^g \sum_{j=1}^b (n_{ij}-1) \cdot s_{ij}^2}{N - (g \cdot b)}$$

The numerator of this quantity is the sum of squares for error (SSE) based on $N-(g \cdot b)$ degrees of freedom.

In the case of a balanced design ($n_{ij} = n$ for all i, j), the effect sums of squares (SS) are computed in a way that is similar to that shown for the *one-way ANOVA*. For example, the sum of squares for ‘Group’ (SSG), which represents the variability among group levels, is based on the sum of squared deviations of the group means from the overall mean. In the same way, the sum of squares for ‘Block’ (SSB), which represents the variability among the block levels, is based on the sum of squared deviations of the block means from the overall mean. The interaction sum of squares (SSGB) is based on the sum of squared deviations of the cell means from the overall mean. The computations are shown in Example 7.1.

7.3 Examples

Example 7.1—Hemoglobin Changes in Anemia

A new synthetic erythropoietin-type hormone, Rebligen, which is used to treat chemotherapy-induced anemia in cancer patients, was tested in a study of 48 adult cancer patients undergoing chemo-therapeutic treatment. Half the patients received low-dose administration of Rebligen via intramuscular injection three times at 2-day intervals; half the patients received a placebo in a similar fashion. Patients were stratified according to their type of cancer: cervical, prostate, or colorectal. For study admission, patients were required to have a baseline hemoglobin less than 10 mg/dl and a decrease in hemoglobin of at least 1 mg/dl following the last chemotherapy. Changes in hemoglobin (in mg/dl) from pre-first injection to one week after last injection (as shown in Table 7.3) were obtained for analysis. Does Rebligen have any effect on the hemoglobin (Hgb) levels?

TABLE 7.3 Raw Data for Example 7.1

Cancer Type	— ACTIVE —		— PLACEBO —	
	Patient Number	Hgb Change	Patient Number	Hgb Change
CERVICAL	1	1.7	2	2.3
	3	-0.2	4	1.2
	6	1.7	5	-0.6
	7	2.3	8	1.3
	10	2.7	9	-1.1
	12	0.4	11	1.6
	13	1.3	14	-0.2
	15	0.6	16	1.9
PROSTATE	22	2.7	21	0.6
	24	1.6	23	1.7
	26	2.5	25	0.8
	28	0.5	27	1.7
	29	2.6	30	1.4
	31	3.7	32	0.7
	34	2.7	33	0.8
	36	1.3	35	1.5
COLORECTAL	42	-0.3	41	1.6
	45	1.9	43	-2.2
	46	1.7	44	1.9
	47	0.5	48	-1.6
	49	2.1	50	0.8
	51	-0.4	53	-0.9
	52	0.1	55	1.5
	54	1.0	56	2.1

Solution

You use a two-way ANOVA, with main effects ‘Treatment’ and ‘Cancer Type’, and the interaction ‘Treatment-by-Type’. Treatment has two levels: Active and Placebo. Cancer Type has three levels: Cervical, Prostate, and Colorectal. Of primary interest is whether the Active treatment shows any effect on hemoglobin relative to any effects shown by the Placebo group.

First, obtain the summary statistics. These are shown in a table of cell and marginal means in Table 7.4. Notice the balanced nature of the layout with n=8 for each cell.

TABLE 7.4 Summary Statistics by Treatment Group for Example 7.1

Cancer Type	Treatment Group		Row Mean N
	ACTIVE	PLACEBO	
CERVICAL	1.313 (0.988) N=8	0.800 (1.258) N=8	1.056 16
	2.200 (1.004) N=8	1.150 (0.469) N=8	1.675 16
PROSTATE	0.825 (0.998) N=8	0.400 (1.707) N=8	0.613 16
	Column Mean N	1.446 24	0.783 24
			1.115 48

Entries in Table 7.4 are Cell mean, (SD), and sample size, N

The sum of squares for the main effects, Treatment and Cancer Type, are computed as

$$\begin{aligned} \text{SS(TRT)} &= 24 \cdot (1.446 - 1.115)^2 + \\ &\quad 24 \cdot (0.783 - 1.115)^2 = 5.27 \end{aligned}$$

$$\begin{aligned} \text{SS(TYPE)} &= 16 \cdot (1.056 - 1.115)^2 + \\ &\quad 16 \cdot (1.675 - 1.115)^2 + \\ &\quad 16 \cdot (0.613 - 1.115)^2 = 9.11 \end{aligned}$$

The interaction sum of squares can be computed as

$$\begin{aligned}
 \text{SS(TRT-by-TYPE)} &= 8 \cdot (1.313 - 1.115)^2 + \\
 &\quad 8 \cdot (0.800 - 1.115)^2 + \\
 &\quad 8 \cdot (2.200 - 1.115)^2 + \\
 &\quad 8 \cdot (1.150 - 1.115)^2 + \\
 &\quad 8 \cdot (0.825 - 1.115)^2 + \\
 &\quad 8 \cdot (0.400 - 1.115)^2 - \\
 \text{SS(TRT)} - \text{SS(TYPE)} &= 0.92
 \end{aligned}$$

The total sum of squares is simply the numerator of the sample variance based on all observations.

$$\text{SS(TOT)} = (1.7 - 1.115)^2 + (-0.2 - 1.115)^2 + \dots + (2.1 - 1.115)^2 = 69.18$$

Finally, the error sum of squares (SSE) can be found by subtracting the sum of squares of each of the effects from the total sum of squares.

$$\begin{aligned}
 \text{SSE} &= \text{SS(TOT)} - \text{SS(TRT)} - \text{SS(TYPE)} - \text{SS(TRT-by-TYPE)} \\
 &= 69.18 - 5.27 - 9.11 - 0.92 = 53.88
 \end{aligned}$$

As a check, you can also compute SSE from the cell standard deviations.

$$\begin{aligned}
 \text{SSE} &= 7 \cdot (0.988)^2 + \\
 &\quad 7 \cdot (1.258)^2 + \\
 &\quad 7 \cdot (1.004)^2 + \\
 &\quad 7 \cdot (0.469)^2 + \\
 &\quad 7 \cdot (0.998)^2 + \\
 &\quad 7 \cdot (1.707)^2 = 53.88
 \end{aligned}$$

Now you can complete the ANOVA table and compute the F-statistics. See Table 7.5.

TABLE 7.5 ANOVA Summary for Example 7.1

Source	df	SS	MS	F	p-Value
Treatment (TRT)	1	5.27	5.27	4.11	0.049 *
Cancer (TYPE)	2	9.11	4.55	3.55	0.038 *
TRT-by-TYPE	2	0.92	0.46	0.36	0.702
Error	42	53.88	1.28		
Total	47	69.18			

* Significant ($p < 0.05$)

(p-values obtained from SAS)

At a significance level of α , the F-statistic is compared with the critical F-value, which can be obtained from widely tabulated F-tables or from SAS, by using the function, FINV(1- α ,U,L). The upper degrees of freedom (U) correspond to the MS in the numerator; the error degrees of freedom, which correspond to the MSE, are used as the lower degrees of freedom (L). The F-value for Treatment effect in example 7.1 is the ratio, MS(TRT)/MSE, based on 1 upper and 42 lower degrees of freedom.

The ANOVA summary shows a non-significant interaction, which indicates that the differences between Treatment levels are not inconsistent over Cancer Types. The F-test for Treatment is significant ($p = 0.049$), which indicates that the mean hemoglobin response for the Active group differs from that of the Placebo group averaged over all Cancer Types.

The Cancer Type effect is also significant at the 0.05 level, which suggests differing mean response levels among the Cancer Types. Such information is secondary to the study objective and might be useful in designing future studies or in guiding further analyses.

SAS Analysis of Example 7.1

The SAS code and output for analyzing these data with the two-way ANOVA are shown on the next four pages. The cell summary statistics are first printed using the MEANS procedure ①. The GLM procedure is used with the main effects, Treatment (trt) and Cancer Type (type), specified as class variables in the CLASS statement and as factors in the MODEL statement ②. The interaction (trt*type) is also included in the MODEL statement.

For a balanced layout as shown in this example, the SAS Types I, II, III, and IV sums of squares (see Appendix D) are identical. The Type III results ③ (specified by the SS3 option in the MODEL statement) corroborate the results obtained by manual computations.

While the primary concern is the treatment effect (trt), which is seen to be significant ($p=0.0491$) ④, there is also a significant difference in response among Cancer Types ($p=0.0376$) ⑤. Because there are more than two levels of this effect, further analyses are needed to determine where the differences exist. To perform pairwise t-tests for multiple comparisons, you can include the LSMEANS statement with the T option after the MODEL statement in the SAS code ⑥. This provides comparisons between each pair of Cancer Types, as described in Chapter 6. As seen in Output 7.1, mean hemoglobin response differs significantly between the prostate and colorectal Cancer Types ⑦.

The LINES option of the LSMEANS statement is also included to illustrate the pairwise comparisons. When the PDIFF option is used, SAS prints out a matrix of p-values for all pairwise comparisons ❸. As indicated by a p-value less than 0.05, mean responses for the colorectal (no.2 LSMean) and prostate (no.3 LSMean) Cancer Types differ significantly ($p = 0.0112$).

SAS Code for Example 7.1

```

data hgbds;
    input trt $ type $ patno hgbch @@;
    datalines;
ACT C 1 1.7 ACT C 3 -0.2 ACT C 6 1.7
ACT C 7 2.3 ACT C 10 2.7 ACT C 12 0.4
ACT C 13 1.3 ACT C 15 0.6 ACT P 22 2.7
ACT P 24 1.6 ACT P 26 2.5 ACT P 28 0.5
ACT P 29 2.6 ACT P 31 3.7 ACT P 34 2.7
ACT P 36 1.3 ACT R 42 -0.3 ACT R 45 1.9
ACT R 46 1.7 ACT R 47 0.5 ACT R 49 2.1
ACT R 51 -0.4 ACT R 52 0.1 ACT R 54 1.0
PBO C 2 2.3 PBO C 4 1.2 PBO C 5 -0.6
PBO C 8 1.3 PBO C 9 -1.1 PBO C 11 1.6
PBO C 14 -0.2 PBO C 16 1.9 PBO P 21 0.6
PBO P 23 1.7 PBO P 25 0.8 PBO P 27 1.7
PBO P 30 1.4 PBO P 32 0.7 PBO P 33 0.8
PBO P 35 1.5 PBO R 41 1.6 PBO R 43 -2.2
PBO R 44 1.9 PBO R 48 -1.6 PBO R 50 0.8
PBO R 53 -0.9 PBO R 55 1.5 PBO R 56 2.1
;

proc format;
    value $typfmt 'C' = 'CERVICAL '
                  'P' = 'PROSTATE '
                  'R' = 'COLORECTAL' ;
run;

proc sort data = hgbds;
    by trt type;

proc means mean std n; ❶
    var hgbch;
    by trt type;
    format type $typfmt.;
    title1 'Two-Way ANOVA';
    title2 'EXAMPLE 7.1: Hemoglobin Changes in Anemia';
run;

proc glm data = hgbds;
    class trt type;
    model hgbch = trt type trt*type / ss3; ❷
        lsmeans type / pdiff stderr t lines; ❸
        format type $typfmt.;

run;
quit;

```

OUTPUT 7.1 SAS Output for Example 7.1

Two-Way ANOVA
EXAMPLE 7.1: Hemoglobin Changes in Anemia

The MEANS Procedure
Analysis Variable : hgbch

----- trt=ACT type=CERVICAL -----

Mean	Std Dev	N
1.3125000	0.9876921	8

----- trt=ACT type=PROSTATE -----

Mean	Std Dev	N
2.2000000	1.0042766	8

----- trt=ACT type=COLORECTAL -----

Mean	Std Dev	N
0.8250000	0.9982127	8

①

----- trt=PBO type=CERVICAL -----

Mean	Std Dev	N
0.8000000	1.2581165	8

----- trt=PBO type=PROSTATE -----

Mean	Std Dev	N
1.1500000	0.4690416	8

----- trt=PBO type=COLORECTAL -----

Mean	Std Dev	N
0.4000000	1.7071279	8

OUTPUT 7.1 SAS Output for Example 7.1 (continued)

Two-Way ANOVA								
EXAMPLE 7.1: Hemoglobin Changes in Anemia								
The GLM Procedure								
Class Level Information								
Class								
trt								
type								
Number of Observations Read								
Number of Observations Used								
Dependent Variable: hgbch								
Sum of Squares								
Source	DF	Mean Square	F Value	Pr > F				
Model	5	15.29604167	3.05920833	2.38	0.0543			
Error	42	53.88375000	1.28294643					
Corrected Total	47	69.17979167						
R-Square Coeff Var Root MSE hgbch Mean								
0.221106 101.6229 1.132672 1.114583								
Source	DF	Type III SS	Mean Square	F Value	Pr > F			
trt	1	5.26687500	5.26687500	4.11	0.0491	④		
type	2	9.11291667	4.55645833	3.55	0.0376	⑤		
trt*type	2	0.91625000	0.45812500	0.36	0.7018			

③

④

⑤

OUTPUT 7.1 SAS Output for Example 7.1 (continued)

Two-Way ANOVA EXAMPLE 7.1: Hemoglobin Changes in Anemia				
Least Squares Means				
type	hgbch	LSMEAN	Standard Error	Pr > t
CERVICAL	1.05625000	0.28316806	0.0006	1
COLORECTAL	0.61250000	0.28316806	0.0363	2
PROSTATE	1.67500000	0.28316806	<.0001	3

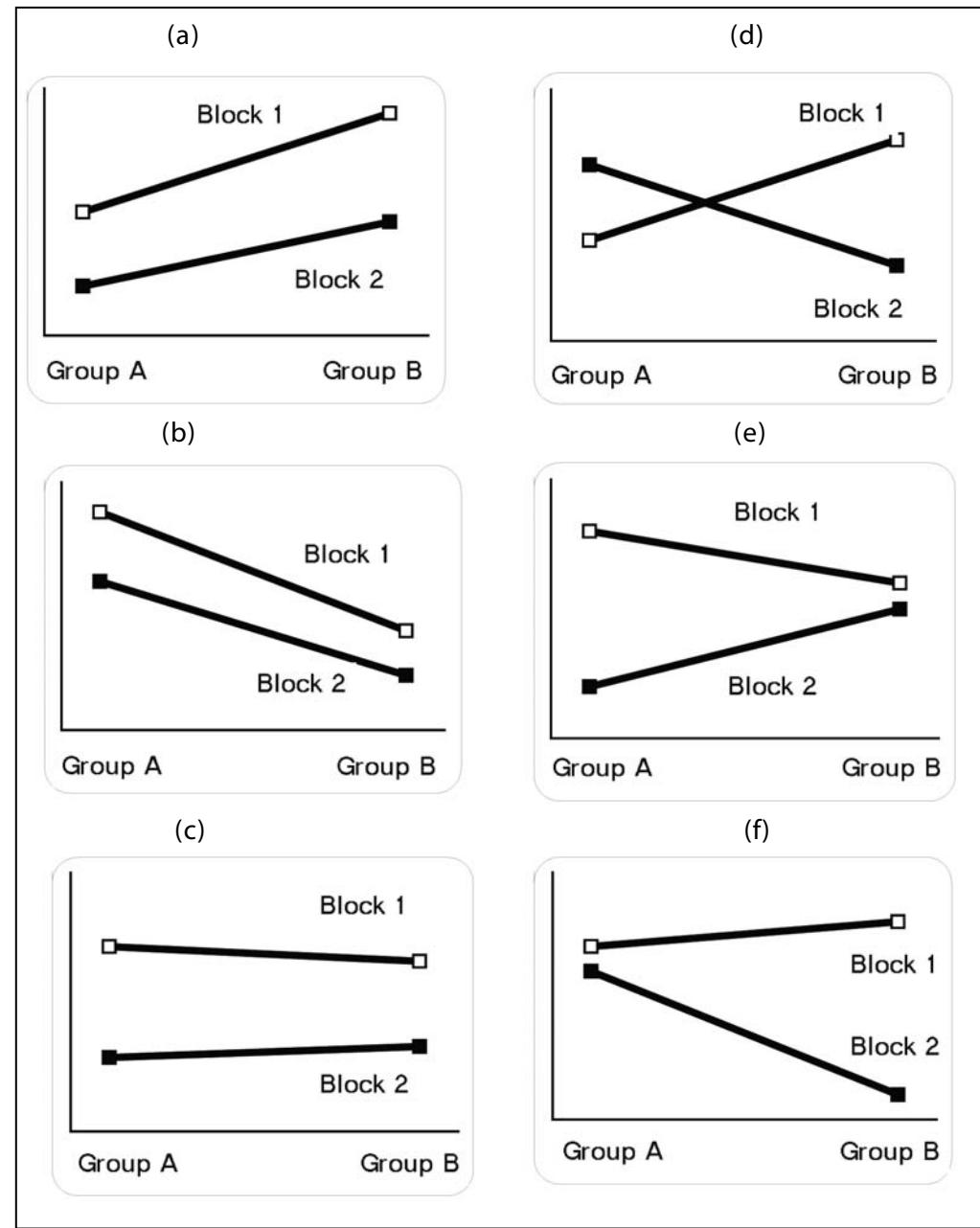
Least Squares Means for Effect type t for H0: LSMean(i)=LSMean(j) / Pr > t				
Dependent Variable: hgbch				
i/j	1	2	3	
1		1.1081 0.2741	-1.5451 0.1298	
2	-1.1081 0.2741		-2.6532 0.0112	⑧
3	1.545098 0.1298	2.653198 0.0112		

T Comparison Lines for Least Squares Means of type LS-means with the same letter are not significantly different.				
	hgbch	type	LSMEAN	Number
A	1.67500	PROSTATE	3	
A				
B	A	1.05625	CERVICAL	1
B				⑦
B		0.61250	COLORECTAL	2

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

To better understand the interaction effect, it is helpful to visualize the trends with graphics. Usually, an interaction is indicated if the response profiles cross or have markedly different slopes among the levels of the blocking factor. In Figure 7.1, graphs a, b, and c show response means that indicate no interaction; graphs d, e, and f depict an interaction between the group and block main effects.

FIGURE 7.1 No Interaction (a, b, c) and Interaction (d, e, f) Effects



Example 7.2 shows the two-way ANOVA for an unbalanced layout with three treatment groups and a significant interaction.

Example 7.2—Memory Function

Two investigators conducted a clinical trial to determine the effect of two doses of a new therapeutic agent on short-term memory function. A single oral dose of the test preparation was administered to subjects, who were then asked to recall items one hour after exposure to a list consisting of 15 items. The number of items correctly identified are shown in Table 7.6. A placebo group was included as a control in a parallel-group design.

TABLE 7.6 Raw Data for Example 7.2

CENTER	DOSE GROUP				
	Placebo	30 mg	60 mg		
Dr. Abel	6	5	8	6	11
	5	6	12	9	7
	6	5	7	6	9
	8	5	8	11	11
	3	7			15
	4	8			9
Dr. Best	7	5	5	8	12
	4	9	6	6	14
	7	11	6	9	15
	6	4	5	11	9
	7	7	3	5	12
	8				13

Solution

This is an unbalanced layout because not all six group \times center cells have the same number of patients. In this case, the computation of the effect sums of squares as illustrated in Example 7.1 might not produce the most appropriate tests. With an unbalanced layout, the SAS Types I, II, and III sums of squares will differ, as discussed in Appendix D. This analysis focuses only on the Type III results.

The summary statistics and ANOVA table are shown in Table 7.7.

TABLE 7.7 Summary Statistics by Dose Group for Example 7.2

CENTER	----- DOSE GROUP -----		
	Placebo	30 mg	60 mg
Dr. Abel	5.67 (1.50) 12	8.38 (2.20) 8	10.11 (2.52) 9
Dr. Best	6.82 (2.09) 11	6.40 (2.32) 10	12.11 (2.03) 9

Entries in Table 7.7 are mean, (SD), and sample size.

SAS Analysis of Example 7.2

The SAS analysis initially uses PROC GLM with a MODEL statement that includes the Dose Group effect (dose), the Center effect (center), and the two-way interaction (dose*center) ⑨. In Output 7.2, the Dose Group effect is seen to be highly significant ($p < 0.0001$) ⑩, which indicates different mean responses among the dose groups.

The LSMEANS statement is included with PROC GLM to provide LSMeans for the Dose Groups ⑪. The pdiff option provides the p-values for pairwise t-tests using the ANOVA MSE to compute the standard errors. The output shows that the 60 mg dose group (LSMean No.3) differs from both the placebo and 30 mg group ⑫, but the placebo-30 mg group comparison is non-significant ($p = 0.0906$).

However, whenever the interaction term is significant, caution must be used in the interpretation of the main effects. In this example, the F-value for the Dose-by-Center interaction is significant ($p = 0.0157$) ⑬, which indicates that differences among dose groups are not the same for each center. This interaction is depicted in Figure 7.2, which was created by the ODS GRAPHICS request shown in the SAS code. The ANOVA summary table, shown in Table 7.8, is taken from the SAS output (Output 7.2).

TABLE 7.8 ANOVA Summary for Example 7.2

Source	df	SS	MS	F	p-Value
Dose	2	251.42	125.71	28.43	<0.001 *
Center	1	2.23	2.23	0.50	0.481
Center-by-Dose	2	39.77	19.88	4.50	0.016 *
Error	53	234.36	4.42		
Total	58	533.93			

* Significant ($p < 0.05$)

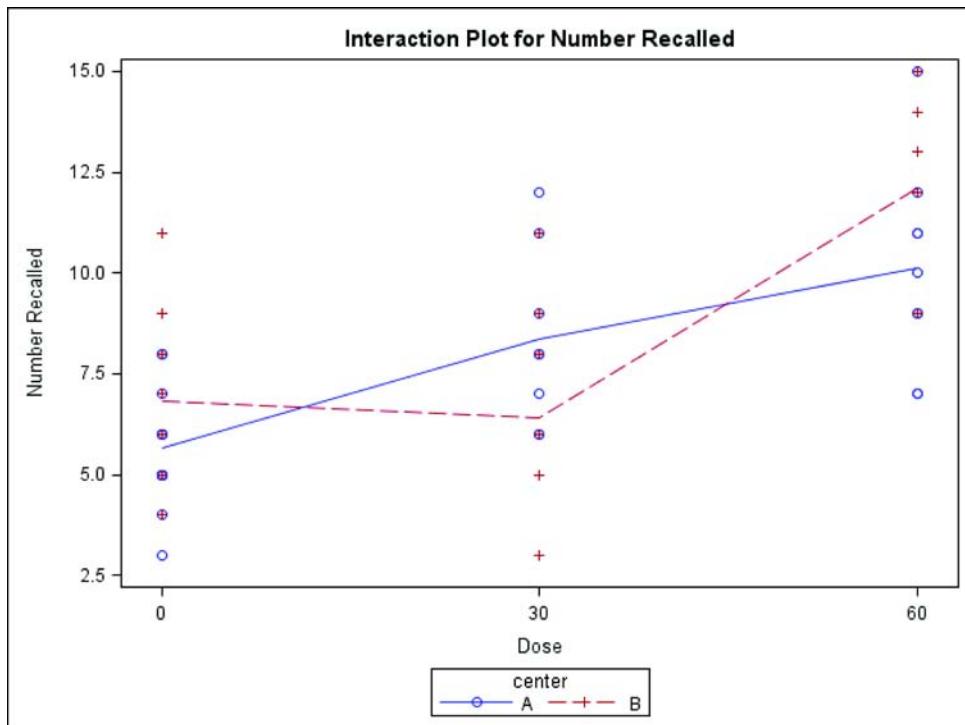
SAS Code for Example 7.2

```

data memry;
    input dose $  center $  y @@;
    datalines;
0 A 6 0 A 5 0 A 6 0 A 8 0 A 3
0 A 4 0 A 5 0 A 6 0 A 5 0 A 5
0 A 7 0 A 8 0 B 7 0 B 4 0 B 7
0 B 6 0 B 7 0 B 8 0 B 5 0 B 9
0 B 11 0 B 4 0 B 7 30 A 8 30 A 12
30 A 7 30 A 8 30 A 6 30 A 9 30 A 6
30 A 11 30 B 5 30 B 6 30 B 6 30 B 5
30 B 3 30 B 8 30 B 6 30 B 9 30 B 11
30 B 5 60 A 11 60 A 7 60 A 7 60 A 11
60 A 9 60 A 10 60 A 12 60 A 9 60 A 15
60 B 9 60 B 12 60 B 13 60 B 9 60 B 13
60 B 12 60 B 14 60 B 15 60 B 12
;
ods graphics on;
proc glm data = memry plots(only)=intplot;
    class dose center;
    model y = dose center center*dose      ⑨
        lsmeans dose/pdiff stderr      ⑫
        label y      = 'Number Recalled'
            center = 'Center'
            dose   = 'Dose';
    title1 'Two-Way ANOVA';
    title2 'EXAMPLE 7.2: Memory Function';
run; quit;
ods graphics off;

```

FIGURE 7.2 Interaction in Example 7.2



OUTPUT 7.2 SAS Output for Example 7.2

```

Two-Way ANOVA
EXAMPLE 7.2: Memory Function

The GLM Procedure
Class Level Information

      Class      Levels      Values
dose            3      0      30      60
center          2      A      B

Number of Observations Read      59
Number of Observations Used     59

Dependent Variable: y      Number Recalled
                    Sum of
Source        DF      Squares      Mean Square      F Value      Pr > F
Model         5      299.5763953    59.9152791     13.55      <.0001
Error        53      234.3558081    4.4218077
Corrected Total 58      533.9322034

      R-Square      Coeff Var      Root MSE      Y Mean
0.561076      26.17421      2.102809      8.033898

Source        DF      Type I SS      Mean Square      F Value      Pr > F
dose          2      256.6302710    128.3151355     29.02      <.0001
center        1      3.1777983      3.1777983     0.72      0.4004
dose*center   2      39.7683260    19.8841630     4.50      0.0157

Source        DF      Type III SS      Mean Square      F Value      Pr > F
dose          2      251.4197307    125.7098653     28.43      <.0001 10
center        1      2.2272995      2.2272995     0.50      0.4810
dose*center   2      39.7683260    19.8841630     4.50      0.0157 11

Least Squares Means

      Standard      LSMEAN
      dose      y LSMEAN      Error      Pr > |t|      Number
0           6.2424242      0.4388811      <.0001      1
30          7.3875000      0.4987251      <.0001      2
60          11.1111111      0.4956369      <.0001      3

Least Squares Means for effect dose
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: y

      i/j      1      2      3      12
      1          0.0906      <.0001
      2          0.0906      <.0001
      3          <.0001      <.0001

```

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

In the presence of an interaction, the next step might be to perform analyses separately within each center. To do this, you could set up contrast statements (see Section 6.4.5) or run a one-way ANOVA (Chapter 6) to test for dose group differences within each center. Using the two-way ANOVA already shown, you could simply include the Dose-by-Center interaction term in an LSMEANS statement as follows:

```
lsmeans dose*center / stderr pdiff;
```

The results, provided in Output 7.3 ⑬, show a significant 30 mg and 60 mg group effect compared with placebo for Dr. Abel's Center A (LSMEAN Numbers 1, 3, and 5) and a significant 60 mg group effect compared with both placebo and the 30 mg dose for Dr. Best's Center B (LSMEANS Numbers 2, 4, and 6). These results are summarized in Table 7.9. You can conclude that a difference exists between the 60 mg dose and the placebo, but the overall efficacy of the 30 mg dose is in question. When significant interactions are found, the analyst can use exploratory analyses to try to better understand or to explain the interaction. In this case, further analyses might reveal, for example, that Dr. Best's patients are older and have greater difficulty with short-term memory, thereby requiring a higher dose of medication.

OUTPUT 7.3 SAS LSMEANS Output for Example 7.2

Two-Way ANOVA EXAMPLE 7.2: Memory Function						
The GLM Procedure Least Squares Means						
dose	center	y LSMEAN	Standard Error	Pr > t	LSMEAN Number	
0	A	5.6666667	0.6070288	<.0001	1	
0	B	6.8181818	0.6340209	<.0001	2	
30	A	8.3750000	0.7434554	<.0001	3	
30	B	6.4000000	0.6649667	<.0001	4	
60	A	10.1111111	0.7009365	<.0001	5	
60	B	12.1111111	0.7009365	<.0001	6	
Least Squares Means for effect dose*center Pr > t for H0: LSMean(i)=LSMean(j)						
Dependent Variable: y						
i/j	1	2	3	4	5	6
1		0.1952	0.0067	0.4190	<.0001	<.0001
2	0.1952		0.1170	0.6509	0.0010	<.0001
3	0.0067	0.1170		0.0529	0.0952	0.0006
4	0.4190	0.6509	0.0529		0.0003	<.0001
5	<.0001	0.0010	0.0952	0.0003		0.0487
6	<.0001	<.0001	0.0006	<.0001	0.0487	
NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.						

⑬

TABLE 7.9 Results of Pairwise Dose Comparisons for Example 7.2

CENTER	----- Pairwise Comparisons -----		
	Placebo vs. 30 mg	Placebo vs. 60 mg	30 mg vs. 60 mg
A (Dr. Abel)	p = 0.0067 *	p < 0.0001 *	p = 0.0952 (NS)
B (Dr. Best)	p = 0.6509 (NS)	p < 0.0001 *	p < 0.0001 *

Asterisk (*)=significant ($p < 0.05$), NS = not significant ($p > 0.05$)

■ “Mixed” Effects

There are two types of effects that can be used in an *ANOVA* model, the ‘fixed’ effect and the ‘random’ effect. All the effects in the examples in this chapter so far have been considered fixed, that is, having pre-specified levels, with the goal of comparing specific levels of that effect. Treatment or dose group is normally a fixed effect, as in Examples 7.1 and 7.2, since you want to compare responses among the specific groups or dose levels. A random effect is one whose levels are randomly selected from a large population of levels. There is no interest in making inferences about specific levels of a random factor, but rather about any level in general.

The blocking factor in a *two-way ANOVA* can be either fixed or random. In Example 7.1, the blocking factor is the type of cancer: cervical, prostate, and colorectal. In this case, the blocking factor would be considered a fixed effect. In Example 7.2, Study Center is considered a fixed blocking effect since there are only two centers, and there may be interest in inferential conclusions about these two centers. However, Study Center is frequently considered a random effect since the centers often represent a sample from a large number of centers available to conduct the study, especially when the study includes many centers.

When there is no interest in the interaction in the *two-way ANOVA*, the analysis of treatment comparisons is identical whether the blocking factor is considered fixed or random. If, however, the blocking factor is considered a random effect and the interaction term is included (interaction also being considered a random effect), then the test for treatment effect changes. In the balanced case, the *F-test* for Treatment Group becomes the ratio of the mean square for Treatment to the mean square for interaction. The mean square for error (MSE) is no longer the appropriate denominator because model assumptions are different for fixed and random effects, and the *F-tests* are based on expected mean squares.

In SAS, you can analyze the *two-way ANOVA* with a fixed treatment effect and a random blocking effect by including the RANDOM statement in PROC GLM to identify the random effects. If you consider Study Center to be a random effect in Example 7.2, you would use the statement:

```
random center dose*center;
```

following the MODEL statement in the SAS code (see SAS Code for Example 7.2).

The output would be identical to that shown in Output 7.2 except that it would also include the forms of the expected mean squares, as seen in Output 7.4. These indicate that the appropriate *F-test* for the dose effect is the ratio of MS(dose) to MS(dose*center) since, under the null hypothesis, the dose effect component (Q(dose)) would be zero and the F-ratio would be 1.

OUTPUT 7.4 SAS Output from Example 7.2 with Random Effects

The GLM Procedure	
Source	Type III Expected Mean Square
dose	Var(Error) + 9.7161 Var(dose*center) + Q(dose)
center	Var(Error) + 9.6546 Var(dose*center) + 28.964 Var(center)
dose*center	Var(Error) + 9.7161 Var(dose*center)

To make the appropriate test in GLM, you must use a TEST statement (following the RANDOM statement). The TEST statement instructs SAS to form the *F-test* as the ratio of mean square for ‘h’ to the mean square for ‘e’, where h is the effect of interest (dose) and e is the appropriate ‘error’ term (dose*center), as follows:

```
test h=dose  e=dose*center;
```

The output (Output 7.5) shows an F-value for dose effect of 6.32, which is not significant ($p = 0.1366$). It is important to note that when testing for a fixed treatment effect, the standard errors of the treatment differences are generally larger when the blocking factor is random than when it is fixed. This observation is consistent with intuition since inferences about treatment differences can only be made for the specific blocks in the fixed case, but can be extended to any randomly selected block when they’re considered random, the latter being a stronger conclusion. You can see that the degrees-of-freedom for the denominator of the *F-test* in the fixed case (MSE) is 53, while the degrees of freedom for the denominator in the random case (MS(dose*center)) is only 2.

OUTPUT 7.5 Results of the TEST Statement

The GLM Procedure					
Dependent Variable: y Number Recalled					
Tests of Hypotheses Using the Type III MS for dose*center as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
dose	2	251.4197307	125.7098653	6.32	0.1366

■ Using PROC MIXED

When using SAS, PROC MIXED is preferable to PROC GLM in many situations (see Section 7.4.11). The SAS code for performing the analysis of Example 7.2 using PROC MIXED is shown below. Here, both Dose Group and Center are considered fixed effects.

SAS Code for Example 7.2 — Using PROC MIXED (fixed effects model)

```
proc mixed data = memry;
  class dose center;
  model y = dose center dose*center;
    lsmeans dose*center / diff; ⑯
    title3 '--- Analysis Using PROC MIXED ---';
  run;
```

Notice that the CLASS, MODEL, and LSMEANS statements for PROC MIXED are all identical to those of PROC GLM for this example. The DIFF option in the LSMEANS statement **⑯** with PROC MIXED is equivalent to PDIFF. The results are shown in Output 7.6.

Under the section titled ‘Dimensions’ **⑯**, there is one covariance parameter. This is the random effect for ‘error’, called ‘Residual’, with an estimate of 4.4218 (under ‘Covariance Parameter Estimates’) **⑯**. This is the same as the MSE obtained from PROC GLM. The ‘Fit Statistics’ are used to evaluate the appropriateness of the covariance matrix. These are discussed further in Chapter 8. Since all effects are considered fixed, the tests **⑰** (“Type 3 Tests of Fixed Effects”) are identical to those obtained from PROC GLM. The LSMEANS and the pairwise comparisons are also the same as those obtained previously. PROC MIXED displays them in a different format, which includes the estimates and standard errors of the differences in Dose Group means **⑯**. These standard errors are not available using PROC GLM.

OUTPUT 7.6 SAS Output for Example 7.2 Using PROC MIXED for Fixed Effects

```
Two-Way ANOVA
EXAMPLE 7.2: Memory Function
--- Analysis Using PROC MIXED ---

The Mixed Procedure

Model Information

Data Set                  WORK.MEMRY
Dependent Variable        Y
Covariance Structure     Diagonal
Estimation Method         REML
Residual Variance Method Profile
Fixed Effects SE Method  Model-Based
Degrees of Freedom Method Residual
```

**OUTPUT 7.6 SAS Output for Example 7.2 Using PROC MIXED for Fixed Effects
(continued)**

Two-Way ANOVA																								
EXAMPLE 7.2: Memory Function																								
--- Analysis Using PROC MIXED ---																								
 Class Level Information																								
<table> <thead> <tr> <th>Class</th> <th>Levels</th> <th>Values</th> <th></th> <th></th> </tr> </thead> <tbody> <tr> <td>dose</td> <td>3</td> <td>0 30 60</td> <td></td> <td></td> </tr> <tr> <td>center</td> <td>2</td> <td>A B</td> <td></td> <td></td> </tr> </tbody> </table>					Class	Levels	Values			dose	3	0 30 60			center	2	A B							
Class	Levels	Values																						
dose	3	0 30 60																						
center	2	A B																						
 Dimensions																								
Covariance Parameters		1	15																					
Columns in X		12																						
Columns in Z		0																						
Subjects		1																						
Max Obs Per Subject		59																						
 Number of Observations																								
Number of Observations Read		59																						
Number of Observations Used		59																						
Number of Observations Not Used		0																						
 Covariance Parameter Estimates																								
Cov Parm		Estimate																						
Residual		4.4218	16																					
 Fit Statistics																								
-2 Res Log Likelihood		242.9																						
AIC (smaller is better)		244.9																						
AICC (smaller is better)		244.9																						
BIC (smaller is better)		246.8																						
 Type 3 Tests of Fixed Effects																								
<table> <thead> <tr> <th>Effect</th> <th>Num DF</th> <th>Den DF</th> <th>F Value</th> <th>Pr > F</th> </tr> </thead> <tbody> <tr> <td>dose</td> <td>2</td> <td>53</td> <td>28.43</td> <td><.0001</td> </tr> <tr> <td>center</td> <td>1</td> <td>53</td> <td>0.50</td> <td>0.4810</td> </tr> <tr> <td>dose*center</td> <td>2</td> <td>53</td> <td>4.50</td> <td>0.0157</td> </tr> </tbody> </table>					Effect	Num DF	Den DF	F Value	Pr > F	dose	2	53	28.43	<.0001	center	1	53	0.50	0.4810	dose*center	2	53	4.50	0.0157
Effect	Num DF	Den DF	F Value	Pr > F																				
dose	2	53	28.43	<.0001																				
center	1	53	0.50	0.4810																				
dose*center	2	53	4.50	0.0157																				

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**OUTPUT 7.6 SAS Output for Example 7.2 Using PROC MIXED for Fixed Effects
(continued)**

Two-Way ANOVA									
EXAMPLE 7.2: Memory Function									
--- Analysis Using PROC MIXED ---									
Least Squares Means									
Effect	dose	center	Estimate	Standard Error	DF	t Value	Pr > t		
dose*center	0	A	5.6667	0.6070	53	9.34	<.0001		
dose*center	0	B	6.8182	0.6340	53	10.75	<.0001		
dose*center	30	A	8.3750	0.7435	53	11.26	<.0001		
dose*center	30	B	6.4000	0.6650	53	9.62	<.0001		
dose*center	60	A	10.1111	0.7009	53	14.43	<.0001		
dose*center	60	B	12.1111	0.7009	53	17.28	<.0001		
Differences of Least Squares Means									
Effect	dose	center	_dose	_center	Estimate	Standard Error	DF	t Value	Pr > t
dose*center	0	A	0	B	-1.1515	0.8778	53	-1.31	0.1952
dose*center	0	A	30	A	-2.7083	0.9598	53	-2.82	0.0067
dose*center	0	A	30	B	-0.7333	0.9004	53	-0.81	0.4190
dose*center	0	A	60	A	-4.4444	0.9273	53	-4.79	<.0001
dose*center	0	A	60	B	-6.4444	0.9273	53	-6.95	<.0001
dose*center	0	B	30	A	-1.5568	0.9771	53	-1.59	0.1170
dose*center	0	B	30	B	0.4182	0.9188	53	0.46	0.6509
dose*center	0	B	60	A	-3.2929	0.9451	53	-3.48	0.0010
dose*center	0	B	60	B	-5.2929	0.9451	53	-5.60	<.0001
dose*center	30	A	30	B	1.9750	0.9975	53	1.98	0.0529
dose*center	30	A	60	A	-1.7361	1.0218	53	-1.70	0.0952
dose*center	30	A	60	B	-3.7361	1.0218	53	-3.66	0.0006
dose*center	30	B	60	A	-3.7111	0.9662	53	-3.84	0.0003
dose*center	30	B	60	B	-5.7111	0.9662	53	-5.91	<.0001
dose*center	60	A	60	B	-2.0000	0.9913	53	-2.02	0.0487

Next, the analysis of Example 7.2 is repeated using PROC MIXED, assuming that Study Center is a random effect. One of the main differences in the syntax for PROC MIXED compared with GLM is that, when random effects are included, they are omitted from the MODEL statement and included instead in a RANDOM statement. As shown in the SAS code that follows, center and dose*center are included in the RANDOM statement ⑯ rather than in the MODEL statement.

SAS Code for Example 7.2 — Using PROC MIXED (mixed model)

```
proc mixed data = memry;
  class dose center;
  model y = dose;
    random center dose*center; 18
    lsmeans dose / diff;
  title3 'Mixed Model Using PROC MIXED';
  run;
```

The output (Output 7.7) shows a non-significant Dose Group effect ($F=8.71$, $p=0.1030$) based on the PROC MIXED analysis 19. There are no tests for Center or the Dose-by-Center interaction since these are now considered random effects. This mixed model analysis enables you to make inferences about the Dose effect that applies to the entire population without concern for the levels of the random effects.

OUTPUT 7.7 Results of Example 7.2 Using PROC MIXED for Mixed Effects

```
Two Way ANOVA
EXAMPLE 7.2: Memory Function
Mixed Model Using PROC MIXED

The Mixed Procedure

Model Information

Data Set           WORK.MEMRY
Dependent Variable Y
Covariance Structure Variance Components
Estimation Method  REML
Residual Variance Method Profile
Fixed Effects SE Method Model-Based
Degrees of Freedom Method Containment

Class Level Information

Class      Levels   Values
dose       3        0 30 60
center     2        A B

Dimensions

Covariance Parameters      3
Columns in X                4
Columns in Z                8
Subjects                     1
Max Obs Per Subject         59
```

**OUTPUT 7.7 Results of Example 7.2 Using PROC MIXED for Mixed Effects
(continued)**

Two Way ANOVA
EXAMPLE 7.2: Memory Function
Mixed Model Using PROC MIXED

Number of Observations

Number of Observations Read	59
Number of Observations Used	59
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	257.42364635	
1	5	254.69935090	0.000002889
2	1	254.69728265	0.00000018
3	1	254.69726874	0.00000000

Convergence criteria met.

Covariance Parameter
Estimates

Cov Parm	Estimate
center	0
dose*center	1.0390
Residual	4.4252

Fit Statistics

-2 Res Log Likelihood	254.7
AIC (smaller is better)	258.7
AICC (smaller is better)	258.9
BIC (smaller is better)	256.1

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F	19
dose	2	2	8.71	0.1030	

OUTPUT 7.7 Results of Example 7.2 Using PROC MIXED for Mixed Effects (continued)

Two Way ANOVA						
EXAMPLE 7.2: Memory Function						
Mixed Model Using PROC MIXED						
Least Squares Means						
Standard						
Effect	dose	Estimate	Error	DF	t Value	Pr > t
dose	0	6.2356	0.8439	2	7.39	0.0178
dose	30	7.3520	0.8760	2	8.39	0.0139
dose	60	11.1111	0.8749	2	12.70	0.0061
Differences of Least Squares Means						
Standard						
Effect	dose	_dose	Estimate	Error	DF	t Value
dose	0	30	-1.1163	1.2164	2	-0.92
dose	0	60	-4.8755	1.2155	2	-4.01
dose	30	60	-3.7592	1.2381	2	-3.04

7.4 Details & Notes

- **7.4.1** In Example 7.1, the main benefit of including Cancer Type as an ANOVA factor in the analysis is to improve the precision of the treatment comparisons by identifying the large variations among Cancer Types and removing them from the estimate of the error variation (MSE). Indeed, if the *one-way ANOVA* had been used to compare treatments (ignoring Cancer Type), you might verify that the result would be non-rejection of the hypothesis of equal treatment means based on an *F-test* of 3.79 ($p = 0.058$). This analysis produces an inflated estimate of the MSE of 1.39, compared with an MSE of 1.28 when using the more appropriate *two-way ANOVA*.
- **7.4.2** In developing the concept of analysis-of-variance in this and other chapters, manual calculations are demonstrated for balanced layouts to emphasize how the within- and between-group variability estimates arise and how the results are interpreted. The computing methods in Example 7.1 show the deviations used in obtaining the effect sums of squares. Formulas similar to those used in the one-way ANOVA (see Section 6.4.10) can also be used for the two-way (and higher) ANOVA with balanced layouts. However, such equations are rarely used. Statistical packages obviate the need for manual calculations in the balanced case and are mandatory for most practical applications that involve unbalanced layouts. Advances in computing power, which efficiently use numerical techniques to solve very complex equations, have opened the door to newer statistical approaches in recent years. These

approaches give the statistician powerful new tools for analyzing complex data layouts with fewer assumptions. Procedures in SAS, like MIXED, GENMOD, GLIMMIX, and NLIN MIXED, have wide application in statistical modeling and are moving into mainstream use.

- **7.4.3** In clinical trials, there is almost always imbalance in two-way layouts, even if the study were designed as a balanced one. This might be due to patients who dropped out or missed visits, data exclusion, or some other reason that results in missing data. In such cases, there might be more than one way to compute the sums of squares for the ANOVA effects, as seen by the different SAS types of sums of squares.

In the unbalanced case, the total sum of squares cannot be broken down as easily into additive components due to the sources of variation. A number of statistical methods have been devised to circumvent the problems that arise with sample size imbalance. The method of ‘fitting constants’ and the method of ‘weighted squares of means’ are two classical approaches (Bancroft, 1968). Each method tests a slightly different hypothesis, as framed in terms of cell means. The method that should be used is the method that corresponds most closely to the assumptions and hypotheses relevant to the given problem.

In SAS, Type III corresponds to the method of weighted squares of means and is often the method of choice for the analysis of clinical data. The hypothesis tested by using Type III results is the equality of treatment group means, where each group mean is the unweighted average of the cell means that comprise that group. The group mean does not depend on the cell sample sizes (LS-mean), as is the case with Types I and II. In cases of extreme imbalance, such as a large number of patients in some centers and a very small number of patients in other centers, the use of Type III results for the treatment effect when Center is used as a blocking factor should be questioned when finite populations must be assumed. LS-means are discussed in Appendix C (Section C.3), and interpretation of the various types of sums of squares is discussed in Appendix D.

- **7.4.4** In a multi-center trial, there might be differences among study centers due to such things as geography, climate, general population characteristics, or specialty differences at specific centers. If you ignore these differences, the variation due to Study Centers will be included in the estimate of experimental error (MSE). As seen in Chapter 6, “One-Way ANOVA”, potential treatment differences become obscured with large values of MSE. Therefore, you want to try to identify all important sources of variation that can be factored out of the experimental error to yield a more precise test for treatment effect. In the case of a multi-center study, ‘Study Center’ is usually considered an important factor in analyzing treatment differences because of known or suspected differences among centers.
- **7.4.5** Sometimes, including a fixed block effect with a two-way ANOVA results in a less precise treatment comparison of the group means than you would obtain by ignoring the blocking factor and using the one-way ANOVA. Because the error degrees of freedom are reduced when including the Block

variable as a source of variation in the ANOVA, the MSE might increase appreciably if among-block variation is small and the number of levels of the blocking factor is large. With an increased MSE, the F-test for GROUP is smaller due to the reduced degrees of freedom for error, therefore, the precision of the group comparisons decreases. Thus, it is counterproductive to use ANOVA in cases that include fixed blocking factors and have a large number of homogeneous levels.

- **7.4.6** Pairwise comparisons of group means are often conducted by using the PDIFF option in the LSMEANS statement in PROC GLM or PROC MIXED, as shown in Examples 7.1 and 7.2. (Note: In PROC MIXED, the DIFF option is equivalent to the PDIFF option). This method compares the LS-means of the levels of the factor specified in the LSMEANS statement using ‘t-test type’ procedures. To avoid greatly altering the tests’ significance levels, only pre-planned comparisons should be made. Multiple comparison procedures controlling for the overall significance level should be used when the number of comparisons becomes large (see Appendix E). You can use the ADJUST= option of the LSMEANS statement to get adjusted p-values for multiple tests. CONTRAST statements, as discussed in Chapter 6 (Section 6.4.5), can also be used to conduct pairwise or custom comparisons.

When there is a significant interaction, you can include the SLICE= option in the LSMEANS statement to obtain a global test for treatment effect within each blocking level. In Example 7.2, you could include the following statement:

```
lsmeans dose*center / slice=center;
```

This would provide an F-test for the Dose Group effect within each center.

- **7.4.7** The MSE is an estimate of the variance among similar patients. Because patients are most similar within Group-by-Block cells, the MSE is an average or pooled variance over all cells of the within-cell variances. However, if each cell has only one measurement ($n_{ij} = 1$ for all i,j), the within-cell variability cannot be estimated. In such cases, the analysis can proceed by assuming there is no interaction and using the interaction sum of squares as the SSE.

Even in layouts with cells that have more than one measurement, if it is known or can be safely assumed that there is no interaction between main effects, the interaction can be ignored as a source in the ANOVA. The sum of squares for interaction is then absorbed into the SSE. Although this increases the SSE, the number of degrees of freedom also increases. If the interaction effect is really insignificant, then increasing the degrees of freedom for MSE might more than offset the SSE increase with the possible net effect of gaining sensitivity in testing the main effects.

- **7.4.8** An ANOVA can be easily extended to include more than two factors with the analyses by following the same pattern as demonstrated in this chapter. For example, you can conduct a three-way ANOVA on the data set

TRIAL in Chapter 3 by using the factors Treatment Group (TRT), Study Center (CENTER), and patient gender (SEX). Using the GLM procedure, the following SAS statements show a model that includes all two-way and the three-way interactions:

```
proc glm data = trial;
  class trt center sex;
  model score = trt center sex
    trt*center trt*sex center*sex
    trt*center*sex;
```

The number of possible interactions increases markedly as new main effects are added to the model. Higher-order interactions and even some two-way interactions might not be meaningful or might be difficult to explain, and including a large number of interactions can decrease the degrees of freedom available for estimation of the error (MSE). For these reasons, only meaningful interactions or interactions that might lead to important subgroup differences should be considered when performing the final analysis.

- **7.4.9** A statistical model associated with a two-way or higher-order ANOVA is called a ‘fixed-effects’ model if all effects are fixed, a ‘random-effects’ model if all effects are random, and a ‘mixed’ model if there is a mixture of fixed and random effects. Since the treatment or dose group used in clinical trials is usually considered a fixed-effect model, most models encountered in clinical research are fixed or mixed.
- **7.4.10** You can see from the analysis of Example 7.2 how important it is to correctly pre-designate the effects in your model as fixed or random. There is a highly significant Dose effect along with a significant interaction when Center is considered fixed, but the Dose effect is no longer significant when Center is considered a random effect.
- **7.4.11** A fixed-effect model contains a fixed (nonrandom) component for each fixed effect, plus a random ‘error’ component (sometimes referred to as ‘measurement error’), which you typically assume has a normal distribution. The covariance structure of that model is determined by the error term. Typically, you assume the errors are normally distributed, are independent, and have the same variance. In that case, the covariance structure is a matrix whose diagonal elements have all the same values, and the off-diagonal elements are all zero. The standard technique for conducting ANOVA is via sums of squares computations, as demonstrated in this and other chapters, and it is the method on which PROC GLM primarily relies.

PROC MIXED can analyze a fixed-effects model using a completely different approach, referred to as ‘likelihood methods’. Simplistically, these methods determine the model parameter estimates that would most likely result in the data set observed, and they do not require the usual restrictive ANOVA assumptions of independence and homogeneous variances. You might be inclined to think of PROC MIXED only for mixed models. However, PROC MIXED can be used very effectively (without the RANDOM statement) in

cases when all effects are fixed, as demonstrated for Example 7.2. Unlike PROC GLM, you can easily use PROC MIXED to analyze a fixed-effects model when the observations are correlated or have different variances. In a mixed model, you include one or more fixed components and one or more random components in addition to the ‘measurement error’ term. The covariance structure is dependent on all the random components in the model, not just the error term. The diagonal elements of the covariance matrix, which are associated with the variability, may be different, and the off diagonal elements, which are associated with the correlation between pairs of observations, may be nonzero.

When one or more factors are random effects, PROC MIXED is recommended over PROC GLM. PROC MIXED is also preferable over GLM in the fixed effects model when observations are correlated. The GLM procedure was originally developed for fixed-effects models with uncorrelated data. The effect of the RANDOM statement in GLM is simply to identify the random effects and instruct SAS to provide the corresponding table of expected mean squares. However, the tests are computed as if all effects were fixed. GLM computations are based on sums of squares and mean squares that produce exact F-tests in the balanced case and approximate F-tests, by using ‘reasonable’ estimates, in the unbalanced case.

PROC MIXED computes the F-tests differently. It uses generalized linear modeling that relies on specification of the covariance structure through the methods of generalized Least Squares (“Least Squares” is mentioned further in Chapter 10). The analysis is based on the covariance structure that SAS builds through the identification of the random effects given in the RANDOM statement in PROC MIXED. The F-tests from PROC MIXED are also approximate tests, but they have been shown to be more robust than those of GLM under a large variety of circumstances. These more advanced topics are discussed in detail in various references, including several books within the SAS Press program, notably *SAS for Linear Models, Fourth Edition* (Littell, Stroup, and Freund, 2002) and *SAS for Mixed Models, Second Edition* (Littell, Milliken, Stroup, Wolfinger, and Schabenberger, 2006).

The important thing to realize is that GLM does not always produce the correct standard errors for treatment comparisons in the presence of random effects when using a CONTRAST or ESTIMATE statement (see Sections 6.4.5 and 6.4.7). Furthermore, you cannot get the standard errors for treatment differences using the LSMEANS statement with PROC GLM. However, PROC MIXED provides the correct standard errors using the DIFF option with LSMEANS (see ⑭ in Example 7.2). Although both GLM and MIXED produce approximate F-tests with unbalanced data, in general, PROC MIXED is able to handle more complex layouts better than PROC GLM, especially when the clinical trial results are plagued with missing data or when severe imbalances in sample sizes exist among cells. You will also see in the next chapter that PROC MIXED can more easily handle correlated data than PROC GLM.

- **7.4.12** You can specify a covariance structure in the RANDOM statement with PROC MIXED using the TYPE= option. The default is the variance component structure (TYPE=VC) with a mixed model, as in Example 7.2 when Center is treated as a random effect. Although the example only illustrates two study centers, when there are many centers, the observations among patients within a center might be correlated, and that correlation structure is built into the covariance matrix used by PROC MIXED. Other types of covariance structures are discussed in Chapter 8.
- **7.4.13** PROC MIXED uses as default a ‘restricted maximum likelihood’ method, or REML, to estimate the variance components. This method can result in negative estimates of the variances. Since we know variances must be nonnegative, REML in PROC MIXED sets the estimate to zero. This is the case for the variance component for center in Example 7.2 (see “Covariance Parameter Estimates” in Output 7.7). When the estimate is set to zero, the estimates of the other parameters are affected, and this can result in biased treatment comparisons. You can select other estimation methods in SAS by specifying the METHOD= option in PROC MIXED, such as ML or TYPE3. Using METHOD=TYPE3 provides the Type III results from PROC GLM when using the RANDOM statement.

CHAPTER 8

Repeated Measures Analysis

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8.1 Introduction

Repeated measures refer to multiple measurements taken from the same experimental unit, such as serial evaluations 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 a *repeated measures* experiment in clinical trials. The data generated are sometimes referred to as ‘longitudinal’ data. These repeated response measurements can be used to characterize a response profile over time. 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 a placebo group. This situation might arise, for example, when trying to determine if the onset of effect or rate of improvement due to a new treatment is faster than that of a competitor’s treatment. Comparison of response profiles can be tested with a single *F-test* from a *repeated measures analysis*.

8.2 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 periods. Letting n_i represent the number of patients in Group i ($i=1,2,\dots,g$), the layout for $g=3$ groups is shown in Table 8.1. In comparative trials, the groups often represent different parallel treatment groups or dose levels of a drug.

TABLE 8.1 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_{1n_11}	y_{1n_12}	...	y_{1n_1t}
2	1	y_{211}	y_{212}	...	y_{21t}
	2	y_{221}	y_{222}	...	y_{22t}

	n_2	y_{2n_21}	y_{2n_22}	...	y_{2n_2t}
3	1	y_{311}	y_{312}	...	y_{31t}
	2	y_{321}	y_{322}	...	y_{32t}

	n_3	y_{3n_31}	y_{3n_32}	...	y_{3n_3t}

There are a number of analytic approaches for handling *repeated measures*. You can examine a ‘univariate’ method that is based on the same *ANOVA* concepts discussed in Chapter 7 and Appendix C. A ‘multivariate’ method, which treats the repeated measurements as a multivariate response vector, may also be used in many circumstances. These approaches have been used successfully in the past, however, they come with restrictive assumptions that are often not satisfied in everyday clinical data analysis settings. More commonly used now are approaches based on general linear modeling techniques, the analysis of which is facilitated by increasingly powerful computing methods. These modeling techniques, which are easily handled with the MIXED, GLIMMIX, and GENMOD procedures in SAS, can accommodate missing data and a rich palette of assumptions regarding the covariance structure. You will want to use these latter methods, primarily. However, it’s instructive to start with a brief discussion of the univariate approach.

The ‘Univariate’ Approach

Consider the Group and Time effects shown in Table 8.1 as two factors in an *ANOVA*. Using the ideas discussed in Chapter 7, you could consider the Group effect as a fixed treatment effect and the Time effect as a blocking factor, and then analyze the data using a *two-way ANOVA*. Recall, however, that one of the assumptions of *two-way ANOVA* is independent observations. Here, the measurements across different patients at any time point can be considered independent, but measurements within a patient are correlated. When you use *two-way ANOVA* methods with correlated data, you may end up with erroneous results.

In this case, you'll need to consider Patient (within each Group) as another effect and make certain assumptions regarding the covariance or correlation structure of the repeated measurements within Patients. Notice that the response might vary among groups, among patients within groups, and among the different measurement times. Therefore, you include a Group effect, a Patient (within-Group) effect, and a 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, you assume normality of the response measurements and variance homogeneity among groups. In addition, the univariate *ANOVA* requires that each pair of repeated measures has the same correlation, a feature known as 'compound symmetry'.

A significant interaction between the Group and Time effects means that changes in response over time differ among groups, i.e., a significant difference in response profiles, as illustrated in Figure 8.1. When the 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 a 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 the simplest case of *repeated measures ANOVA*, which is presented here, the Group and the evaluation Time are cross-classified main effects. However, because of the correlation of measurements over time from the same patient, a Patient effect must be included as a source of variation in the *ANOVA* table.

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

TABLE 8.2 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)		

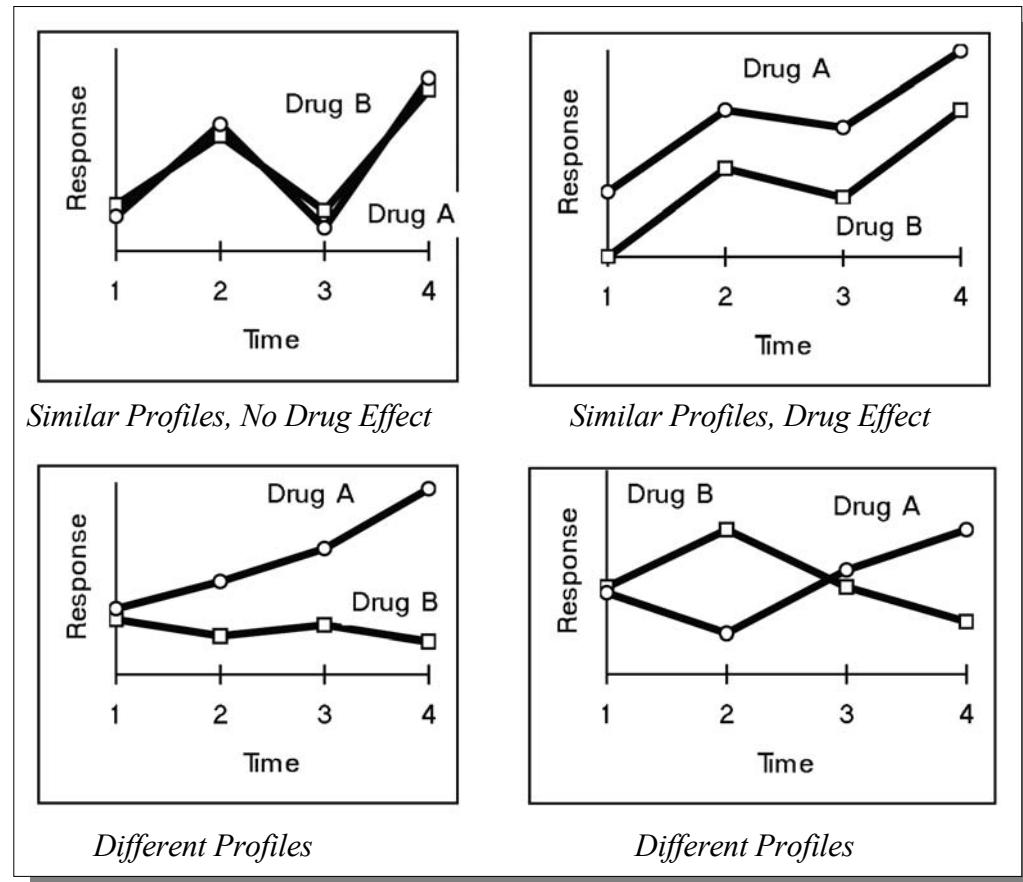
For the balanced layout ($n_1 = n_2 = \dots = n_g$), the sums of squares can be computed in a manner similar to that used for the *two-way ANOVA* (Chapter 7). These computations are shown in Example 8.1. As usual, the mean squares (MS) are found by dividing the sums of squares (SS) by the corresponding 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-group 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 ratio of these independent estimates (F_T) is the F-statistic used to test the hypothesis of no Time effect. Similarly, the interaction mean square, which is also a measure of within-patient variation under H_0 , is compared to the MSE to test for a significant Group-by-Time interaction.

Sample response profiles are shown in Figure 8.1 for 2 drug groups and 4 time periods. As depicted, treatment differences depend on time if the profiles differ.

FIGURE 8.1 Sample Profiles of Drug Response



8.3 Examples

The following example is used to demonstrate the ‘univariate’ approach to the *repeated measures ANOVA* in the balanced case.

Example 8.1—Arthritic Discomfort Following Vaccine

A pilot study was conducted in 8 patients to evaluate the effect of a 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 for 3 months and evaluate their comfort level with routine daily chores during the 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 8.3. Is there any evidence of a difference in response profiles between the active and placebo vaccines?

TABLE 8.3 Raw Data for Example 8.1

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

Solution

Using a ‘univariate’ *repeated measures ANOVA*, you 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 as demonstrated previously (Chapters 6 and 7). First, you obtain the marginal means, as shown in Table 8.4.

TABLE 8.4 Marginal Summary Statistics for Example 8.1

Vaccine	Patient Number	Visit			Mean
		Month 1	Month 2	Month 3	
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:

$$\text{SS(Vaccine)} = (12 \cdot (3.500 - 4.167)^2) + (12 \cdot (4.833 - 4.167)^2) = 10.667$$

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:

$$\begin{aligned} \text{SS(Visit)} &= (8 \cdot (6.375 - 4.167)^2) + \\ &\quad (8 \cdot (3.125 - 4.167)^2) + \\ &\quad (8 \cdot (3.000 - 4.167)^2) = 58.583 \end{aligned}$$

$$\begin{aligned} \text{SS(Vaccine-by-Visit)} &= (4 \cdot (6.25 - 4.167)^2) + \\ &\quad (4 \cdot (2.75 - 4.167)^2) + \\ &\quad (4 \cdot (1.50 - 4.167)^2) + \\ &\quad (4 \cdot (6.50 - 4.167)^2) + \\ &\quad (4 \cdot (3.50 - 4.167)^2) + \\ &\quad (4 \cdot (4.50 - 4.167)^2) - \\ &\quad \text{SS(Vaccine)} - \text{SS(Visit)} \\ &= 77.833 - 10.667 - 58.583 = 8.583 \end{aligned}$$

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, you have 6 degrees of freedom for Patient(Vaccine).

$$\begin{aligned}
 \text{SS(Patient(Vaccine))} &= (3 \cdot (3.000 - 3.500)^2) + \\
 &\quad (3 \cdot (3.667 - 3.500)^2) + \\
 &\quad (3 \cdot (2.333 - 3.500)^2) + \\
 &\quad (3 \cdot (5.000 - 3.500)^2) + \\
 &\quad (3 \cdot (5.333 - 4.833)^2) + \\
 &\quad (3 \cdot (6.333 - 4.833)^2) + \\
 &\quad (3 \cdot (4.000 - 4.833)^2) + \\
 &\quad (3 \cdot (3.667 - 4.833)^2) \\
 \\
 &= 25.333
 \end{aligned}$$

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.

$$\begin{aligned}
 \text{SS(Total)} &= ((6 - 4.167)^2) + \\
 &\quad ((3 - 4.167)^2) + \\
 &\quad ((0 - 4.167)^2) + \\
 &\quad ((7 - 4.167)^2) + \\
 &\quad \dots + \\
 &\quad ((3 - 4.167)^2) \\
 \\
 &= 115.333
 \end{aligned}$$

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

$$\begin{aligned}
 \text{SSE} &= \text{SS(Total)} \\
 &\quad - \text{SS(Vaccine)} \\
 &\quad - \text{SS(Patient(Vaccine))} \\
 &\quad - \text{SS(Visit)} \\
 &\quad - \text{SS(Vaccine-by-Visit)} \\
 \\
 &= 115.333 - 10.667 - 25.333 - 58.583 - 8.583 \\
 &= 12.167
 \end{aligned}$$

The ANOVA table can now be completed as shown in Table 8.5. 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 squares to the appropriate error associated with that effect.

TABLE 8.5 ANOVA Summary for Example 8.1

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 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 squares for these effects to the mean square error (MSE = 1.014).

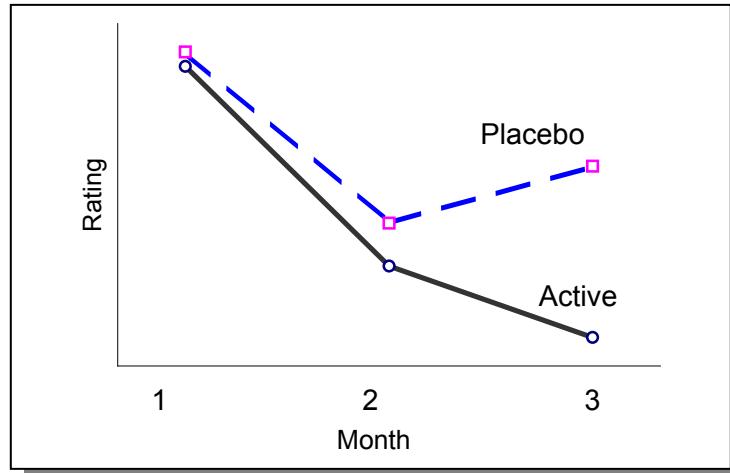
The null hypothesis of similar response profiles over time for each of the vaccine groups is tested by the Vaccine-by-Visit interaction. For two treatment groups as used in this example, the hypothesis can be expressed as the simultaneous equality of Treatment Group differences at each time point. That 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 $j = 1, 2$, or 3, then the test summary can be expressed as

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

This interaction indicates that the Vaccine Group differences depend on the time point considered. Further analyses can be performed by depicting the mean responses over time in graphical format (see Figure 8.2) and by using some of the methods illustrated in Chapter 6, such as 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.

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 responses 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.

FIGURE 8.2 Response Profiles (Example 8.1)



SAS Analysis of Example 8.1

In the SAS code for analyzing the data for Example 8.1 (shown below), the input data set arthr is transformed to a new format with 1 observation per record in the data set discom. This data set is displayed by using PROC PRINT ❶ (see Output 8.1).

PROC GLM 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. These effects must be designated as class variables in the CLASS statement ❷. The interaction is specified in the MODEL statement as vacgrp*visit. The SS3 option is used in the MODEL statement to request only the Type III sums of squares. The RANDOM statement identifies patient as a random effect and signals SAS that patient is nested within the groups (pat(vacgrp)). SAS prints the forms of the expected mean squares when the RANDOM statement is included.

As shown in Output 8.1, the MSE verifies the computation 1.014 ❸. Notice that SAS automatically computes 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 ❹. The resulting F-value of 2.53 ❺ is the appropriate *F-test* for the null hypothesis of no Vaccine Group effect. The F-value 10.52 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, even though Patient is a random effect, PROC GLM computes the sums-of-squares as if it were a fixed effect. The significant Vaccine-by-Visit interaction ❶ suggests that the difference between treatments is time-related, as was previously discovered and as illustrated in Figure 8.2.

SAS Code for Example 8.1—‘Univariate’ Approach

```

data arthr;
    input vacgrp $  pat mol 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
;

data discom; set arthr;
    keep vacgrp pat visit score;
    score = mol; visit = 1; output;
    score = mo2; visit = 2; output;
    score = mo3; visit = 3; output;
run;

proc print data = discom;                                ❶
    var vacgrp pat visit score;
    title1 'Repeated-Measures ANOVA';
    title2 'Example 8.1: Arthritic Discomfort Following Vaccine';
run;

proc glm data = discom;
    class vacgrp pat visit;                            ❷
    model score = vacgrp pat(vacgrp) visit vacgrp*visit/ss3;
    random pat(vacgrp);
    test h=vacgrp e=pat(vacgrp);                     ❸
    quit;
run;

```

OUTPUT 8.I SAS Output for Example 8.I

Repeated-Measures ANOVA
Example 8.1: Arthritic Discomfort Following Vaccine

Obs	vacgrp	pat	visit	score
1	ACT	101	1	6
2	ACT	101	2	3
3	ACT	101	3	0
4	ACT	103	1	7
5	ACT	103	2	3
6	ACT	103	3	1
7	ACT	104	1	4
8	ACT	104	2	1
9	ACT	104	3	2
10	ACT	107	1	8
11	ACT	107	2	4
12	ACT	107	3	3
13	PBO	102	1	6
14	PBO	102	2	5
15	PBO	102	3	5
16	PBO	105	1	9
17	PBO	105	2	4
18	PBO	105	3	6
19	PBO	106	1	5
20	PBO	106	2	3
21	PBO	106	3	4
22	PBO	108	1	6
23	PBO	108	2	2
24	PBO	108	3	3

❶

Repeated-Measures ANOVA
Example 8.1: Arthritic Discomfort Following Vaccine

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	❸	
Corrected Total	23	115.3333333			

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

OUTPUT 8.1 SAS Output for Example 8.1 (continued)

Repeated-Measures ANOVA						
Example 8.1: Arthritic Discomfort Following Vaccine						
The GLM Procedure						
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	❶ 0.0406	
Source	Type III Expected Mean Square					
vacgrp	Var(Error) + 3 Var(pat(vacgrp)) + ❷ 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)					
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	❸ 0.1631	

The PROC MIXED Approach

The assumption of compound symmetry required by the ‘univariate’ approach just discussed means that the correlation between a measurement at Time i and any other measurement within the same patient, say at Time j, is the same for all i and j. This is a very restrictive assumption that is not likely to be satisfied in most cases, especially when there are many measurement times. When using PROC MIXED in the SAS analysis, you can do away with all assumptions regarding the correlations by designating an ‘unstructured’ covariance type. This designation also allows for heterogeneous variances across the time points.

As shown in the SAS code below for the analysis of Example 8.1 using PROC MIXED, include the fixed effects (vacgrp, visit) in the MODEL statement ❸, and include a REPEATED statement ❹ to indicate that the visits represent repeated measures within pat(vacgrp). You do not need to include a RANDOM statement for the random patient effect since it is included in the REPEATED statement. To request the unstructured covariance, specify TYPE = UN in the REPEATED statement.

Looking at Output 8.2, you see the Vaccine-by-Visit interaction is no longer significant. The p-value is 0.0878 ❽ with the unstructured covariance compared with p=0.0406 ❻ based on the univariate approach under the compound symmetry assumption. The Section of Output 8.2 entitled “Null Model Likelihood Ratio Test” shows a chi-square value of 5.65 with a p-value of 0.3414 ❾. This test is an

indication of whether the covariance structure used, in this case ‘unstructured’, provides a better fit compared with the ANOVA independent errors case (i.e., zero correlation). In this case it is not significant, indicating that there is no advantage to using the more general unstructured covariance. However, keep in mind that this example has only 8 patients and 3 time points, so the power for any statistical tests using this data set will be low. SAS provides this method and others to help determine the best covariance structure, which you will see in the next example.

SAS Code for Example 8.1—‘Unstructured Covariance’

```

proc mixed data = discom;
  class vacgrp pat visit;
  model score = vacgrp visit vacgrp*visit; ❸
    repeated visit / subject=pat(vacgrp) type=un; ❹
  title3 'PROC MIXED with Unstructured Covariance';
  run;

```

OUTPUT 8.2 SAS Output for Example 8.1 Using PROC MIXED

```

Repeated-Measures ANOVA
Example 8.1: Arthritic Discomfort Following Vaccine

PROC MIXED with Unstructured Covariance

The Mixed Procedure

Model Information

Data Set                      WORK.DISCOM
Dependent Variable            score
Covariance Structure          Unstructured
Subject Effect                pat(vacgrp)
Estimation Method              REML
Residual Variance Method      None
Fixed Effects SE Method       Model-Based
Degrees of Freedom Method     Between-Within

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

Dimensions

  Covariance Parameters           6
  Columns in X                   12
  Columns in Z                   0
  Subjects                        8
  Max Obs Per Subject             3

Number of Observations

  Number of Observations Read    24
  Number of Observations Used    24
  Number of Observations Not Used 0

```

OUTPUT 8.2 SAS Output for Example 8.1 Using PROC MIXED (continued)

```

Repeated-Measures ANOVA
Example 8.1: Arthritic Discomfort Following Vaccine
PROC MIXED with Unstructured Covariance
The Mixed Procedure
Iteration History
Iteration   Evaluations    -2 Res Log Like      Criterion
0           1             72.61099851
1           1             66.95765383       0.00000000
Convergence criteria met.

Covariance Parameter Estimates
Cov Parm   Subject      Estimate
UN(1,1)     pat(vacgrp)  2.9583
UN(2,1)     pat(vacgrp)  1.3750
UN(2,2)     pat(vacgrp)  1.6250
UN(3,1)     pat(vacgrp)  1.0833
UN(3,2)     pat(vacgrp)  0.7500
UN(3,3)     pat(vacgrp)  1.6667

Fit Statistics
-2 Res Log Likelihood          67.0
AIC (smaller is better)        79.0
AICC (smaller is better)       86.6
BIC (smaller is better)        79.4

Null Model Likelihood Ratio Test
DF      Chi-Square      Pr > ChiSq
5       5.65            0.3414  ⑫

Type 3 Tests of Fixed Effects
Effect      Num DF      Den DF      F Value      Pr > F
vacgrp      1         6         2.53        0.1631  ⑪
visit       2         6         26.39       0.0011
vacgrp*visit 2         6         3.75        0.0878  ⑩

```

The choice of the unstructured covariance requires estimation of a large number of parameters, which decreases power. There are other sets of assumptions that can be made about the covariance structures that might be better. Also, additional factors can be used in the *repeated measures analysis*. Example 8.2 shows a stratification blocking effect in an unbalanced *repeated measures* layout and further illustrates selection of the covariance structure for use with PROC MIXED.

Example 8.2—Treadmill Walking Distance in Intermittent Claudication

Patients were randomly assigned to receive either the new drug Novafylline, thought to reduce the symptoms of intermittent claudication, or a placebo in a 4-month double-blind study. The primary measurement of efficacy is the walking distance on a treadmill until discontinuation due to claudication pain. A total of 38 patients underwent treadmill testing at baseline (Month-0) and at each of 4 monthly, follow-up visits. The treadmill walking distances (in meters) are shown in Table 8.6. Patients were stratified by sex. Is there any distinction in exercise tolerance profiles between patients who receive Novafylline and those on placebo?

TABLE 8.6 Raw Data for Example 8.2

Novafylline Group		Placebo Group									
Pat. No. / Sex	Treatment Month					Pat. No. / Sex	Treatment Month				
	0	1	2	3	4		0	1	2	3	4
101 / M	190	212	213	195	248	104 / M	187	177	200	190	206
105 / M	98	137	185	215	225	111 / M	205	230	172	196	232
109 / M	155	145	196	189	176	114 / M	165	142	195	185	170
112 / M	245	228	280	274	260	118 / M	256	232	252	326	292
117 / M	182	205	218	194	193	125 / M	197	182	160	210	185
122 / M	140	138	187	195	205	127 / M	134	115	150	165	170
123 / M	196	185	185	227	180	131 / M	196	166	166	188	205
128 / M	162	176	192	230	215	133 / M	167	144	176	155	158
129 / M	195	232	199	185	200	135 / M	98	102	89	128	130
132 / M	167	187	228	192	210	102 / F	167	175	122	162	125
134 / M	123	165	145	185	215	106 / F	123	136	147	130	135
136 / M	105	144	119	168	165	110 / F	95	102	154	105	112
103 / F	161	177	162	185	192	116 / F	181	177	140	212	230
107 / F	255	242	330	284	319	120 / F	237	232	245	193	245
108 / F	144	195	180	184	213	121 / F	144	172	163	158	188
113 / F	180	218	224	165	200	130 / F	182	202	254	185	173
115 / F	126	145	173	175	140	137 / F	165	140	153	180	155
119 / F	175	155	154	164	154	138 / F	196	195	204	188	178
124 / F	227	218	245	235	257						
126 / F	175	197	195	182	193						

Solution

A summary of the mean walking distances (in meters) and sample sizes is shown in Table 8.7. Typical analyses would include treatment comparisons at each visit and changes in mean walking distances from baseline within and between treatment groups. You could approach these analyses using the methods of Chapters 5-7. The analysis shown here will concentrate on the changes over time, and in particular, the Treatment-by-Visit interaction from a *repeated measures analysis* to compare response profiles between the active and placebo groups.

TABLE 8.7 Summary Statistics for Example 8.2

Treatment	Sex	Month				
		0	1	2	3	4
Active	M	163.2 12	179.5 12	195.6 12	204.1 12	207.7 12
	F	180.4 8	193.4 8	207.9 8	196.8 8	208.5 8
	Combined	170.1 20	185.1 20	200.5 20	201.2 20	208.0 20
Placebo	M	178.3 9	165.6 9	173.3 9	193.7 9	194.2 9
	F	165.6 9	170.1 9	175.8 9	168.1 9	171.2 9
	Combined	171.9 18	167.8 18	174.6 18	180.9 18	182.7 18
	Total	38	38	38	38	38

Because of the sample size imbalance, the methods illustrated in Example 8.1 for manually computing the sums of squares cannot be used. In such cases, the Type III sums of squares are most efficiently computed by using matrix algebra with a program such as SAS. A ‘multivariate’ approach using PROC GLM would work fine for this example since there are no missing data. However, to allow greater latitude in assumptions regarding the correlations in measurements across visits, PROC MIXED is a more efficient method, as shown below.

SAS Analysis of Example 8.2

As seen in the SAS code that follows, the data are input in a multivariate format, and then converted to a new data set, WDUNI, with one observation per patient-visit ⑯. PROC MIXED is used initially with an unstructured covariance matrix. Effects included in the MODEL statement are Treatment (treatment), Sex (sex), Study Month (month), and the Treatment-by-Sex and Treatment-by-Month interactions ⑰. The time effect (month) is specified in the REPEATED statement as the factor on which repeated observations are made ⑱. Designating pat(treatment) in the REPEATED statement tells SAS that pat is a random effect

whose levels represent independent subjects and that measures within each subject are the repeated measures. The TYPE=UN option requests the unstructured covariance.

The output from PROC MIXED is shown in Output 8.3. The “Model Information” section ⑯ includes the data set (WDUNI) and response variable (wd) that are used in the analysis, the covariance structure (unstructured), and the methodology (REML). REML, shorthand for restricted maximum likelihood, is the default for PROC MIXED and the most commonly used method for estimating the model parameters.

You can scan the “Class Level Information” section ⑰ to be sure all the effects and their levels are being picked up correctly. The “Dimensions” section ⑱ shows that there are 15 covariance parameters and corroborates that there are, at most, 5 measurements for each of the 38 patients. SAS estimates the covariances for each pair of time points (Month 0 vs. Month 1, Month 0 vs. Month 2, ..., Month 3 vs. Month 4), a total of 10, plus separate variances at each of the 5 time points. This accounts for the 15 covariance parameters.

The estimated R and R Correlation matrices are produced by the R and RCORR options in the REPEATED statement ⑲. You can examine these matrices to get an idea of any patterns that might exist in the correlations across time. It is often the case in a *repeated measures* experiment that observations taken closer together in time have a higher correlation than those taken farther apart. Such a trend is not found in these data. In fact, the estimated correlation matrix shows high positive correlations among all pairs of visits (0.63 to 0.88) with no pattern of a decreasing correlation between visits as they get farther apart. For example, the estimated correlation between the response at Month 0 and Month 4 is 0.7464, and between Month 1 and Month 2 is 0.7175.

SAS Code for Example 8.2

```
data wdvis;
  input treatment $ pat sex $ wd0 wd1 wd2 wd3 wd4;
  datalines;
  ACT 101 M 190 212 213 195 248
  ACT 105 M 98 137 185 215 225
  ACT 109 M 155 145 196 189 176
  ACT 112 M 245 228 280 274 260

  . . . (more data lines) . .

  PBO 137 F 165 140 153 180 155
  PBO 138 F 196 195 204 188 178
; run;

data wduni; set wdvis;
  keep treatment pat sex month wd;
  wd = wd0; month = 0; output;
  wd = wd1; month = 1; output;
  wd = wd2; month = 2; output;
```

```

wd = wd3; month = 3; output;
wd = wd4; month = 4; output;
run;

proc mixed data = wduni;
  class treatment sex month pat;
  model wd = treatment sex treatment*sex month treatment*month; ⑭
  repeated month / subject=pat(treatment) type=un r rcorr; ⑮
  title1 'Repeated-Measures ANOVA';
  title2 'Example 8.2: Treadmill Walking Distance in
    Intermittent Claudication';
  title3 'PROC MIXED using Unstructured Covariance';
run;

```

OUTPUT 8.3 SAS Output for Example 8.2

<p>Repeated-Measures ANOVA Example 8.2: Treadmill Walking Distance in Intermittent Claudication PROC MIXED using Unstructured Covariance</p> <p>The Mixed Procedure</p> <p>Model Information ⑯</p> <table border="0"> <tr><td>Data Set</td><td>WORK.WDUNI</td></tr> <tr><td>Dependent Variable</td><td>wd</td></tr> <tr><td>Covariance Structure</td><td>Unstructured</td></tr> <tr><td>Subject Effect</td><td>pat(treatment)</td></tr> <tr><td>Estimation Method</td><td>REML</td></tr> <tr><td>Residual Variance Method</td><td>None</td></tr> <tr><td>Fixed Effects SE Method</td><td>Model-Based</td></tr> <tr><td>Degrees of Freedom Method</td><td>Between-Within</td></tr> </table> <p>Class Level Information ⑰</p> <table border="0"> <thead> <tr><th>Class</th><th>Levels</th><th>Values</th></tr> </thead> <tbody> <tr><td>treatment</td><td>2</td><td>ACT PBO</td></tr> <tr><td>sex</td><td>2</td><td>F M</td></tr> <tr><td>month</td><td>5</td><td>0 1 2 3 4</td></tr> <tr><td>pat</td><td>38</td><td>101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138</td></tr> </tbody> </table> <p>Dimensions ⑱</p> <table border="0"> <tr><td>Covariance Parameters</td><td>15</td></tr> <tr><td>Columns in X</td><td>24</td></tr> <tr><td>Columns in Z</td><td>0</td></tr> <tr><td>Subjects</td><td>38</td></tr> <tr><td>Max Obs Per Subject</td><td>5</td></tr> </table> <p>Number of Observations</p> <table border="0"> <tr><td>Number of Observations Read</td><td>190</td></tr> <tr><td>Number of Observations Used</td><td>190</td></tr> <tr><td>Number of Observations Not Used</td><td>0</td></tr> </table>	Data Set	WORK.WDUNI	Dependent Variable	wd	Covariance Structure	Unstructured	Subject Effect	pat(treatment)	Estimation Method	REML	Residual Variance Method	None	Fixed Effects SE Method	Model-Based	Degrees of Freedom Method	Between-Within	Class	Levels	Values	treatment	2	ACT PBO	sex	2	F M	month	5	0 1 2 3 4	pat	38	101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138	Covariance Parameters	15	Columns in X	24	Columns in Z	0	Subjects	38	Max Obs Per Subject	5	Number of Observations Read	190	Number of Observations Used	190	Number of Observations Not Used	0
Data Set	WORK.WDUNI																																														
Dependent Variable	wd																																														
Covariance Structure	Unstructured																																														
Subject Effect	pat(treatment)																																														
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sex	2	F M																																													
month	5	0 1 2 3 4																																													
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Covariance Parameters	15																																														
Columns in X	24																																														
Columns in Z	0																																														
Subjects	38																																														
Max Obs Per Subject	5																																														
Number of Observations Read	190																																														
Number of Observations Used	190																																														
Number of Observations Not Used	0																																														

OUTPUT 8.3 SAS Output for Example 8.2 (continued)

Repeated-Measures ANOVA
 Example 8.2: Treadmill Walking Distance in Intermittent Claudication
 PROC MIXED using Unstructured Covariance

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	1870.50053229	
1	2	1695.50589078	0.000000024
2	1	1695.50572682	0.000000000

Convergence criteria met.

Estimated R Matrix for pat(treatmnt) 101 ACT

Row	Col1	Col2	Col3	Col4	Col5
1	1851.77	1449.58	1561.05	1352.32	1421.97
2	1449.58	1471.93	1278.77	994.81	1250.65
3	1561.05	1278.77	2157.91	1275.98	1470.46
4	1352.32	994.81	1275.98	1666.44	1512.20
5	1421.97	1250.65	1470.46	1512.20	1959.72

Estimated R Correlation Matrix for pat(treatmnt) 101 ACT

Row	Col1	Col2	Col3	Col4	Col5
1	1.0000	0.8780	0.7809	0.7698	0.7464
2	0.8780	1.0000	0.7175	0.6352	0.7364
3	0.7809	0.7175	1.0000	0.6729	0.7151
4	0.7698	0.6352	0.6729	1.0000	0.8368
5	0.7464	0.7364	0.7151	0.8368	1.0000

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	pat(treatmnt)	1851.77
UN(2,1)	pat(treatmnt)	1449.58
UN(2,2)	pat(treatmnt)	1471.93
UN(3,1)	pat(treatmnt)	1561.05
UN(3,2)	pat(treatmnt)	1278.77
UN(3,3)	pat(treatmnt)	2157.91
UN(4,1)	pat(treatmnt)	1352.32
UN(4,2)	pat(treatmnt)	994.81
UN(4,3)	pat(treatmnt)	1275.98
UN(4,4)	pat(treatmnt)	1666.44
UN(5,1)	pat(treatmnt)	1421.97
UN(5,2)	pat(treatmnt)	1250.65
UN(5,3)	pat(treatmnt)	1470.46
UN(5,4)	pat(treatmnt)	1512.20
UN(5,5)	pat(treatmnt)	1959.72

23

Fit Statistics

-2 Res Log Likelihood	1695.5
AIC (smaller is better)	1725.5
AICC (smaller is better)	1728.5
BIC (smaller is better)	1750.1

OUTPUT 8.3 SAS Output for Example 8.2 (continued)

Repeated-Measures ANOVA				
Example 8.2: Treadmill Walking Distance in Intermittent Claudication				
PROC MIXED using Unstructured Covariance				
Null Model Likelihood Ratio Test 22				
DF Chi-Square Pr > ChiSq				
14 174.99 <.0001				
Type 3 Tests of Fixed Effects				
Effect		Num DF	Den DF	F Value Pr > F
treatment	1	34	1.94	0.1729
sex	1	34	0.00	0.9914
treatment*sex	1	34	0.00	0.9928
month	4	34	7.14	0.0003
treatment*month	4	34	4.12	0.0079

Based on this preliminary look, it appears that the data might support the assumption of compound symmetry, i.e., similar correlations among any two time points. The same MODEL is run in PROC MIXED using the TYPE=CS option in the REPEATED statement to obtain results under compound symmetry19.

SAS Code for Example 8.2—Compound Symmetry

```
proc mixed data = wduni;
  class treatment sex month pat;
  model wd = treatment sex treatment*sex month treatment*month;
  repeated month / subject=pat(treatment) type=cs r; 19
    lsmeans treatment*month / slice=month; 25
    estimate 'Month 4 Change from Baseline: ACT v. PBO' 28
      treatment*month -1 0 0 0 1 1 0 0 0 -1;
    title3 'PROC MIXED using Compound Symmetric Covariance';
  run;
```

In Output 8.4, you can see the covariance structure is “Compound Symmetry”20. Under “Dimensions”, there are only 2 covariance parameters, corresponding to (1) the common variances at each time point and (2) the correlation between each pair of time points, the assumptions of compound symmetry. These parameter estimates are shown as 1350.28 and 464.78 21.

The *F*-tests are shown under “Type 3 Tests for Fixed Effects” 22. The main interest lies in the Treatment-by-Month interaction that is significant ($F=2.63$, $p=0.037$), implying that the response profiles over time differ between treatment groups. There appears to be no difference between males and females, and no Treatment-by-Sex interaction.

The next step might be to perform pairwise comparisons using the LSMEANS statement 23 to list the least squares mean estimates by treatment group at each month, along with the standard errors 24. If you include the DIFF option in

LSMEANS, you get pairwise comparisons for all 10 Treatment x Month combinations (55 comparisons), many of which are of no particular interest. Instead, you can use the SLICE=MONTH option in the LSMEANS statement, which requests that SAS perform a test for a significant Treatment effect at each Month ²⁷. Since there are only 2 Treatment groups, a significant test would not require further multiple comparisons. In this case, none of the ‘sliced’ comparisons are significant, although marginal significance is seen at Month 2 ($p=0.0578$) and Month 4 ($p=0.0642$).

One comparison of particular interest might be the improvement in mean walking distances from Baseline to the end of the study (Month 4) between the Active and Placebo groups. Examining the LSMEANS output ²⁸, you see the mean walking distance improved from 170.8 meters at Baseline (Month 0) to 208.7 meters at Month 4 for the active group, and from 171.9 to 182.7 for the placebo group, increases of 38.0 and 10.8, respectively. You can compare these differences using the ESTIMATE statement shown in the SAS code ²⁹. The output shows ²⁹ a mean difference in those improvements of $38.0 - 10.8 = 27.2$ meters ($t=2.74$, $p=0.0069$), indicating a significant difference in the mean pre- to poststudy walking distance improvement between the two groups.

OUTPUT 8.4 SAS Output for Example 8.2—Compound Symmetry

```
Repeated-Measures ANOVA
Example 8.2: Treadmill Walking Distance in Intermittent Claudication
PROC MIXED using Compound Symmetric Covariance
```

The Mixed Procedure

Model Information

Data Set	WORK.WDUNI
Dependent Variable	wd
Covariance Structure	Compound Symmetry 20
Subject Effect	pat(treatmnt)
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Between-Within

Class Level Information

Class	Levels	Values
treatmnt	2	ACT PBO
sex	2	F M
month	5	0 1 2 3 4
pat	38	101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138

Dimensions

Covariance Parameters	2
Columns in X	24
Columns in Z	0
Subjects	38
Max Obs Per Subject	5

**OUTPUT 8.4 SAS Output for Example 8.2—Compound Symmetry
(continued)**

```

Repeated-Measures ANOVA
Example 8.2: Treadmill Walking Distance in Intermittent Claudication
PROC MIXED using Compound Symmetric Covariance

Number of Observations
Number of Observations Read 190
Number of Observations Used 190
Number of Observations Not Used 0

Iteration History
Iteration Evaluations -2 Res Log Like Criterion
0 1 1870.50053229
1 1 1727.30713522 0.00000000

Convergence criteria met.

Estimated R Matrix for pat(treatment) 101 ACT
Row Col1 Col2 Col3 Col4 Col5
1 1815.06 1350.28 1350.28 1350.28 1350.28
2 1350.28 1815.06 1350.28 1350.28 1350.28
3 1350.28 1350.28 1815.06 1350.28 1350.28
4 1350.28 1350.28 1350.28 1815.06 1350.28
5 1350.28 1350.28 1350.28 1350.28 1815.06

Covariance Parameter Estimates 21
Cov Parm Subject Estimate
CS pat(treatment) 1350.28
Residual 464.78

Fit Statistics 23
-2 Res Log Likelihood 1727.3
AIC (smaller is better) 1731.3
AICC (smaller is better) 1731.4
BIC (smaller is better) 1734.6

Null Model Likelihood Ratio Test 22
DF Chi-Square Pr > ChiSq
1 143.19 <.0001

Type 3 Tests of Fixed Effects 24
Effect Num DF Den DF F Value Pr > F
treatment 1 34 2.11 0.1556
sex 1 34 0.02 0.8894
treatment*sex 1 34 0.54 0.4693
month 4 144 8.49 <.0001
treatment*month 4 144 2.63 0.0370

```

**OUTPUT 8.4. SAS Output for Example 8.2—Compound Symmetry
(continued)**

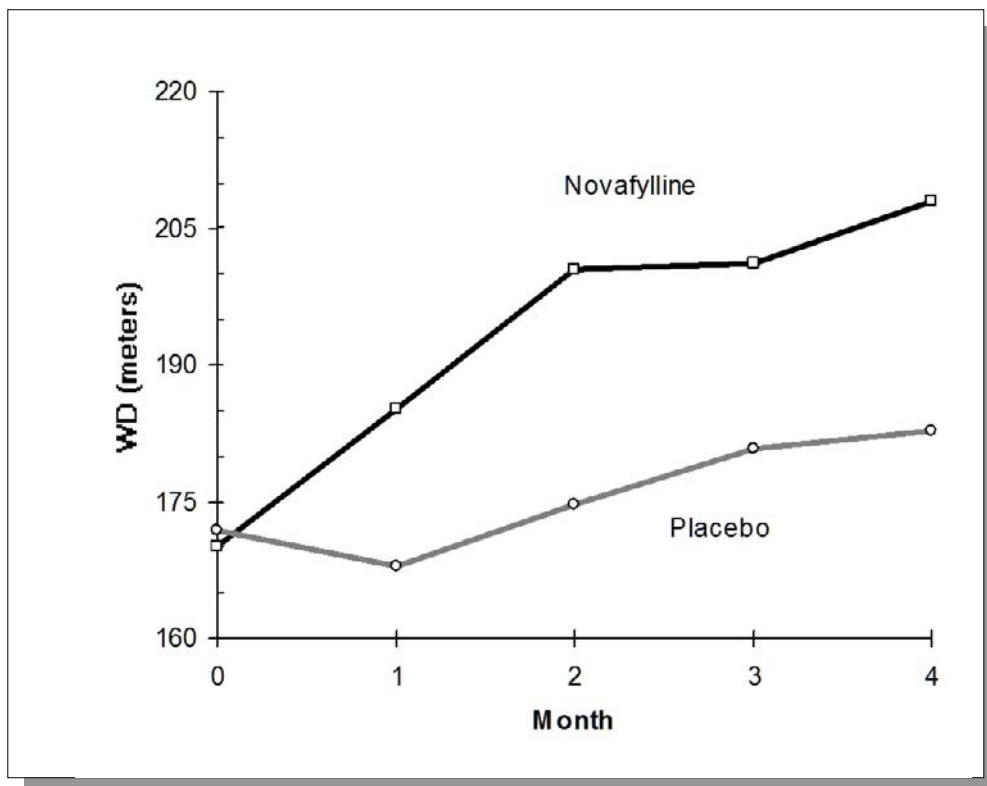
Repeated-Measures ANOVA
Example 8.2: Treadmill Walking Distance in Intermittent Claudication
PROC MIXED using Compound Symmetric Covariance

Label	Estimates					29
	Estimate	Standard Error	DF	t Value	Pr > t	
Baseline to Mo.4 Change: ACT v. PBO	27.1722	9.9055	144	2.74	0.0069	

Effect	Least Squares Means					26
	treatment	month	Estimate	Standard Error	DF	
treatment*month	ACT	0	170.79	9.6830	144	17.64 <.0001
treatment*month	ACT	1	185.79	9.6830	144	19.19 <.0001
treatment*month	ACT	2	201.24	9.6830	144	20.78 <.0001
treatment*month	ACT	3	201.89	9.6830	144	20.85 <.0001
treatment*month	ACT	4	208.74	9.6830	144	21.56 <.0001
treatment*month	PBO	0	171.94	10.0417	144	17.12 <.0001
treatment*month	PBO	1	167.83	10.0417	144	16.71 <.0001
treatment*month	PBO	2	174.56	10.0417	144	17.38 <.0001
treatment*month	PBO	3	180.89	10.0417	144	18.01 <.0001
treatment*month	PBO	4	182.72	10.0417	144	18.20 <.0001

Effect	Tests of Effect Slices					27
	month	Num DF	Den DF	F Value	Pr > F	
treatment*month	0	1	144	0.01	0.9340	
treatment*month	1	1	144	1.66	0.2001	
treatment*month	2	1	144	3.66	0.0578	
treatment*month	3	1	144	2.27	0.1344	
treatment*month	4	1	144	3.48	0.0642	

FIGURE 8.3 Mean Walking Distances for Example 8.2



Missing Data

A number of reasons can lead to missing data in most clinical studies, including dropouts, missed visits, and erroneous or invalidated measurements. In SAS, analysis of *repeated measures* with missing data is better handled using PROC MIXED than PROC GLM. Example 8.3 demonstrates a *repeated measures* analysis in the presence of missing values and explores further options for the covariance structure with PROC MIXED.

Example 8.3—Disease Progression in Alzheimer’s Trial

Patients were randomized to receive one of two daily doses (L=low dose or H=high dose) of a new treatment for Alzheimer’s disease (AD) or a placebo (P) in a parallel study design. Each patient was to return to the clinic every 2 months for 1 year for assessment of disease progression based on cognitive measurements on the Alzheimer’s Disease Assessment Scale (ADAS-cog). This test evaluates memory, language, and praxis function, and is based on the sum of scores from an 11-item scale, with a potential range of 0 to 70, higher scores indicative of greater disease severity. The primary goal is to determine if the rate of disease progression is slowed with active treatment compared with a placebo. The data are shown in Table 8.8 (a decimal point (.) represents missing values). Is there a difference in response profiles over time among the three groups?

TABLE 8.8 Raw Data for Example 8.3

Treatment Group	Patient Number	Study Month					
		2	4	6	8	10	12
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
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

TABLE 8.8 Raw Data for Example 8.3 (continued)

Treatment Group	Patient Number	Study Month					
		2	4	6	8	10	12
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
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

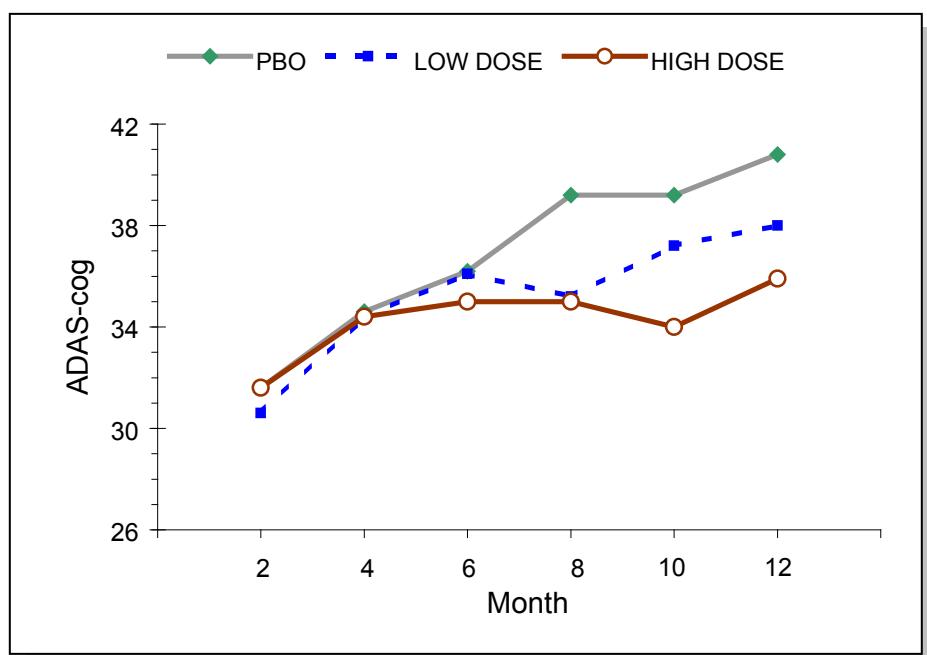
Solution

The mean ADAS-cog scores are shown by Treatment and Month in Table 8.9 and Figure 8.4. Notice that there are 26 missing observations in Table 8.8. Mean scores show an increasing trend across time within each Treatment Group, indicative of disease progression. The question is whether the rate of increase has been slowed by using an active treatment compared with a placebo. Statistically, this is addressed by examining the Treatment-by-Month interaction effect.

TABLE 8.9 Summary Statistics for Example 8.3

Treatment		Month					
		2	4	6	8	10	12
Placebo (N=30)	Mean	31.6	34.6	36.2	39.2	39.2	40.8
	(SD)	(8.4)	(8.0)	(7.7)	(8.4)	(7.4)	(9.2)
	N	29	28	29	28	29	26
Low Dose (N=26)	Mean	30.6	34.4	36.1	35.2	37.2	38.0
	(SD)	(7.6)	(8.5)	(9.1)	(10.0)	(10.7)	(10.3)
	N	25	23	25	25	25	24
High Dose (N=24)	Mean	31.6	34.4	35.0	35.0	34.0	35.9
	(SD)	(10.0)	(9.1)	(9.0)	(9.5)	(7.3)	(9.4)
	N	24	23	22	24	23	22
Combined (N=80)	Mean	31.3	34.5	35.8	36.6	37.0	38.4
	(SD)	(8.6)	(8.4)	(8.5)	(9.4)	(8.7)	(9.7)
	N	78	74	76	77	77	72

FIGURE 8.4 ADAS-cog Response Profiles (Example 8.3)



SAS Analysis of Example 8.3

PROC MIXED is used on the data set UNIALZ in a manner similar to Example 8.2, as shown in the SAS code below. The Treatment (treat), Month (month), and Treatment-by-Month interaction are included in the MODEL statement **30**. Initially, the analysis is conducted using the compound symmetric covariance by specifying TYPE=CS in the REPEATED statement **31**.

SAS Code for Example 8.3

```
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

. . . (more data lines) . . .

P 71 32 34 27 30 36 35
P 74 . 50 52 56 52 54
P 77 24 32 31 37 35 30
;

data unialz; set alzhmrs;
    keep treat pat month adascog;
    month = 2; adascog = adas02; output;
    month = 4; adascog = adas04; output;
    month = 6; adascog = adas06; output;
    month = 8; adascog = adas08; output;
    month = 10; adascog = adas10; output;
    month = 12; adascog = adas12; output;
run;

proc mixed data = unialz;
    class treat month pat;
    model adascog = treat month treat*month;          30
    repeated / subject=pat(treat) type=cs rcorr;       31
    title1 'Repeated-Measures ANOVA';
    title2 '';
    title3 "Example 8.3: Disease Progression in Alzheimer's";
    title4 'PROC MIXED Using Compound Symmetric Covariance (CS)';
run;
```

Output 8.5 confirms the number of missing observations is 26 under “Number of Observations Not Used” in the section titled Number of Observations **32**. The “Null Model Likelihood Ratio Test” tests whether the covariance specified, in this case compound symmetry, fits better than the assumption of independent observations, which is used in the *analysis-of-variance*. The significant chi-square value ($p<0.0001$) **33** shows that compound symmetry indeed is a better assumption than independent errors.

OUTPUT 8.5 SAS Output for Example 8.3—Compound Symmetry

Repeated-Measures ANOVA

Example 8.3: Disease Progression in Alzheimer's
PROC MIXED Using Compound Symmetric Covariance (CS)

The Mixed Procedure

Model Information

Data Set	WORK.UNIALZ
Dependent Variable	adascog
Covariance Structure	Compound Symmetry
Subject Effect	pat(treat)
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Between-Within

Class Level Information

Class	Levels	Values
treat	3	H L P
month	6	2 4 6 8 10 12
pat	80	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80

Dimensions

Covariance Parameters	2
Columns in X	28
Columns in Z	0
Subjects	80
Max Obs Per Subject	6

Number of Observations

Number of Observations Read	480
Number of Observations Used	454
Number of Observations Not Used	26

**OUTPUT 8.5 SAS Output for Example 8.3—Compound Symmetry
(continued)**

Repeated-Measures ANOVA					
Example 8.3: Disease Progression in Alzheimer's PROC MIXED Using Compound Symmetric Covariance (CS)					
The Mixed Procedure					
Iteration History					
Iteration	Evaluations	-2 Res Log Like		Criterion	
0	1	3199.50437651			
1	2	2743.52120526		0.00004119	
2	1	2743.47961563		0.00000016	
3	1	2743.47945798		0.00000000	
Convergence criteria met.					
Estimated R Correlation Matrix for pat(treat) 2 H					
Row	Col1	Col2	Col3	Col4	Col5
1	1.0000	0.8038	0.8038	0.8038	0.8038
2	0.8038	1.0000	0.8038	0.8038	0.8038
3	0.8038	0.8038	1.0000	0.8038	0.8038
4	0.8038	0.8038	0.8038	1.0000	0.8038
5	0.8038	0.8038	0.8038	0.8038	1.0000
6	0.8038	0.8038	0.8038	0.8038	1.0000
Covariance Parameter Estimates					
Cov Parm	Subject		Estimate		
CS	pat(treat)		64.6927		
Residual			15.7919		
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					
Null Model Likelihood Ratio Test					
DF	Chi-Square		Pr > ChiSq		
1	456.02		<.0001		33
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	34

Output 8.5 shows a significant ($p = 0.0125$) Treatment-by-Month interaction using the compound symmetry assumption ④. The section entitled “Fit Statistics” provides an indication of relative goodness of fit of the covariance structure, 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 help determine the most appropriate one. A vast array of different user-selected covariance structures is available in SAS. (For more information about PROC MIXED, see the *SAS/STAT User’s Guide*).

To illustrate this process, re-run the analysis using several other commonly used covariance structures: autoregressive (AR(1)), Toeplitz (TOEP), first order autoregressive moving average (ARMA(1,1)), and the unstructured (UN) covariance structures by replacing the REPEATED statement with the statements below. These covariance structures are good candidates when the measurement times are equally spaced, as in this example. The results are shown in Output 8.6 – 8.9. Note that the ODS statements shown were used to include only selected output:

```
ods select Mixed.ModelInfo
      Mixed.Dimensions
      Mixed.FitStatistics
      Mixed.LRT
      Mixed.Tests3 ;
```

SAS Code for Example 8.3 (PROC MIXED) (continued)

```
...
      repeated / subject=pat(treat) type=ar(1); ⑤
      title4 'PROC MIXED Using First-Order Auto-Regressive
              Covariance (AR(1))';
...
      repeated / subject=pat(treat) type=toep; ⑥
      title4 'PROC MIXED Using Toeplitz Covariance (TOEP)';
...
      repeated / subject=pat(treat) type=arma(1,1); ⑦
      title4 'PROC MIXED Using 1st-Order Auto-Regressive
              Moving Average Covariance (ARMA(1,1))';
...
      ods select Mixed.RCorr;
      repeated / subject=pat(treat) type=un rcorr; ⑧
      title4 'PROC MIXED Using Unstructured Covariance (UN)';
run;
```

OUTPUT 8.6 SAS Output for Example 8.3—AR(1) Covariance

Repeated-Measures ANOVA				
Example 8.3: Disease Progression in Alzheimer's				
PROC MIXED Using 1st-Order Auto-Regressive Covariance (AR(1))				
The Mixed Procedure				
35				
Model Information				
Data Set	WORK.UNIALZ			
Dependent Variable	adascog			
Covariance Structure	Autoregressive			
Subject Effect	pat(treat)			
Estimation Method	REML			
Residual Variance Method	Profile			
Fixed Effects SE Method	Model-Based			
Degrees of Freedom Method	Between-Within			
Dimensions				
Covariance Parameters	2			
Columns in X	28			
Columns in Z	0			
Subjects	80			
Max Obs Per Subject	6			
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			
Null Model Likelihood Ratio Test				
DF	Chi-Square	Pr > ChiSq		
1	501.35	<.0001	39	
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

OUTPUT 8.7 SAS Output for Example 8.3—Toeplitz (TOEP) Covariance

Repeated-Measures ANOVA				
Example 8.3: Disease Progression in Alzheimer's				
PROC MIXED Using Toeplitz Covariance (TOEP)				
The Mixed Procedure				
Model Information				
Data Set	WORK.UNIALZ	36	36	36
Dependent Variable	adascog			
Covariance Structure	Toeplitz			
Subject Effect	pat(treat)			
Estimation Method	REML			
Residual Variance Method	Profile			
Fixed Effects SE Method	Model-Based			
Degrees of Freedom Method	Between-Within			
Dimensions				
Covariance Parameters	6	40	40	40
Columns in X	28			
Columns in Z	0			
Subjects	80			
Max Obs Per Subject	6			
Fit Statistics				
-2 Res Log Likelihood	2671.1	41	41	41
AIC (smaller is better)	2683.1			
AICC (smaller is better)	2683.3			
BIC (smaller is better)	2697.4			
Null Model Likelihood Ratio Test				
DF	Chi-Square	39	39	39
5	528.44			
	<.0001			
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	2.06	0.0266

OUTPUT 8.8 SAS Output for Example 8.3—ARMA Covariance

Repeated-Measures ANOVA				
Example 8.3: Disease Progression in Alzheimer's				
PROC MIXED Using 1st-Order Auto-Regressive-Moving Average				
Covariance (ARMA(1,1))				
The Mixed Procedure				
Model Information				
Data Set	WORK.UNIALZ			
Dependent Variable	adascog			
Covariance Structure	Autoregressive			
	Moving Average			
Subject Effect	pat(treat)			
Estimation Method	REML			
Residual Variance Method	Profile			
Fixed Effects SE Method	Model-Based			
Degrees of Freedom Method	Between-Within			
Dimensions				
Covariance Parameters	3			
Columns in X	28			
Columns in Z	0			
Subjects	80			
Max Obs Per Subject	6			
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			
Null Model Likelihood Ratio Test				
DF	Chi-Square	Pr > ChiSq		
2	508.35	<.0001	39	
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

OUTPUT 8.9 SAS Output for Example 8.3—Unstructured Covariance

Repeated-Measures ANOVA

Example 8.3: Disease Progression in Alzheimer's PROC MIXED Using an Unstructured Covariance (UN)

The Mixed Procedure

38

Model Information

Data Set	WORK.UNIALZ
Dependent Variable	adascog
Covariance Structure	Unstructured
Subject Effect	pat(treat)
Estimation Method	REML
Residual Variance Method	None
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Between-Within

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

Null Model Likelihood Ratio Test

DF Chi-Square Pr > ChiSq
20 549.61 <.0001

Type 3 Tests of Fixed Effects

Effect	Num	Den	F Value	Pr > F
	DF	DF		
treat	2	77	0.94	0.3967
month	5	77	21.55	<.0001
treat*month	10	77	2.08	0.0357

The likelihood-ratio tests are significant for each of these covariance structures ⑨, indicating that each of these alternative choices provides a better fit than that of the independence structure.

In the Fit Statistics sections of the output, AIC stands for Akaike's Information Criterion, which is a measure of model fit. AICC is a 'corrected' version of the AIC, and BIC stands for Bayesian Information Criterion. These are all adjusted values of the -2 residual log likelihood values based on the number of covariance parameters that need to be estimated. The goal is to find the simplest model with the smallest AIC, AICC, and BIC.

Based on the output, the Toeplitz structure provides the best fit of the five covariance structures considered, based on the AIC, AICC, and BIC fit parameters (smallest values) as seen in Table 8.10. The unstructured covariance has a lower -2 residual log likelihood value, but it is also the most complex structure requiring the most parameter estimates (21). The Toeplitz structure assumes the same correlations for time points with the same lag (see Section 8.4.11). For example, adjacent visits (2 months apart) have a lag of 1 (i.e., they would all have the same correlation, ρ_1). Similarly, visits 4 months apart have a lag of 2 (i.e., they would all have the same correlation, ρ_2), etc. You can see how this pattern fits, approximately, by scanning the off-diagonal elements of the estimated correlation matrix shown in Output 8.9. Since the maximum lag is 5, there are 5 correlation parameters for this structure, plus one for the variances making 6, as shown in Output 8.7 ⑩.

TABLE 8.10 Summary of PROC MIXED Results for Example 8.3 for Various Correlation Structures

Covariance Structure	No. of Parameters	SAS Name	-2 Res Log Likeli-hood	AIC	AICC	BIC
Compound Symmetry	2	CS	2743.5	2747.5	2747.5	2752.2
Autoregressive	2	AR(1)	2698.2	2702.2	2702.2	2706.9
Toeplitz	6	TOEP	2671.1	2683.1	2683.3	2697.4
Autoregressive MA	3	ARMA(1)	2691.2	2697.2	2697.2	2704.3
Unstructured	21	UN	2649.9	2691.9	2694.1	2741.9

Proceeding with the analysis using the Toeplitz covariance structure, you see a significant Treatment-by-Month interaction ⑪ ($p=0.0266$), which is indicative of different response profiles over time among the treatment groups.

8.4 Details & Notes

- **8.4.1** The manual computing methods demonstrated in Example 8.1 apply only to balanced designs (i.e., same number of patients per group). This demonstration is used to show which deviations are represented by each of the effect sum of squares and is not recommended in practice. Even if a suitable computer package

is unavailable, easier computing formulas are available for manual computations with a balanced layout (see e.g., Winer, 1971).

- **8.4.2** For the balanced case, the *repeated measures ANOVA* error sum of squares (SSE) is simply the sum of the SSEs found by using *two-way ANOVAs* (with no interaction) *within* each treatment group. In Example 8.1, the *two-way ANOVA* tables within each Vaccine Group are as follows:

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 be 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 fixed Time effect (SS(Visit)) are not additive (see Table 8.5).

- **8.4.3** In the ‘univariate’ analysis of Example 8.1, Patient-within-Vaccine is considered a random effect because patients are selected from a large population of eligible patients, and you want to make inferences about that population, not just the selected patients. To indicate this, the RANDOM statement is used following the MODEL statement in the SAS code. As discussed in Chapter 7, whenever the RANDOM statement is included in PROC GLM, SAS prints the form of the expected mean squares as functions of the variance components attributed to each source of variation. Output 8.1⑦ shows that the expected mean square for the fixed Vaccine effect is composed of a component due to the Vaccine (i.e., VACGRP) and two additional components due to the random effects.

Under the null hypothesis of no Vaccine effect, the quadratic form involving Vaccine (i.e., Q(VACGRP,VACGRP*VISIT)) would be 0, reducing it to the same form as the expected mean square for Patient(Vaccine). Therefore, when H_0 is true, the ratio of these two mean squares would be 1, which confirms that this is the appropriate test for Vaccine.

In more complex *repeated measures* designs and those involving missing data, the structures of the expected mean squares are important in helping to determine how to construct the *F-tests* when using PROC GLM. As an exact test might not exist, the analyst must examine the variance structures to determine the ratio for the most appropriate *F-tests* as discussed above, and then use the TEST statement to carry out the test. As previously recommended, however, PROC MIXED, which uses a

different computational approach, circumvents this expected-mean-squares approach.

- **8.4.4** In a balanced layout like Example 8.1, the error variation for between-group comparisons (averaged over time) is estimated by the mean square for Patients-within-Group (MSP(G)) with $N-g$ degrees of freedom. The within-Patient error variation (over all groups) is estimated by MSE, with $(N-g)(t-1)$ degrees of freedom. The error variation within the Group-by-Time cells is the pooled combination of these two sometimes disparate error estimates, namely,

$$\hat{\sigma}_{\text{cell}}^2 = \frac{\text{SSP}(G) + \text{SSE}}{(N - g) + (N - g)(t - 1)} = \frac{\text{SSP}(G) + \text{SSE}}{t \cdot (N - g)}$$

This within-cell error variation is the within-time, among-patient variation, averaged over all time points. That is, if MSE_j represents the MSE from a *one-way ANOVA* conducted at time period j , the within-cell variance of the *repeated measures* layout is the average of these MSEs:

$$\hat{\sigma}_{\text{cell}}^2 = \frac{\sum_{j=1}^t \text{MSE}_j}{t}$$

This within-cell error variation can be used to compare cell means (e.g., Group i vs. Group j) for a specific time point or to form 95% confidence intervals for the cell means.

- **8.4.5** As mentioned in Section 8.2, you can analyze a *repeated measures* data set using a ‘multivariate’ approach with PROC GLM. The data set has the repeated measurements for each patient in the same record, as shown in data sets WDVIS in Example 8.2 and ALZHMRS in Example 8.3. The SAS code for the multivariate approach for Example 8.2 would include a REPEATED statement as follows:

```
proc glm data = wdvvis;
  class treatment sex;
  model wd0 wd1 wd2 wd3 wd4 =
    treatment sex treatment*sex / ss3;
  repeated month;
run;
```

This approach accounts for the correlation among the repeated measurements, wd0, wd1, wd2, wd3, and wd4. However, unlike with PROC MIXED, the presence of only one missing value results in the omission of the entire patient from the analysis.

- **8.4.6** The use of generalized estimating equations (GEE) is another method for analyzing *repeated measures* data sets. 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 modeling methodology will usually produce good estimates of the model parameters even if the correlation

structure is misspecified. In this sense, a GEE analysis is more robust than using the general linear modeling methods of PROC GLM or PROC MIXED.

The SAS statements below illustrate how to conduct a GEE analysis of the data in Example 8.3 by using the autoregressive (TYPE = AR(1)) working correlation matrix. 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 8.3—PROC GENMOD

```
ods select
    genmod.type3;

proc genmod data = unialz;
    class treat month pat;
    model adascog = treat month treat*month / type3;
        repeated subject = pat / type = ar(1);
    title4 'GEE Analysis Using PROC GENMOD';
    title5 'Autoregressive Correlation (AR(1)) Working
    Correlation';
run;
```

OUTPUT 8.10 SAS Output for Example 8.3—PROC GENMOD

Repeated-Measures ANOVA			
Example 8.3: Disease Progression in Alzheimer's			
GEE Analysis Using PROC GENMOD			
Autoregressive Correlation (AR(1)) Working Correlation			
The GENMOD Procedure			
Score Statistics For Type 3 GEE Analysis			
Chi-			
Source	DF	Square	Pr > Chisq
treat	2	2.02	0.3639
month	5	46.14	<.0001
treat*month	10	21.76	0.0164

The GEE approach does not depend so heavily on the correct specification of the correlation structure. However, PROC GENMOD cannot handle random effects. In the analysis above, GENMOD treats patient as a fixed effect, whereas PROC MIXED treats patient as a random effect in Output 8.5.

- **8.4.7** The *repeated measures analysis* is not recommended when there are many missing values. A *repeated measures* layout with two time periods is similar to the paired-difference situation (Chapter 3). Patients who have a missing value at one of the two time periods might contribute information regarding between-patient variability, but nothing regarding the patients' response profile (within-patient variability). Similar problems, though not as extreme, occur when patients have missing data in layouts with more than two time periods.

When there are missing values, the ‘univariate’ approach to *repeated measures analysis* using SAS confounds hypotheses regarding treatment effects with other model effects that result in complex hypotheses and interpretation difficulties. Unless the analyst can clearly re-state the statistical hypothesis in clinical terms, this approach to *repeated measures analysis* should be avoided when the data set is plagued with missing data (just a few missing values in a large study should not present interpretation difficulties).

The ‘multivariate’ approach using SAS eliminates patients who have missing values from the analysis, thereby decreasing the test’s power. Much of the data will be excluded from evaluation when using this analysis method if there are a large number of patients each having just one missing value.

PROC MIXED is capable of handling missing values and unbalanced cases for *repeated measures analysis*, but it assumes values are missing at random (MAR). Results will be biased whenever missing values have a systematic pattern. 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 8.3 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.

Estimates can sometimes be computed for missing values, and these can then be used in the analysis as if they were observed. The simplest estimate and one that is often used in clinical trials is the patient’s observation from the previous time point. This has been referred to as the ‘last-observation-carried-forward’ (LOCF) technique, but it is not universally endorsed. Lachin (2000), for example, refers to this method as ‘clearly ridiculous’ in clinical trials that involve disease states that might progress or deteriorate during the study. Other estimates of missing values might be based on averages of adjacent values, or on row and column means, or based on regression techniques accounting for explanatory covariates. However, such estimation techniques also rely heavily on the MAR assumption to avoid bias.

If the repeated measures model assumptions are not satisfied and imputation techniques are not tenable in data sets plagued with missing values, one may deem the most appropriate analysis to be separate ANOVAs at each time point. Generally, if large amounts of missing data are expected, the repeated measures ANOVA should not even be considered as an analysis tool. However, if large amounts of missing data unexpectedly occur in a study for which you plan to use a repeated measures analysis, you might consider revising the hypothesis and conducting a new study. Some authors (e.g., Gillings and Koch, 1991) suggest that the credibility of an entire study might be in question if it unexpectedly contains a

large number of missing values. In any case, the occurrence of missing data in a repeated measures setting calls for the application of alternative statistical methods to confirm the results of the method selected.

For further reading, Dmitrienko, Molenberghs, Chuang-Stein, and Offen offer a detailed discussion of analyses involving incomplete data, including imputation methods for missing values in their book, *Analysis of Clinical Trials Using SAS: A Practical Guide*, published by SAS Press.

- **8.4.8** When there are no missing values, PROC MIXED, using the compound symmetric covariance structure (TYPE=CS), produces the same *F*-tests as PROC GLM in the ‘univariate’ approach.
- **8.4.9** When using PROC MIXED, there is no problem including random effects or numeric covariates in the model if there is sufficient sample size. Covariates (discussed in Chapter 11) are specified directly in the MODEL statement (but not the CLASS statement). Random effects can be included in the RANDOM statement. However, the results of PROC MIXED analyses might not be reliable for smaller data sets with a larger number of covariates and other effects. Generally, if there are no more than 3 or 4 explanatory variables (including ‘Treatment Group’), you should have a minimum sample size of 50 to 100 patients.
- **8.4.10** Example 7.3 demonstrates some techniques for selecting an appropriate covariance structure. You can also use PROC GLIMMIX in SAS with the COVTEST statement to test covariance structures. Please refer to the *SAS/STAT User’s Guide* on PROC GLIMMIX for details.
- **8.4.11** 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 according to how you think measurements across time are related. 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 than measurements taken over lengthy 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 without regard to how far apart they occur.

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 , to measurements that are taken 2 visits apart; ρ_3 , to measurements that are taken 3 visits apart, etc. Each of these

(except the unstructured) assumes equal variances at all measurement times.

By using the RCORR option in the REPEATED statement in PROC MIXED, SAS will provide the estimated correlation matrix based on the type of structure assumed. The estimated correlation matrix for the unstructured type is shown in Output 8.9 for Example 8.3 (PROC MIXED).

- **8.4.12** It is important to select the right covariance structure when using PROC MIXED. Inferences from PROC MIXED may become faulty or result in low power if the incorrect covariance structure is used. As seen in Example 8.3, the significance of the Treatment-by-Month interaction is very much dependent on the covariance structure used. On the other hand, if the structure is approximately correct, the analysis is somewhat robust.

One of the problems in using PROC MIXED is that, if you have no information about the covariance structure, you will need to estimate it from the study data, and then use it for the final inferences. This is a data-driven technique which cannot be pre-specified in your Statistical Analysis Plan. Often PROC MIXED and other modeling techniques are used for exploratory and secondary analyses. When used for a primary analysis in a pivotal study, you should be able to corroborate the results using other pre-specified methods.

When selecting a covariance structure, you should first exclude from consideration those that don't make sense and include as candidates those that are consistent with expectation about how the correlations should act over the time course of the study and by time lag. If visits are equally spaced, you should consider the TOEP, ARMA(1,1), AR(1), UN, or CS structures, and if unequally spaced, one of the spatial structures. (For more information about spatial and other covariance structures, see the documentation for PROC MIXED in the *SAS/Stat User's Guide*). Usually for a Phase III pivotal study, you will have some knowledge about the most appropriate covariance structures from previous studies.

- **8.4.13** If you recall from Chapter 5, Satterthwaite's method was introduced to perform an approximate *two-sample t-test* when the variances differ between the two groups. The method consists of adjusting the degrees of freedom in order to control the bias from using estimates in place of the unknown variances. PROC MIXED performs analyses using estimates of the covariance parameters, and these can differ from one time point to another. These methods can also be improved by using adjusted degrees of freedom, especially the degrees of freedom in the denominator of the F-tests, which are affected by the covariance structure.

SAS offers several methods for adjusting the denominator degrees of freedom by using the DDFM= option in the MODEL statement of PROC MIXED. Among them are the Kenward-Roger (DDFM=KR) method and Satterthwaite's (DDFM=SAT) method. Without the adjustment, the Type I error probability may be increased. In Example 8.3 using the Toeplitz covariance structure, the p-value for the Treatment-by-Month interaction was found to be 0.0266 ④ using the default denominator degrees of freedom. The p-values using KR and SAT are 0.0334 and 0.0321, respectively.

You can also specify your own degrees of freedom using the DDF= option. For further details, refer to the documentation for PROC MIXED in the *SAS/STAT User's Guide*.

- **8.4.14** The term ‘Time’ has been used in this chapter to describe the study visit or time of measurement of the response variable during a trial. This is perhaps the most common *repeated measures* situation, especially for comparative drug studies. In general, however, the repeated factor need not refer to time at all. The repeated factor could be a set of experimental conditions, each applied to the same set of patients in random order, such as subjecting each patient to each dose level of a test drug. The dose levels in this example represent the repeated measure, and the ‘profiles’ are the dose-response curves.

The *crossover* design (Chapter 9) is an example of this and can be analyzed with the *repeated measures ANOVA*. In the popular two-period *crossover*, the treatment (A or B) is the repeated factor, because both treatments A and B are given to each patient. The sequence group, representing the order in which patients receive the treatments (A-B or B-A), would represent the ‘GROUP’ effect. Patients are nested within sequence group, and the analysis would be carried out as in Example 8.1. A significant Treatment-by-Sequence group interaction would suggest a ‘period effect’, in which case, the analyst would perform separate analyses for each treatment period by using the *two-sample t-test*.

- **8.4.15** When no assumptions can be made about the correlation structure, some analysts prefer to use techniques that consolidate the multiple response measurements for each patient into a single response and then analyze those measures using univariate analyses with independent data. If there is no reason to believe measurements should change over time, analyses might use the mean response for each patient (averaged over all visits), for example. Another example is to use the methods of Chapter 10 (“Linear Regression”) to estimate the slope of the response over time for each patient in a repeated measures trial under the assumption of a monotonic response, and then conduct traditional *ANOVA* techniques on the slopes, including tests for comparing slopes among treatment groups. In addition to sidestepping the issue of correlation structures, this method can also be somewhat effective in handling missing values (MAR), assuming there are sufficient time points to work with. There are obvious drawbacks to this technique, such as the assumption of certain time trends or none at all. One must also be cautious about lack of homogeneity of the variances associated with the estimated consolidated statistic (mean, slope, etc.) when using this method.
- **8.4.16** This chapter focuses on response measurements assumed to be normally distributed. Non-normal data are also frequently collected in a *repeated measures* layout. Simple examples include studies in which the response measure is whether the patient is improving (binary response) or showing a categorical degree of improvement (e.g., ‘none’, ‘some’, ‘complete’) at each visit during the trial.

Analyses of non-normal data can often be handled by using categorical techniques. When the analysis includes additional factors or covariates, more sophisticated modeling using Weighted Least Squares (WLS) or Generalized Estimating

Equations (GEE) can be employed. These techniques make use of the SAS procedures CATMOD and GENMOD, which can handle very complex designs. PROC GLIMMIX, a new procedure in SAS 9.2, can handle generalized linear mixed models, including any of the types of data already discussed: normal and certain types of non-normal data (including binomial responses), independent and correlated data, and fixed or random effects models. Its usage is very similar to that of PROC MIXED, and it has some features that make it easy to pinpoint specific comparisons or contrasts of interest. For more information, see the *SAS/STAT User's Guide*.

Note: Several different approaches to the analysis of repeated measures for categorical data, including WLS and GEE methodology, are illustrated in the excellent reference entitled, *Categorical Data Analysis Using the SAS System, Second Edition*, by Stokes, Davis, and Koch. For examples of the use of PROC GLIMMIX in repeated measures, see *SAS for Mixed Models, Second Edition* by Littell, Milliken, Stroup, Wolfinger, and Schabenberger. Both of these books are published under the SAS Press program.

- **8.4.17** Simple usage of the ODS statement to customize the SAS output is demonstrated in this and other chapters. Each section of the SAS output has an associated ODS label and pathname. ODS can be used to select or exclude specific sections of the output simply by identifying its name. Complete names can be found in the SAS documentation, or from the SAS log by including the statement ODS TRACE ON as a statement within the SAS program before the procedure of interest.

Output Delivery System (ODS) is a component of SAS that allows you to get customized SAS output with minimal programming. When you run SAS programs in the SAS Windowing Environment, as was done for this book, the traditional output is provided in text format in the SAS Output window. If you want your SAS text output presented in formats other than plain text, you can use ODS to send your SAS output to RTF (Microsoft Word Rich Text Format), PDF, and HTML formats. Beginning with SAS 9.2, the ODS system also includes the ability to easily obtain supportive graphics output for many procedures, as first referenced in Chapter 7 and illustrated in several examples in this book. For more information and details on ODS Graphics and the ODS system in general, refer to Jack Shostak's book, *SAS Programming in the Pharmaceutical Industry* published under the SAS Press program, or "Statistical Graphics Using ODS" in the *SAS/STAT 9.2 User's Guide*.

The SAS code in supplemental analyses for Example 8.3, for example, uses the ODS SELECT feature to tell SAS to include in the output only the sections named: Model Information, Dimensions, Fit Statistics, Likelihood Ratio Tests, and the Type 3 Tests. Alternatively, you can use the ODS EXCLUDE statement to exclude specific parts of the output. Obviously, these are very simple examples of using the powerful ODS features in SAS. Additional uses of the ODS SELECT and ODS EXCLUDE statements, as well as the new ODS Graphics are used in other examples throughout this book. (Note: A license for SAS/GRAFH software is required to run ODS Graphics.)

CHAPTER 9

The Crossover Design

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9.1 Introduction

The *crossover design* is used to compare the mean responses of two or more treatments when each patient receives each treatment over successive time periods. Typically, patients are randomized to treatment sequence groups which determine the order of treatment administration.

While responses among treatments in a parallel study are considered independent, responses among treatments in a *crossover* study are correlated because they are measured on the same patient, much like the *repeated measures* setup discussed in Chapter 8. In fact, the *crossover* is a special case of a *repeated measures* design. Procedures for treatment comparisons must consider this correlation when analyzing data from a *crossover* design. In addition to treatment comparisons, the *crossover* analysis can also investigate period effects, sequence effects, and carryover effects.

Because the *crossover* study features within-patient control among treatment groups, fewer patients are generally required than with a parallel study. This advantage is often offset, however, by an increase in the length of the study. For example, when there are two treatments, the study will be at least twice as long as its parallel-design counterpart because treatments are studied in each of two successive periods. The addition of a wash-out period between treatments, which is usually required in a *crossover* study, tends to further lengthen the trial duration.

The *crossover* design is normally used in studies with short treatment periods, most frequently in pre-clinical and early-phase clinical studies, such as bioavailability, dose-ranging, bio-equivalence, and pharmacokinetic trials. If it is thought that the patient's condition will not be the same at the beginning of each treatment period, the *crossover* design should be avoided. Lengthier treatment periods provide

increased opportunities for changing clinical conditions and premature termination. For the same reasons, crossover designs should limit the number of study periods as much as possible. The focus in this chapter is on the 2-period crossover design, although an example that uses 4 periods with carryover is also presented. Responses are assumed to be normally distributed.

9.2 Synopsis

For the two-way *crossover* design, each patient receives two treatments, A and B, in one of two sequences, that is, A followed by B (A-B) or B followed by A (B-A). Samples of n_1 and n_2 patients are randomly assigned to each of the two sequence groups, A-B and B-A, respectively (usually, $n_1 = n_2$). Response measurements (y_{ijk}) are taken for each patient following each treatment for a total of $N = 2n_1 + 2n_2$ measurements, where y_{ijk} is the measurement for the k^{th} patient in the sequence group for which Treatment i ($i=1$ for Treatment A, $i=2$ for Treatment B) is given in Period j ($j=1, 2$). A sample layout is shown in Table 9.1.

TABLE 9.1 Crossover Layout

Sequence	Patient Number	Period 1	Period 2
A-B	1	(Treatment A) y_{111}	(Treatment B) y_{221}
	3	y_{112}	y_{222}
	6	y_{113}	y_{223}
	7	y_{114}	y_{224}
	.	.	.
	.	.	.
	.	.	.
	.	.	.
B-A	2	(Treatment B) y_{211}	(Treatment A) y_{121}
	4	y_{212}	y_{122}
	5	y_{213}	y_{123}
	8	y_{214}	y_{124}
	.	.	.
	.	.	.
	.	.	.
	.	.	.

The data can be analyzed using *analysis of variance* methods with the following factors: Treatment Group, Period, Sequence, and Patient-within-Sequence. Computations proceed as shown in Chapters 6, 7, and 8 to obtain the ANOVA summary shown in Table 9.2.

TABLE 9.2 ANOVA Summary Table for a 2-Period Crossover

Source	df	SS	MS	F
Treatment (T)	1	SST	MST	$F_T = MST / MSE$
Period (P)	1	SSP	MSP	$F_P = MSP / MSE$
Sequence (S)	1	SSS	MSS	$F_S = MSS / MSP(S)$
Patient-within-Sequence (P(S))	n_1+n_2-2	SSP(S)	MSP(S)	
Error	n_1+n_2-2	SSE	MSE	
Total	N-1	TOT(SS)		

The Treatment sum of squares, SST, is proportional to the sum of squared deviations of each Treatment mean from the overall mean. Likewise, SSP is computed from the sum of squared deviations of each Period mean from the overall mean, etc. The mean squares (MS) are found by dividing the sums of squares (SS) by their respective degrees of freedom (df). Computations are shown in Example 9.1.

The hypothesis of equal treatment means is tested at a two-tailed significance level, α , by comparing the *F-test* statistic, F_T , with the critical F-value based on 1 upper and n_1+n_2-2 lower degrees of freedom, denoted by $F_{n_1+n_2-2}^1(\alpha)$. With μ_i representing the mean response for Treatment i ($i = A, B$), the test summary for no treatment effect is

null hypothesis:	$H_0: \mu_A = \mu_B$
alt. hypothesis:	$H_A: \mu_A \neq \mu_B$
test statistic:	$F_T = \frac{MST}{MSE}$
decision rule:	reject H_0 if $F_T > F_{n_1+n_2-2}^1(\alpha)$.

The Period and Sequence effects can also be tested in a similar manner. As in the *repeated measures ANOVA* (Chapter 8), the test for Sequence effect uses the mean square for Patient-within-Sequence in the denominator because patients are nested within sequence groups. When the hypothesis of no Sequence Group effect is true, variation between sequence groups simply reflects patient-to-patient variation.

9.3 Examples

Example 9.1—Diaphoresis Following Cardiac Medication

A preliminary study was conducted on 16 normal subjects to examine the time to perspiration following administration of a single dose of a new cardiac medication with known diaphoretic effects. Subjects were asked to walk on a treadmill at the clinic following dosing with the new medication (A), and again on a separate clinic visit following placebo (B). A two-period crossover design was used with 8 subjects randomized to each of the two sequence groups. Time in minutes until the appearance of perspiration beads on the forehead was recorded as shown in Table 9.3. Is there any difference in perspiration times?

TABLE 9.3 Raw Data for Example 9.1

Sequence	Subject Number	Period 1	Period 2
A-B		(A)	(B)
	1	6	4
	3	8	7
	5	12	6
	6	7	8
	9	9	10
	10	6	4
	13	11	6
B-A		(B)	(A)
	2	5	7
	4	9	6
	7	7	11
	8	4	7
	11	9	8
	12	5	4
	14	8	9
	16	9	13

Solution

Using the two-way *crossover ANOVA*, you want to compare mean perspiration times between treatment groups, controlling for treatment period and sequence (order) of administration. For a balanced design, the sum of squares for each of these sources can be computed in the same manner as demonstrated previously (Chapters 6, 7, 8). First, obtain the marginal means, as shown in Table 9.4.

TABLE 9.4 Summary of Marginal Means for Example 9.1

Sequence	Subject Number	Period 1	Period 2	Subject Means
A-B		(A)	(B)	
	1	6	4	5.0
	3	8	7	7.5
	5	12	6	9.0
	6	7	8	7.5
	9	9	10	9.5
	10	6	4	5.0
	13	11	6	8.5
Sequence Means	15	8	8	8.0
		8.375	6.625	7.5000
B-A		(B)	(A)	
	2	5	7	6.0
	4	9	6	7.5
	7	7	11	9.0
	8	4	7	5.5
	11	9	8	8.5
	12	5	4	4.5
	14	8	9	8.5
Sequence Means	16	9	13	11.0
		7.000	8.125	7.5625
Overall Means		7.6875	7.3750	7.53125

Treatment A Mean: 8.2500

Treatment B Mean: 6.8125

Now, you can compute the sum of squares as follows:

$$\begin{aligned} \text{SS(Treatment)} &= (16 \cdot (8.2500 - 7.53125)^2) + \\ &\quad (16 \cdot (6.8125 - 7.53125)^2) \\ &= 16.53125 \end{aligned}$$

$$\begin{aligned} \text{SS(Period)} &= (16 \cdot (7.6875 - 7.53125)^2) + \\ &\quad (16 \cdot (7.3750 - 7.53125)^2) \\ &= 0.78125 \end{aligned}$$

$$\begin{aligned} \text{SS(Sequence)} &= (16 \cdot (7.5000 - 7.53125)^2) + \\ &\quad (16 \cdot (7.5625 - 7.53125)^2) \\ &= 0.03125 \end{aligned}$$

$$\begin{aligned} \text{TOTAL(SS)} &= ((6 - 7.53125)^2 + \\ &\quad (8 - 7.53125)^2 + \dots + \\ &\quad (13 - 7.53125)^2) \\ &= 167.96875 \end{aligned}$$

SS(Patients-within-Sequence)

$$\begin{aligned}
 &= ((5.0 - 7.5000)^2 + (7.5 - 7.5000)^2 + \dots + (8.0 - 7.5000)^2 + \\
 &\quad (6.0 - 7.5625)^2 + (7.5 - 7.5625)^2 + \dots + (11.0 - 7.5625)^2) \\
 &= 103.4375
 \end{aligned}$$

Because this is a balanced design, the error sum of squares can be found by subtraction,

$$\begin{aligned}
 \text{SS(Error)} &= \text{TOTAL(SS)} - (\text{SS(TRT)} + \text{SS(PD)} + \text{SS(SEQ)} + \text{SS(PAT(SEQ)))}) \\
 &= 167.96875 - (16.53125 + 0.78125 + 0.03125 + 103.4375) \\
 &= 47.1875
 \end{aligned}$$

Completing the ANOVA table, you obtain the following:

Table 9.5 ANOVA Summary for Example 9.1

Source	df	SS	MS	F
Treatment (T)	1	16.53125	16.53125	4.90
Period (P)	1	0.78125	0.78125	0.23
Sequence (S)	1	0.03125	0.03125	0.004
Patient-within Sequence (P(S))	14	103.4375	7.38839	
Error	14	47.1875	3.37054	
Total	31	167.96875		

With μ_i representing the mean response for Treatment i ($i = A, B$), the test summary for Treatment effect becomes

null hypothesis: $H_0: \mu_A = \mu_B$

alt. hypothesis: $H_A: \mu_A \neq \mu_B$

test statistic: $F_T = 4.90$

decision rule: reject H_0 if $F_T > F_{14}^1(0.05) = 4.60$

conclusion: Because $4.90 > 4.60$, you reject H_0 and conclude that the new medication and placebo have different diaphoretic profiles, based on a significance level of $\alpha = 0.05$.

SAS Analysis of Example 9.1

The manual calculations show how to compute the mean squares and F-tests in an *analysis-of-variance* approach and can easily be duplicated using PROC GLM. The SAS code would be as follows:

SAS Code for Example 9.1

```
data xover;
    input pat seq $ trt $ pd y @@;
    datalines;
1 AB A 1   6     3 AB A 1   8     5 AB A 1 12     6 AB A 1   7
9 AB A 1   9     10 AB A 1   6    13 AB A 1 11    15 AB A 1   8
1 AB B 2   4     3 AB B 2   7     5 AB B 2   6     6 AB B 2   8
9 AB B 2 10   10 AB B 2   4    13 AB B 2   6    15 AB B 2   8
2 BA A 2   7     4 BA A 2   6     7 BA A 2 11    8 BA A 2   7
11 BA A 2   8    12 BA A 2   4    14 BA A 2   9    16 BA A 2 13
2 BA B 1   5     4 BA B 1   9     7 BA B 1   7    8 BA B 1   4
11 BA B 1   9    12 BA B 1   5    14 BA B 1   8    16 BA B 1   9
;

proc glm data = xover;
    class seq trt pd pat;
    model y = seq pat(seq) trt pd; ①
    test h=seq e=pat(seq); ②
    title1 'Crossover Design'; ③
    title2 'Example 9.1: Diaphoresis Following Cardiac
Medication';
    run;
```

All four sources of variation (Sequence, Treatment, Period, and Patient) must appear in the CLASS statement ① in PROC GLM. Noting that the Treatment effect (trt) is the same as the Sequence-by-Period interaction (seq*pd) (see Section 9.4.2), the MODEL statement ② is identical to that shown in Example 8.1 (univariate approach to *repeated measures* analysis). The Sequence (seq) represents the two randomized groups, and patients are nested within Sequence Group. Therefore, the TEST statement can be used to conduct the appropriate *F-test* for the Sequence effect using the random effect pat(seq) as the error term ③, as discussed in Chapters 7 and 8.

Also, as mentioned previously, PROC MIXED is usually a better choice than PROC GLM when using SAS, especially when there are missing values, and when it comes to performing pairwise tests for treatment differences or using CONTRAST or ESTIMATE statements. The SAS code and output for the analysis using PROC MIXED is shown below. Here, the layout is balanced with no missing data, so the results are identical to those that would be obtained using PROC GLM.

```
proc mixed data = xover;
    class seq trt pd pat;
    model y = seq trt pd;
    lsmeans trt / diff;
    random pat(seq);
    run;
```

The F-values for the effects Sequence (seq), Treatment (trt) and Period (pd) are shown in Output 9.1 ❸ and agree with the manual calculations. The Treatment effect has a significant p-value of 0.0439 ❹, which indicates a departure from the null hypothesis of equal Treatment means. Neither the Sequence effect (which tests for differences in the order of administration) nor the Period effect (which tests for differences between periods) are significant ($p=0.9491$ and 0.6376 , respectively). The LSMEANS statement for trt shows the Treatment means 8.25 and 6.8125, and the difference of 1.4375 minutes ❺.

OUTPUT 9.1 SAS Output for Example 9.1

Crossover Design			
Example 9.1: Diaphoresis Following Cardiac Medication			
The Mixed Procedure			
Model Information			
Data Set	WORK.XOVER	Dependent Variable	Y
Covariance Structure	Variance Components	Estimation Method	REML
Residual Variance Method	Profile	Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment		
Class Level Information			
Class	Levels	Values	
seq	2	AB BA	
trt	2	A B	
pd	2	1 2	
pat	16	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16	
Dimensions			
Covariance Parameters	2	Columns in X	7
Columns in Z	16	Subjects	1
Max Obs Per Subject	32		
Number of Observations			
Number of Observations Read	32	Number of Observations Used	32
Number of Observations Not Used	0		
Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	136.27710462	
1	1	134.17436545	0.00000000
Convergence criteria met.			

OUTPUT 9.1 SAS Output for Example 9.1 (continued)

Crossover Design						
Example 9.1: Diaphoresis Following Cardiac Medication						
Covariance Parameter Estimates						
Cov Parm	Estimate					
pat(seq)	2.0089					
Residual	3.3705					
Fit Statistics						
-2 Res Log Likelihood	134.2					
AIC (smaller is better)	138.2					
AICC (smaller is better)	138.7					
BIC (smaller is better)	139.7					
Type 3 Tests of Fixed Effects						
Effect		Num DF	Den DF	④ F Value	Pr > F	
seq		1	14	0.00	0.9491	
trt		1	14	4.90	0.0439	
pd		1	14	0.23	0.6376	
Least Squares Means						
Effect	⑥ trt	Estimate	Standard Error	DF	t Value	
trt	A	8.2500	0.5798	14	14.23	
trt	B	6.8125	0.5798	14	11.75	
Differences of Least Squares Means						
Effect	trt	_trt	Estimate	Standard Error	DF	t Value
trt	A	B	1.4375	0.6491	14	2.21
						0.0439

The next example illustrates the analysis of a 4-period *crossover* design balanced for residuals (see Section 9.1), including a test for carryover effect using SAS.

❖ Example 9.2—Antibiotic Blood Levels Following Aerosol Inhalation

Normal subjects were enrolled in a pilot study to compare blood levels among 4 doses (A, B, C, and D) of an antibiotic using a new aerosol delivery formulation. The response measure is area-under-the-curve (AUC) up to 6 hours after dosing. A 4-period crossover design was used with a 3-day wash-out period between doses. Three subjects were randomized to each of four sequence groups, as shown in Table 9.6 along with the data (given as log-AUCs). Are there any differences in blood levels among the 4 doses?

TABLE 9.6 Raw Data for Example 9.2

Dosing Sequence	Subject Number	Period 1	Period 2	Period 3	Period 4
A-B-D-C	102	2.31	3.99	11.75	4.78
	106	3.95	2.07	7.00	4.20
	109	4.40	6.40	9.76	6.12
B-C-A-D	104	6.81	8.38	1.26	10.56
	105	9.05	6.85	4.79	4.86
	111	7.02	5.70	3.14	7.65
C-D-B-A	101	6.00	4.79	2.35	3.81
	108	5.25	10.42	5.68	4.48
	112	2.60	6.97	3.60	7.54
D-A-C-B	103	8.15	3.58	8.79	4.94
	107	12.73	5.31	4.67	5.84
	110	6.46	2.42	4.58	1.37

Solution

You can proceed with an ANOVA approach by computing Dose Group, Period, and Sequence effects in the same way as presented for Example 9.1 since this is a balanced layout with no missing values.

In SAS (PROC GLM or PROC MIXED), you can include a carryover effect in the model and adjust the tests for Dose Group effects for the carryover effect. The carryover effect is somewhat entangled with the Dose effect, so the Dose Group means cannot be estimated free of carryover effects. However, differences in the Dose Group means are estimable (see Section 9.4.7).

SAS Analysis of Example 9.2

The data are input in data lines according to Sequence Group (seqgrp) and Patient (pat), as shown in the inhal1 data set in the SAS code for Example 9.2. The Dose Group (dose) and Period (per) variables are added in the data set inhal2 ⑦ using the OUTPUT statement in the DATA step. The final data set for analysis, inhal, is created to include a character-valued variable for the carryover effect, co ⑧, whose values represent the dose group from the previous period. A value of '0' is assigned to co for the first period. The printout of the data set inhal shows the values of each of these carryover effects ⑨.

PROC MIXED is used to analyze the data. The MODEL statement ⑩ includes effects for Sequence Group (seqgrp), Dose Group (dose), Period (per) and the overall carryover (co). Patient-within-Sequence Group (pat(seqgrp)) is included in a RANDOM statement.

The carryover effect has a p-value of 0.0916 ⑪, which is not significant, but may deserve further attention if it makes sense clinically due to its marginal significance. The Dose Group effect adjusted for carryover is highly significant ($p=0.0002$). No Sequence Group ($p=0.5113$) or Period effects ($p=0.9929$) are evident.

The LSMEANS statement is included to get the estimates of mean AUC differences between Dose Groups ⑫. While the Dose LSMEANS are not estimable, their differences are in this case, since the carryover effect is assumed to be the same for each dose level. As seen in Output 9.2, the mean response for Dose Group D differs significantly ($p<0.05$) from those of the other three (A, B, and C) ⑬.

SAS Code for Example 9.2

```
data inhal1;
  input seqgrp $ pat auc1 auc2 auc3 auc4 @@;
  datalines;
    ABDC 102  2.31  3.99 11.75  4.78
    ABDC 106  3.95  2.07  7.00  4.20
    ABDC 109  4.40  6.40  9.76  6.12
    BCAD 104  6.81  8.38  1.26 10.56
    BCAD 105  9.05  6.85  4.79  4.86
    BCAD 111  7.02  5.70  3.14  7.65
    CDBA 101  6.00  4.79  2.35  3.81
    CDBA 108  5.25 10.42  5.68  4.48
    CDBA 112  2.60  6.97  3.60  7.54
    DACB 103  8.15  3.58  8.79  4.94
    DACB 107 12.73  5.31  4.67  5.84
    DACB 110  6.46  2.42  4.58  1.37
  ;
run;
```

```

data inhal2; set inhal1;                                7
  dose = substr(seqgrp,1,1); per = 1; auc = auc1; output;
  dose = substr(seqgrp,2,1); per = 2; auc = auc2; output;
  dose = substr(seqgrp,3,1); per = 3; auc = auc3; output;
  dose = substr(seqgrp,4,1); per = 4; auc = auc4; output;
run;

proc sort data = inhal2; by pat per;

data inhal; set inhal2;
  keep pat seqgrp dose per auc co;
  co = lag(dose); if per = 1 then co = '0';           8
run;

proc sort data = inhal; by seqgrp per pat;
proc print data = inhal;
  title1 'CrossOver Design';
  title2 'Example 9.2: Antibiotic Blood Levels Following
          Aerosol Inhalation';
run;

proc mixed data = inhal;
  class seqgrp dose per co;                            10
  model auc = seqgrp dose per co;
  random pat(seqgrp);
  lsmeans dose / diff;                             12
run;
quit;

```

OUTPUT 9.2 SAS Output for Example 9.2

CrossOver Design Example 9.2: Antibiotic Blood Levels Following Aerosol Inhalation						
Obs	seqgrp	pat	dose	per	auc	co
❾ 1	ABDC	102	A	1	2.31	0
2	ABDC	106	A	1	3.95	0
3	ABDC	109	A	1	4.40	0
4	ABDC	102	B	2	3.99	A
5	ABDC	106	B	2	2.07	A
6	ABDC	109	B	2	6.40	A #
7	ABDC	102	D	3	11.75	B
8	ABDC	106	D	3	7.00	B
9	ABDC	109	D	3	9.76	B
10	ABDC	102	C	4	4.78	D #
11	ABDC	106	C	4	4.20	D
12	ABDC	109	C	4	6.12	D
13	BCAD	104	B	1	6.81	0
14	BCAD	105	B	1	9.05	0
15	BCAD	111	B	1	7.02	0
16	BCAD	104	C	2	8.38	B
17	BCAD	105	C	2	6.85	B
18	BCAD	111	C	2	5.70	B
19	BCAD	104	A	3	1.26	C
20	BCAD	105	A	3	4.79	C
21	BCAD	111	A	3	3.14	C
22	BCAD	104	D	4	10.56	A
23	BCAD	105	D	4	4.86	A
24	BCAD	111	D	4	7.65	A #
25	CDBA	101	C	1	6.00	0
26	CDBA	108	C	1	5.25	0
27	CDBA	112	C	1	2.60	0
28	CDBA	101	D	2	4.79	C
29	CDBA	108	D	2	10.42	C
30	CDBA	112	D	2	6.97	C
31	CDBA	101	B	3	2.35	D
32	CDBA	108	B	3	5.68	D
33	CDBA	112	B	3	3.60	D
34	CDBA	101	A	4	3.81	B
35	CDBA	108	A	4	4.48	B
36	CDBA	112	A	4	7.54	B
37	DACB	103	D	1	8.15	0
38	DACB	107	D	1	12.73	0
39	DACB	110	D	1	6.46	0
40	DACB	103	A	2	3.58	D #
41	DACB	107	A	2	5.31	D
42	DACB	110	A	2	2.42	D
43	DACB	103	C	3	8.79	A
44	DACB	107	C	3	4.67	A
45	DACB	110	C	3	4.58	A #
46	DACB	103	B	4	4.94	C
47	DACB	107	B	4	5.84	C
48	DACB	110	B	4	1.37	C

Randomly selected observations that were excluded for reanalysis (see Section 9.4.6).

OUTPUT 9.2 SAS Output for Example 9.2 (continued)

```
CrossOver Design
Example 9.2: Antibiotic Blood Levels Following Aerosol Inhalation

The Mixed Procedure

Model Information

Data Set                      WORK.INHAL
Dependent Variable             auc
Covariance Structure          Variance Components
Estimation Method              REML
Residual Variance Method      Profile
Fixed Effects SE Method       Model-Based
Degrees of Freedom Method     Containment

Class Level Information

Class      Levels      Values
seqgrp     4          ABDC BCAD CDBA DACB
dose       4          A B C D
per        4          1 2 3 4
co         5          0 A B C D

Dimensions

Covariance Parameters          2
Columns in X                   18
Columns in Z                   4
Subjects                        1
Max Obs Per Subject            48

Number of Observations

Number of Observations Read    48
Number of Observations Used   48
Number of Observations Not Used 0

Iteration History

Iteration    Evaluations    -2 Res Log Like    Criterion
0           1             177.82196406
1           1             177.82196406        0.00000000

Convergence criteria met.

Covariance Parameter
Estimates

Cov Parm      Estimate
pat(seqgrp)    0
Residual      4.3881

Fit Statistics

-2 Res Log Likelihood          177.8
AIC (smaller is better)        179.8
AICC (smaller is better)       179.9
BIC (smaller is better)        179.2
```

OUTPUT 9.2 SAS Output for Example 9.2 (continued)

CrossOver Design Example 9.2: Antibiotic Blood Levels Following Aerosol Inhalation					
Type 3 Tests of Fixed Effects					
Effect		Num DF	Den DF	F Value	Pr > F
seqgrp		3	31	0.79	0.5113
dose		3	31	8.97	0.0002
per		2	31	0.01	0.9929
co		3	31	2.35	0.0916

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Least Squares Means						
Effect	dose	Estimate	Standard Error	DF	t Value	Pr > t
dose	A	Non-est
dose	B	Non-est
dose	C	Non-est
dose	D	Non-est

Differences of Least Squares Means							
Effect	dose	_dose	Estimate	Standard Error	DF	t Value	Pr > t
dose	A	B	-1.5465	0.8969	31	-1.72	0.0946
dose	A	C	-1.6106	0.8969	31	-1.80	0.0823
dose	A	D	-4.5472	0.8969	31	-5.07	<.0001
dose	B	C	-0.06408	0.8969	31	-0.07	0.9435
dose	B	D	-3.0007	0.8969	31	-3.35	0.0022
dose	C	D	-2.9367	0.8969	31	-3.27	0.0026

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9.4 Details & Notes

- **9.4.1** Study resources might be wasted by conducting *crossover* studies that are likely to yield inconsistent treatment differences among periods. This scenario could arise from a carryover effect of certain treatments, improper wash-out periods between treatments, or the inability of patients to be restored to their original baseline conditions at the start of each period.

Designs balanced for residual effects should be used whenever possible with *crossover* studies. Such schemes have the property that each treatment is preceded by every other treatment the same number of times. The presence of treatment carryover effects can easily be investigated with these types of designs. The layout used in Example 9.2 is that of a 4-treatment design balanced for residuals. In this design, four sequence groups are used, and each treatment is preceded by each of the other treatments exactly once.

An example of a 3-treatment (A, B, and C), 3-period *crossover* design balanced for residuals is shown in Table 9.7. Six sequence groups are used,

and each treatment is preceded by every other treatment exactly twice. Note that such balance would not be possible with only three sequence groups. In general, if k represents the number of treatments, the minimum number of sequence groups needed for complete residual balance is k , if k is even, and $2k$, if k is odd.

TABLE 9.7 A 3-Period Crossover Design Balanced for Residuals

Sequence	Period 1	Period 2	Period 3
1	A	B	C
2	B	C	A
3	C	A	B
4	A	C	B
5	B	A	C
6	C	B	A

Study design features should also include an adequate wash-out duration between treatment periods. As previously mentioned, the length of the trial and the number of periods should also be limited to minimize changing clinical conditions of the patients during the study.

- **9.4.2** In a two-period *crossover design* under the assumption of no carryover effect (such as in Example 9.1), the Period effect is completely confounded with the Treatment-by-Sequence Group interaction. In other words, any differences between Sequence Groups in the Treatment comparisons are indistinguishable from Period differences. The results of Example 9.1 would be identical if the pd effect is replaced with the trt*seq interaction in the MODEL statement in PROC GLM.

Similarly, a Treatment effect implies that response differences between Period 1 and Period 2 are in opposite directions for the two Sequence Groups, i.e., a Period-by-Sequence Group interaction. The Treatment (trt) effect in the MODEL statement in PROC GLM used in Example 9.1 could be replaced with this interaction (pd*seq) with the same results. When the MODEL statement is written in this way, it is identical to the form discussed for the general *repeated measures ANOVA* of Chapter 8 (refer to ‘univariate’ approach using GLM).

The preceding interactions cannot be used interchangeably with main effects in *crossovers* that involve more than two periods or those in which a carryover effect might exist.

- **9.4.3** In the *crossover* analysis, the presence of a Treatment-by-Period interaction generally requires separate analyses within each period using, for example, the *two-sample t-test* (Chapter 5). In such a situation, many analysts accept only the results of the first period.

- **9.4.4** The two-period *crossover* design (A-B/B-A) can also be analyzed in SAS using PROC TTEST with SAS 9.2 and later, assuming there is no carryover effect. This design requires a data set in the form of one patient per observation with four variables, two that identify the treatment used in each period (say trt_pd1, trt_pd2) and two that give the response measurements for each period (say y_pd1, y_pd2). In Example 9.1, you would invoke the TTEST procedure using the crossover option as follows:

```
proc ttest data = xover;
var y1 y_pd2 / crossover = (trt_pd1 trt_pd2);
run;
```

where the data set xover has the form:

Obs	pat	trt_pd1	trt_pd2	y_pd1	y2_pd2
1	1	A	B	6	4
2	2	B	A	5	7
3	3	A	B	8	7
4	4	B	A	9	6
5	5	A	B	12	6
6	6	A	B	7	8
7	7	B	A	7	11
8	8	B	A	4	7
9	9	A	B	9	10
10	10	A	B	6	4
11	11	B	A	9	8
12	12	B	A	5	4
13	13	A	B	11	6
14	14	B	A	8	9
15	15	A	B	8	8
16	16	B	A	9	13

- **9.4.5** Dropouts following the first period will create bias in the analysis of the *crossover* study. For example, in the two-way *crossover*, the ‘within-patient control’ advantage of the *crossover* is lost when a patient has data from the first period, but not from the second. Whether that patient is dropped from the analysis or the analysis is adjusted for the missing value (e.g., use of imputation), the results will likely have some unknown bias. If there are many dropouts, the primary analysis might use just the data from the first period. Because dropout rates usually increase with lengthier studies, this problem can be controlled by using a small number of short treatment periods whenever a *crossover* design is contemplated.

- **9.4.6** You may also get biased estimates in a crossover analysis with missing data. You can still make inferences in a crossover analysis with missing data, assuming there are a limited number of missing values occurring at random. Inferences using PROC MIXED are not as serious a problem as with PROC GLM in the presence of such missing data. The results with PROC MIXED are valid as long as the data are missing at random.

Example 9.2 was reanalyzed after excluding five randomly selected observations as if they were missing, as denoted by the number symbol (#) in Output 9.2. The results of PROC MIXED are shown in Output 9.3 (partial output). Comparing these with Output 9.2, the overall test results are only slightly affected. While the standard errors for the LSMEAN differences are larger, PROC MIXED still identifies the significant comparisons (A vs. D, B vs. D, and C vs. D).

OUTPUT 9.3 SAS Output for Example 9.2 with Missing Data (#)

CrossOver Design							
Example 9.2: Antibiotic Blood Levels Following Aerosol Inhalation							
The Mixed Procedure							
Number of Observations							
Number of Observations Read 48							
Number of Observations Used 43							
Number of Observations Not Used 5							
Type 3 Tests of Fixed Effects							
Effect		Num DF	Den DF	F Value	Pr > F		
seqgrp		3	26	0.95	0.4307		
dose		3	26	7.73	0.0007		
per		2	26	0.06	0.9450		
co		3	26	2.06	0.1298		
Differences of Least Squares Means							
Effect	dose	_dose	Estimate	Standard Error	DF	t Value	Pr > t
dose	A	B	-1.3148	0.9879	26	-1.33	0.1948
dose	A	C	-1.6526	1.0246	26	-1.61	0.1188
dose	A	D	-4.6369	0.9878	26	-4.69	<.0001
dose	B	C	-0.3378	1.0279	26	-0.33	0.7451
dose	B	D	-3.3221	1.0176	26	-3.26	0.0031
dose	C	D	-2.9843	1.0253	26	-2.91	0.0073

- **9.4.7** The term ‘balanced residuals’ implies that, if the carryover effect is the same for each treatment, these effects will be cancelled when looking at differences in treatment means, and thus, the true treatment difference is free of any carryover effects. Biased estimates of treatment differences will result if the residuals are not balanced across treatment groups.
- **9.4.8** Example 9.2 includes a carryover effect (co), which is assumed to be the same for all doses. You can include several carryover or residual effects if you believe the carryover may differ for each dose level. An example of this using SAS is shown in Littell, Stroup, and Freund (2002). To check for different degrees of carryover among treatments, a preliminary test for carryover effects can be made to check model assumptions, often conducted at a significance level greater than 0.05, perhaps in the 0.10 to 0.20 range. If non-

significant, the carryover effect may be dropped from the model. If you conduct further analyses of the carryover effect in Example 9.2, it becomes evident that Dose Group B has a larger carryover effect than the other doses, which might have a clinically relevant interpretation.

When a significant carryover effect is found, caution must be used in the interpretation of the Treatment effects. Significant carryover effects might provide useful information for designing future studies (e.g., using a longer observation period and/or a longer wash-out period).

- **9.4.9** Although this chapter introduces the *crossover design* for normal response data, *crossover designs* can also be performed with non-normal responses, which include categorical and rank data. Using binary responses and non-parametric approaches to the analysis of non-normal responses in *crossovers* are discussed in Jones and Kenward (1989), and examples using SAS are included in Stokes, Davis, and Koch (2000).

CHAPTER 10

Linear Regression

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10.1 Introduction

Regression analysis is used to analyze the relationship between a response, y , and a quantitative factor, x . Knowledge of this relationship can be important for predicting unmeasured responses from a known x -value.

Examples where regression analysis might be useful in clinical data analysis include the modeling of blood pressure response (y) on the dose of a new anti-hypertensive drug (x), cholesterol level (y) on patient's age (x), pain relief (y) on time after dosing with an anti-inflammatory treatment (x), or degree of wound healing (y) on the baseline surface area of a burn wound (x).

While a number of potential relationships between x and y might be considered, in this chapter, the focus is on linear relationships only. *Simple linear regression* methodology provides an estimate of the best-fitting line through a set of data points, $(x_1, y_1), (x_2, y_2), \dots, (x_n, y_n)$. You can then determine the significance of the linear relationship or correlation, predict future responses, estimate mean responses for expected x -values, and make inferences regarding the slope of the regression line.

10.2 Synopsis

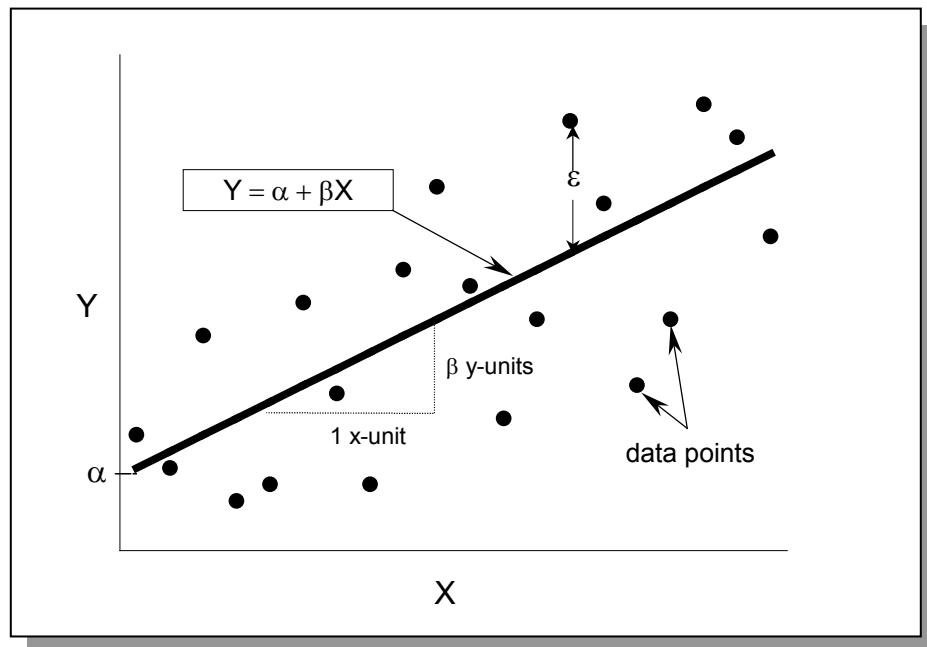
Given a number of observed values of a normally distributed response variable, (y_1, y_2, \dots, y_n) , the mean, \bar{y} , represents the best estimate of a future response. The idea behind regression analysis is to improve this estimate by using the value of some related factor, x . If x and y have a known linear relationship, the best estimate of y will be a linear function of the known value, x .

A linear relationship between a response, y , and an independent variable, x , can be expressed as

$$y = \alpha + \beta x + \varepsilon$$

where α is the intercept and β is the slope, as shown in Figure 10.1. Because the response is subject to random measurement error, y might differ in repeated sampling for the same value of x . The ε accounts for this random nature of the response y .

FIGURE 10.1 Simple Linear Regression of y on x



Typically, you have n pairs of coordinates, $((x_i, y_i)$, for $i = 1, 2, \dots, n$), and assume that the y_i 's are independent, normally distributed, and have the same variance, σ^2 , for all x values. From these data, you estimate the model parameters α and β by a and b , respectively, in such a way that the resulting ‘prediction equation’

$$\hat{y} = a + bx$$

is the ‘best-fitting’ line through the measured coordinates. In this sense, the prediction equation represents the best estimate of the unknown regression model.

You seek values for a and b such that the predicted response, \hat{y} , is as close as possible to the observed response, y , for all measured data points. One way to

satisfy this requirement is to minimize the sum of squared differences between y and \hat{y}

$$SSE = \sum_{i=1}^n (y_i - \hat{y}_i)^2$$

This is known as the Least Squares criterion. SSE is the sum of squared errors or deviations between the actual and predicted responses. By using differential calculus, the SSE can be minimized with respect to a and b , with the following solution.

Define the quantities S_{yy} , S_{xx} , and S_{xy} :

$$S_{yy} = \sum_{i=1}^n (y_i - \bar{y})^2 = \sum_{i=1}^n y_i^2 - n \cdot (\bar{y})^2$$

$$S_{xx} = \sum_{i=1}^n (x_i - \bar{x})^2 = \sum_{i=1}^n x_i^2 - n \cdot (\bar{x})^2$$

and

$$S_{xy} = \sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y}) = \sum_{i=1}^n x_i \cdot y_i - (n \cdot \bar{x} \cdot \bar{y})$$

The ‘best-fitting’ line based on the Least Squares criterion is given by

$$\hat{y} = a + b x$$

where $b = S_{xy} / S_{xx}$

and $a = \bar{y} - b \bar{x}$

The best estimate of the variance, σ^2 , is $s^2 = SSE / (n-2)$, where SSE can be calculated from the formula:

$$SSE = \frac{S_{xx} \cdot S_{yy} - S_{xy}^2}{S_{xx}}$$

based on $n-2$ degrees of freedom.

The main question posed by *simple linear regression* concerns the significance of the slope parameter. If the slope β is 0, then the value of x will not improve the prediction of y over the ordinary predictor, \bar{y} . A significant slope β , indicating a linear relationship between x and y , means that knowledge of the x -values will significantly improve your prediction ability.

The statistical test is based on a function of the slope estimate, b , which has the t-distribution when the null hypothesis of ‘0 slope’ is true. The test summary is

null hypothesis:	$H_0: \beta = 0$
alt. hypothesis:	$H_A: \beta \neq 0$
test statistic:	$t = \frac{b}{s / \sqrt{S_{xx}}}$
decision rule:	reject H_0 if $ t > t_{\alpha/2, n-2}$

If the slope is meaningful, you can estimate the mean response at a given value of x , say x_0 , with a 95% confidence interval by

$$\hat{y}_{x_0} \pm t_{0.025, n-2} \cdot s \cdot \sqrt{\frac{1}{n} + \frac{(x_0 - \bar{x})^2}{S_{xx}}}$$

where $\hat{y}_{x_0} = a + b x_0$

10.3 Examples

¶ Example 10.1—Anti-Anginal Response vs. Disease History

Treadmill stress tests were administered to patients with angina pectoris before and 4 weeks after once-daily dosing with an experimental anti-anginal medication. The investigator wanted to know if the improvement in exercise duration is related to the patient's disease history. Disease duration since initial diagnosis (in years) and percent-improvement in treadmill walking times are shown in Table 10.1 for a study with 20 patients enrolled. Is there a significant linear relationship between improvement on medication and disease duration?

TABLE 10.1 Raw Data for Example 10.1

Patient Number	(x) Disease Duration (years)	(y) % Improvement	Patient Number	(x) Disease Duration (years)	(y) % Improvement
1	1	40	11	1	60
2	1	90	12	4	0
3	3	30	13	2	50
4	2	30	14	2	110
5	1	80	15	3	20
6	5	60	16	3	70
7	1	10	17	5	-30
8	4	-10	18	3	20
9	2	50	19	1	40
10	6	40	20	6	0

Solution

Using the formulas presented, compute

$$\sum x_i = 56 \quad \sum y_i = 760 \quad \sum x_i \cdot y_i = 1570$$

$$\sum x_i^2 = 212 \quad \sum y_i^2 = 52,200 \quad n = 20$$

so that

$$S_{xx} = 212 - \frac{56^2}{20} = 55.2$$

$$S_{yy} = 52,200 - \frac{760^2}{20} = 23,320$$

$$S_{xy} = 1570 - \frac{56 \times 760}{20} = -558$$

The estimated regression equation is found as follows:

$$b = \frac{-558}{55.2} = -10.109$$

and

$$a = \frac{760}{20} - (-10.109) \cdot \frac{56}{20} = 66.304$$

which yield

$$\hat{y} = 66.304 - 10.109 x$$

In addition, you find

$$SSE = \frac{(55.2)(23,320) - (-558)^2}{55.2} = 17,679.35$$

and

$$s = \sqrt{\frac{17,679.35}{18}} = 31.34$$

The regression slope, which represents the average change in y for a one-unit change in x , is the best estimate of the rate of improvement in treadmill performance for each one-year increase in disease duration, i.e., -10.109% per year. The statistical significance is determined by the *t-test*, summarized as follows:

null hypothesis:	$H_0: \beta = 0$
alt. hypothesis:	$H_A: \beta \neq 0$
test statistic:	$t = \frac{-10.109}{31.34 / \sqrt{55.2}} = -2.40$
decision rule:	reject H_0 if $ t > t_{0.025,18}$ ($=2.101$)
conclusion:	Because $2.40 > 2.101$, you reject H_0 and conclude that treadmill improvement has a significant linear relationship to disease duration.

Given the duration of angina history, you can also estimate the improvement in exercise time. For example, the mean improvement in treadmill exercise time for the average patient with a 5-year history of angina is computed as

$$\hat{y}_5 = 66.304 - 10.109(5) = 15.76\%$$

The 95% confidence interval for this mean is

$$\begin{aligned} 15.76 &\pm 2.101 \cdot (31.34) \cdot \sqrt{\frac{1}{20} + \frac{(5-2.8)^2}{55.2}} \\ &= 15.76 \pm 2.101 \cdot (11.629) \\ &= 15.76 \pm 24.43 \end{aligned}$$

or (-8.67% to 40.19%). Because this interval contains 0, you would be inclined to conclude that the average treated patient with a 5-year history of angina does not have significant improvement in exercise tolerance.

SAS Analysis of Example 10.1

The SAS code for analyzing the data set in this example is shown below, and the resulting output is shown in Output 10.1. A printout of the data set is first obtained by using PROC PRINT ❶, followed by the summary statistics for the x and y values using PROC MEANS ❷.

For this example, the regression analysis is performed using PROC GLM, although PROC REG can also be used. When PROC GLM is used without a CLASS statement, SAS assumes that the independent variables in the MODEL statement are quantitative and performs a regression analysis.

SAS Code for Example 10.1

```
data angina;
  input pat x_dur y_impr @@;
  datalines;
  1 1 40      2 1 90      3 3 30      4 2 30
  5 1 80      6 5 60      7 1 10      8 4 -10
  9 2 50      10 6 40     11 1 60     12 4 0
  13 2 50     14 2 110    15 3 20     16 3 70
  17 5 -30    18 3 20     19 1 40     20 6 0
;

proc sort data = angina; by x_dur y_impr;
proc print data = angina; ❶
  var pat x_dur y_impr;
```

```

title1 'Linear Regression & Correlation';
title2 'Example 10.1: Improvement in Angina vs.
        Disease Duration';
run;

proc means mean std n; ❷
  var x_dur y_impr;
run;

proc glm data = angina;
  model y_impr = x_dur / p clm ss1; ❸
run;
quit;

```

The regression estimates, **a** and **b**, are printed in the Estimate column in Output 10.1 ❸. Note the *t-test* for slope is -2.40 with a p-value of 0.0276 ❹, which confirms the analysis using the calculating formulas.

The P and CLM options specified in the MODEL statement ❸ request the predicted values based on the regression equation for each data point, along with 95% confidence intervals for the mean response at the corresponding x values. For x=5 (Observations 17 and 18), the predicted response (15.7608) has a corresponding 95% confidence interval of -8.67 to 40.19 ❺, which confirms the manual calculations.

OUTPUT 10.1 SAS Output for Example 10.1

Linear Regression & Correlation			
Example 10.1: Improvement in Angina vs. Disease Duration			
Obs	pat	x_dur	y_impr
1	7	1	10
2	1	1	40
3	19	1	40
4	11	1	60
5	5	1	80
6	2	1	90
7	4	2	30
8	9	2	50
9	13	2	50
10	14	2	110
11	15	3	20
12	18	3	20
13	3	3	30
14	16	3	70
15	8	4	-10
16	12	4	0
17	17	5	-30
18	6	5	60
19	20	6	0
20	10	6	40

❶

OUTPUT 10.1 SAS Output for Example 10.1 (continued)

Linear Regression & Correlation
Example 10.1: Improvement in Angina vs. Disease Duration

The MEANS Procedure

Variable	Mean	Std Dev	N
<hr/>			
x_dur	2.8000000	1.7044833	20
y_impr	38.0000000	35.0338182	20

②

The GLM Procedure

Number of Observations Read 20
Number of Observations Used 20

Dependent Variable: y_impr

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	5640.65217	5640.65217	5.74	0.0276
Error	18	17679.34783	982.18599		
Corrected Total	19	23320.00000			

R-Square 0.241880 ⑧ Coeff Var 82.47328 Root MSE 31.33985 y_impr Mean 38.00000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
x_dur	1	5640.652174	5640.652174	5.74	⑦ 0.0276

Parameter	Estimate	Standard Error	t Value	Pr > t	
Intercept	66.30434783	③ 13.73346931	4.83	0.0001	
x_dur	-10.10869565	4.21820157	-2.40	0.0276	④

OUTPUT 10.1 SAS Output for Example 10.1 (continued)

Linear Regression & Correlation Example 10.1: Improvement in Angina vs. Disease Duration						
The GLM Procedure						
Observation	Observed	Predicted	Residual	95% Confidence Limits for Mean Predicted Value		
1	10.0000000	56.1956522	-46.1956522	34.4879982	77.9033062	
2	40.0000000	56.1956522	-16.1956522	34.4879982	77.9033062	
3	40.0000000	56.1956522	-16.1956522	34.4879982	77.9033062	
4	60.0000000	56.1956522	3.8043478	34.4879982	77.9033062	
5	80.0000000	56.1956522	23.8043478	34.4879982	77.9033062	
6	90.0000000	56.1956522	33.8043478	34.4879982	77.9033062	
7	30.0000000	46.0869565	-16.0869565	29.7460282	62.4278848	
8	50.0000000	46.0869565	3.9130435	29.7460282	62.4278848	
9	50.0000000	46.0869565	3.9130435	29.7460282	62.4278848	
10	110.0000000	46.0869565	63.9130435	29.7460282	62.4278848	
11	20.0000000	35.9782609	-15.9782609	21.1491101	50.8074117	
12	20.0000000	35.9782609	-15.9782609	21.1491101	50.8074117	
13	30.0000000	35.9782609	-5.9782609	21.1491101	50.8074117	
14	70.0000000	35.9782609	34.0217391	21.1491101	50.8074117	
15	-10.0000000	25.8695652	-35.8695652	7.7076388	44.0314916	
16	0.0000000	25.8695652	-25.8695652	7.7076388	44.0314916	
17	-30.0000000	15.7608696	-45.7608696	-8.6702890	40.1920281	6
18	60.0000000	15.7608696	44.2391304	-8.6702890	40.1920281	
19	0.0000000	5.6521739	-5.6521739	-26.3006276	37.6049755	
20	40.0000000	5.6521739	34.3478261	-26.3006276	37.6049755	
				Sum of Residuals	-0.00000	
				Sum of Squared Residuals	17679.34783	
				Sum of Squared Residuals - Error SS	0.00000	
				PRESS Statistic	22187.33121	
				First Order Autocorrelation	-0.05364	
				Durbin-Watson D	1.91983	

Multiple Linear Regression

Simple linear regression refers to a single response variable, y , modeled on a single predictor variable, x . Sometimes, several quantitative factors (x_1, x_2, x_3, \dots) are thought to affect a response, y . These predictors can be used simultaneously in a *multiple linear regression* equation of the form

$$y = b_0 + b_1x_1 + b_2x_2 + \dots + b_kx_k$$

assuming k factors. Like the estimate b of β in the simple linear model, b_i estimates the unknown parameter, β_i , and is determined by the amount of contribution x_i makes to the prediction of y (for $i = 1, 2, \dots, k$). Interpretation and estimation of the β_i 's is the same as in the *simple linear regression* case. β_i represents the expected

increase in response, y , for a 1-unit increase in x_i (when all other x 's are held constant). These β_i 's are estimated by using the Least Squares method. The REG procedure in SAS is used to illustrate this analysis, as shown in Example 10.2.

Example 10.2—Symptomatic Recovery in Pediatric Dehydration

A study was conducted to determine the degree of recovery that takes place 90 minutes following treatment of 36 children diagnosed at the clinic with moderate to severe dehydration. Upon diagnosis and study entry, patients were treated with an electrolytic solution at various dose levels (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mEq/l) in a frozen, flavored, ice popsicle. The degree of rehydration was determined by using a subjective scale based on physical examination and parental input. These scores were converted to a 0 to 100 point scale, representing the percent of recovery. The child's age and weight were also recorded. Is recovery related to electrolyte dose based on the data shown in Table 10.2?

Solution

The regression procedure (PROC REG) in SAS is used to perform a *multiple linear regression* analysis, regressing the percent recovery at 90 minutes (y) on the concomitant variables Dose Level (X_1), Age (X_2), and Weight (X_3). Although, PROC GLM can also be used for this analysis, PROC REG has diagnostic and model building advantages for regression analysis when there are no class variables. In addition, multiple MODEL statements can be used with PROC REG.

In this example, the model is written

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \varepsilon$$

where x_1 = dose (mEq/l), x_2 = patient's age (years), and x_3 = patient's weight (lbs.) are the independent or 'explanatory' variables. The parameters are β_0 , which represents the overall average response, and β_i , which represents the average increase in the degree of recovery (y) for a one-unit increase in x_i ($i = 1, 2, 3$). The objective is to investigate the effect of Dose Level on response and how age and weight might influence that effect.

TABLE 10.2 Raw Data for Example 10.2

Patient Number	Degree of Recovery (%) (Y)	Dose (mEq/l) (X ₁)	Age (yrs) (X ₂)	Weight (lbs) (X ₃)
1	77	0.0	4	28
2	65	1.5	5	35
3	75	2.5	8	55
4	63	1.0	9	76
5	75	0.5	5	31
6	82	2.0	5	27
7	70	1.0	6	35
8	90	2.5	6	47
9	49	0.0	9	59
10	72	3.0	8	50
11	67	2.0	7	50
12	100	2.5	7	46
13	75	1.5	4	33
14	58	3.0	8	59
15	58	1.5	6	40
16	55	0.0	8	58
17	80	1.0	7	55
18	55	2.0	10	76
19	44	0.5	9	66
20	62	1.0	6	43
21	60	1.0	6	48
22	75	2.5	7	50
23	77	1.5	5	29
24	80	2.5	11	64
25	68	3.0	9	61
26	71	2.5	10	71
27	90	1.5	4	26
28	80	2.0	3	27
29	70	0.0	9	56
30	58	2.5	8	57
31	88	1.0	3	22
32	68	0.5	5	37
33	60	0.5	6	44
34	90	3.0	5	45
35	79	1.5	8	53
36	90	2.0	4	29

SAS Analysis of Example 10.2

The SAS code and output for analyzing the data set in this example are shown on the following pages. A printout of the data set is first obtained using PROC PRINT ⑩. The CORR option in PROC REG requests the inclusion of the correlation coefficients between each pair of variables in the output ⑪. Notice that Age and Weight are highly correlated ($r=0.9367$), and that the degree of recovery at 90 minutes after treatment (y) is positively correlated with dose and negatively correlated with Age and Weight.

The F-value 9.03 ⑫ for the MODEL statement is a global test of the hypothesis,

$$H_0: \beta_1 = \beta_2 = \beta_3 = 0$$

vs.

$$H_a: \beta_1 = 0, \text{ or } \beta_2 = 0, \text{ or } \beta_3 = 0$$

This is highly significant ($p=0.0002$) indicating that the response is linearly related to at least one of the independent variables.

The prediction model can be written from the parameter estimates ⑬ as

$$\hat{y} = 85.48 + (6.17 \times \text{Dose}) + (0.28 \times \text{Age}) - (0.54 \times \text{Weight})$$

The β_i 's can be tested individually for equality to 0 using the *t-test*, as shown in Example 10.1. SAS prints these tests with the corresponding p-values as shown in Output 10.2 ⑭. Dose Level is significant ($p = 0.0016$), while Age ($p=0.9043$) and Weight ($p=0.1032$) are not, based on this model.

One method to check the ‘goodness-of-fit’ of a regression model is by its coefficient of determination, R^2 . You see an R^2 of 0.4584 ⑮, which indicates that about 46% of the variability of the measured responses can be accounted for by the explanatory variables, Dose Level, Age, and Weight. Sometimes there can be other (unmeasured) explanatory variables that might increase R^2 by their inclusion in the model which leads to a better fit, or the relationship might be a non-linear one. Many times, much of the unexplained variation is simply due to measurement error or the random nature of the responses that cannot be measured.

Multiple MODEL statements can be used in PROC REG. These are labeled consecutively in the SAS output (MODEL1, MODEL2, etc.) according to the order specified in the PROC REG syntax. The second MODEL statement (see SAS code for Example 10.2) requests a *simple linear regression* and uses Dose Level as the only explanatory variable. The output shows a significant linear relationship between Dose and response ($p = 0.0313$) when Age and Weight are not included. However, the R^2 has been reduced to only 12.9% ⑯, which indicates a relatively poor fit compared to the full model, despite the lack of significance found for Age and Weight in MODEL1.

This finding, combined with the high correlation between Age and Weight ⑪, suggests the possibility of collinearity, a condition in which two or more independent variables, both of which measure similar underlying effects, are included in the regression model. By using redundant model information, the parameter estimates (b_i 's) might be inappropriate and/or the variance of these estimates might be inflated, which results in fewer significant findings.

Results for two additional models are shown. Age and Weight are separately included with Dose Level in MODEL3 ⑫ and MODEL4 ⑬, respectively. A coefficient of determination of $R^2 = 41.1\%$ ⑭ is obtained by using Dose Level and Age, and an $R^2 = 45.8\%$ ⑮ is found with Dose Level and Weight. These results indicate that if Age or Weight is used in the model, including the other does not help much.

The final model used is MODEL4 because it has the highest R^2 . With this model, Weight is a highly significant explanatory variable ($p < 0.0001$).

SAS Code for Example 10.2

```

data dehyd;
    input pat y dose age wt @@;
    datalines;
1 77 0.0 4 28   2 65 1.5 5 35   3 75 2.5 8 55
4 63 1.0 9 76   5 75 0.5 5 31   6 82 2.0 5 27
7 70 1.0 6 35   8 90 2.5 6 47   9 49 0.0 9 59
10 72 3.0 8 50  11 67 2.0 7 50  12 100 2.5 7 46
13 75 1.5 4 33 14 58 3.0 8 59  15 58 1.5 6 40
16 55 0.0 8 58 17 80 1.0 7 55  18 55 2.0 10 76
19 44 0.5 9 66 20 62 1.0 6 43  21 60 1.0 6 48
22 75 2.5 7 50 23 77 1.5 5 29  24 80 2.5 11 64
25 68 3.0 9 61 26 71 2.5 10 71 27 90 1.5 4 26
28 80 2.0 3 27 29 70 0.0 9 56 30 58 2.5 8 57
31 88 1.0 3 22 32 68 0.5 5 37 33 60 0.5 6 44
34 90 3.0 5 45 35 79 1.5 8 53 36 90 2.0 4 29
;

proc print data = dehyd;                                ⑩
    var pat y dose age wt;
    title1 'Multiple Linear Regression';
    title2 'Example 10.2: Recovery in Pediatric
            Dehydration';
run;

proc reg corr data = dehyd;                            ⑪
    model y = dose age wt / ss1 ss2 vif collinoint;
    model y = dose      ;
    model y = dose age  ;                             ⑫
    model y = dose wt   ;                             ⑬
run;
quit;

```

OUTPUT 10.2 SAS Output for Example 10.2

Multiple Linear Regression
Example 10.2: Recovery in Pediatric Dehydration

Obs	pat	Y	dose	age	wt
1	1	77	0.0	4	28
2	2	65	1.5	5	35
3	3	75	2.5	8	55
4	4	63	1.0	9	76
5	5	75	0.5	5	31
6	6	82	2.0	5	27
7	7	70	1.0	6	35
8	8	90	2.5	6	47
9	9	49	0.0	9	59
10	10	72	3.0	8	50
11	11	67	2.0	7	50
12	12	100	2.5	7	46
13	13	75	1.5	4	33
14	14	58	3.0	8	59
15	15	58	1.5	6	40
16	16	55	0.0	8	58
17	17	80	1.0	7	55
18	18	55	2.0	10	76
19	19	44	0.5	9	66
20	20	62	1.0	6	43
21	21	60	1.0	6	48
22	22	75	2.5	7	50
23	23	77	1.5	5	29
24	24	80	2.5	11	64
25	25	68	3.0	9	61
26	26	71	2.5	10	71
27	27	90	1.5	4	26
28	28	80	2.0	3	27
29	29	70	0.0	9	56
30	30	58	2.5	8	57
31	31	88	1.0	3	22
32	32	68	0.5	5	37
33	33	60	0.5	6	44
34	34	90	3.0	5	45
35	35	79	1.5	8	53
36	36	90	2.0	4	29

⑩

OUTPUT 10.2 SAS Output for Example 10.2 (continued)

Multiple Linear Regression Example 10.2: Recovery in Pediatric Dehydration					
The REG Procedure					
Number of Observations Read 36					
Number of Observations Used 36					
Correlation 11					
Variable	dose	age	wt	y	
dose	1.0000	0.1625	0.1640	0.3595	
age	0.1625	1.0000	0.9367	-0.4652	
wt	0.1640	0.9367	1.0000	-0.5068	
y	0.3595	-0.4652	-0.5068	1.0000	
The REG Procedure 12					
Model: MODEL1					
Dependent Variable: y					
Number of Observations Read 36					
Number of Observations Used 36					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2667.66870	889.22290	9.03	0.0002
Error	32	3151.22019	98.47563		
Corrected Total	35	5818.88889			
Root MSE	9.92349	R-Square	0.4584	15	
Dependent Mean	71.55556	Adj R-Sq	0.4077		
Coeff Var	13.86823				
Parameter Estimates					
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr> t Type I SS
Intercept	1	85.47636	5.96528	14.33	<.0001 184327
dose	1	6.16969	1.79081	3.45	0.0016 752.15170
age	1	0.27695	2.28474	0.12	0.9043 638.51013
wt	1	-0.54278	0.32362	-1.68	0.1032 277.00687
Parameter Estimates					
Variable	DF	Type II SS	Variance Inflation		
Intercept	1	20219	0		
dose	1	1168.84352	1.02833		
age	1	1.44701	8.16330		
wt	1	277.00687	8.16745		

OUTPUT 10.2 SAS Output for Example 10.2 (continued)

Multiple Linear Regression					
Example 10.2: Recovery in Pediatric Dehydration					
The REG Procedure					
Model: MODEL1					
Dependent Variable: y					
Collinearity Diagnostics (intercept adjusted) 21					
Number	Eigenvalue	Condition Index	Proportion of Variation---		
			dose	age	wt
1	1.99054	1.00000	0.02518	0.02918	0.02918
2	0.94617	1.45044	0.97480	0.00337	0.00330
3	0.06329	5.60804	0.00002134	0.96745	0.96752
Model: MODEL2					
Dependent Variable: y					
Number of Observations Read 36					
Number of Observations Used 36					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	752.15170	752.15170	5.05	0.0313
Error	34	5066.73719	149.02168		
Corrected Total	35	5818.88889			
Root MSE 12.20744 R-Square 0.1293 16					
Dependent Mean 71.55556 Adj R-Sq 0.1037					
Coeff Var 17.06009					
Parameter Estimates					
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > t
Intercept	1	63.89576	3.97040	16.09	<.0001
dose	1	4.88058	2.17242	2.25	0.0313

OUTPUT 10.2 SAS Output for Example 10.2 (continued)

Multiple Linear Regression Example 10.2: Recovery in Pediatric Dehydration					
The REG Procedure Model: MODEL3					
Dependent Variable: y					
Number of Observations Read 36					
Number of Observations Used 36					
17					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2390.66183	1195.33092	11.51	0.0002
Error	33	3428.22706	103.88567		
Corrected Total	35	5818.88889			
Root MSE		10.19243	R-Square	0.4108	19
Dependent Mean		71.55556	Adj R-Sq	0.3751	
Coeff Var		14.24408			
Parameter Estimates					
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > t
Intercept	1	84.07228	6.06631	13.86	<.0001
dose	1	6.06709	1.83827	3.30	0.0023
age	1	-3.30580	0.83240	-3.97	0.0004
Model: MODEL4					
Dependent Variable: y					
Number of Observations Read 36					
Number of Observations Used 36					
18					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2666.22169	1333.11084	13.95	<.0001
Error	33	3152.66720	95.53537		
Corrected Total	35	5818.88889			
Root MSE		9.77422	R-Square	0.4582	20
Dependent Mean		71.55556	Adj R-Sq	0.4254	
Coeff Var		13.65962			
Parameter Estimates					
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > t
Intercept	1	85.59416	5.79705	14.77	<.0001
dose	1	6.17526	1.76329	3.50	0.0013
wt	1	-0.50610	0.11307	-4.48	<.0001

10.4 Details & Notes

- **10.4.1** In *simple linear regression*, the hypothesis of ‘zero regression slope’ is equivalent to the hypothesis that the covariate is not an important predictor of response. The covariate, x , can be viewed as an ANOVA model factor with 1 degree of freedom with the following ANOVA summary:

ANOVA

SOURCE	df	SS	MS	F
X	1	SSX	MSX	$F_x = MSX/MSE$
Error	$n-2$	SSE	MSE	
Total	$n-1$	TOT(SS)		

The SSX and MSX can be computed as

$$SSX = MSX = \frac{S_{xy}^2}{S_{xx}}$$

The F-value for the null hypothesis that the covariate is unimportant is $F = MSX/MSE$, with 1 upper and $n-2$ lower degrees of freedom. Algebraically, this is equivalent to the square of the *t-test* for the ‘zero slope’ hypothesis because

$$F = \frac{MSX}{MSE} = \frac{\left(\frac{S_{xy}}{S_{xx}}\right)^2}{s^2} = \frac{\left(\frac{S_{xy}}{S_{xx}}\right)^2}{s^2/S_{xx}} = \left(\frac{b}{s/\sqrt{S_{xx}}}\right)^2 = t^2$$

The F-value for the covariate effect in Example 10.1 is seen in Output 10.1 to be 5.74 $\textcircled{7}$, which is the square of the t-value (-2.40).

An ANOVA table can be prepared in a similar manner for each model effect, x_i , in a *multiple linear regression* analysis. There is 1 degree of freedom for each new parameter (β_i), so that the error degrees of freedom decrease by 1 for each explanatory variable.

- **10.4.2** Another equivalent method of making inferences about the covariate effect in *simple linear regression* is with the correlation coefficient. A positive regression slope indicates a positive correlation, i.e., y increases as x increases. A negative slope indicates a negative correlation, i.e., y decreases as x increases. The Pearson-product-moment correlation coefficient is often used as a measure of the degree of linear correlation between two variables, x and y . The correlation coefficient, ρ , is a unitless measure between -1 and +1. A correlation of +1 indicates a perfect positive correlation, a correlation of -1

indicates a perfect negative correlation, and a 0 indicates no linear correlation. The population parameter, ρ , is estimated by the sample correlation coefficient, r

$$r = \frac{S_{xy}}{\sqrt{S_{xx} \cdot S_{yy}}}$$

A *t-test* for significant linear correlation is

null hypothesis:	$H_0: \rho = 0$
alt. hypothesis:	$H_A: \rho \neq 0$

test statistic:	$t = \frac{r \cdot \sqrt{n-2}}{\sqrt{1-r^2}}$
------------------------	---

decision rule:	reject H_0 if $ t > t_{\alpha/2, n-2}$
-----------------------	---

Expressing the slope b , the standard deviation s , and the correlation coefficient r , in terms of S_{xx} , S_{yy} , and S_{xy} , it is easy to show that the *t-test* based on the correlation is identical to the *t-test* based on the slope. Notice the equivalency of the hypotheses, $H_0: \beta = 0$ and $H_0: \rho = 0$.

The correlation coefficient for Example 10.1 is

$$r = -558 / (55.2(23,320))^{1/2} = -0.492$$

The t-statistic for significant correlation is

$$t = \frac{(-0.492) \cdot \sqrt{18}}{\sqrt{1 - (-0.492)^2}} = -2.4$$

which is the same t-value computed from the slope estimate b . The SAS output for Example 10.1 prints r^2 ($-0.492^2 = 0.242$) ❸ (R-Square), which is a measure of goodness-of-fit.

- **10.4.3** The coefficient of determination for a *multiple linear regression* model, R^2 , represents the percentage reduction in error variability by using the explanatory variables as predictors of y over just using the mean, \bar{y} . Thus, you can write

$$R^2 = (TSS - SSE) / TSS$$

where TSS is the total sum of squares. In this case, the SSE is a measure of error variation associated with the ‘full’ model, which includes all explanatory variables, and TSS is a measure of error variation associated with the ‘reduced’ model, which includes none of the explanatory variables. The difference is the ‘model’ sum of squares. In Output 10.2 for MODEL1, the R-Square value 0.4584 ⑯ can be found as Model SS / Total SS = 2667.67 / 5818.89.

Similarly, you can compute ‘partial’ correlation coefficients (r_j) between the response (y) and any independent variable (x_j) adjusted for all other variables in the model. The coefficient of determination associated with x_j is r_j^2 , a measure of the reduction in variability accounted for by including x_j in the model. This can be found by using the partial sum of squares, labeled Type II SS in Output 10.2, which represent the sums of squares associated with x_j adjusted for all other independent variables in the model.

If SSE represents the error sum of squares based on the ‘full’ model, which includes k explanatory variables, and SSE(j) is the error sum of squares based on the set of $k-1$ variables, which excludes x_j , then the reduction in variability due to x_j is the Type II sum of squares for x_j . Thus, $\text{SSE}(j) = \text{Type II SS}_{X_j} + \text{SSE}$, and the partial coefficient of determination can be computed as

$$r_j^2 = (\text{SSE}(j) - \text{SSE}) / \text{SSE}(j)$$

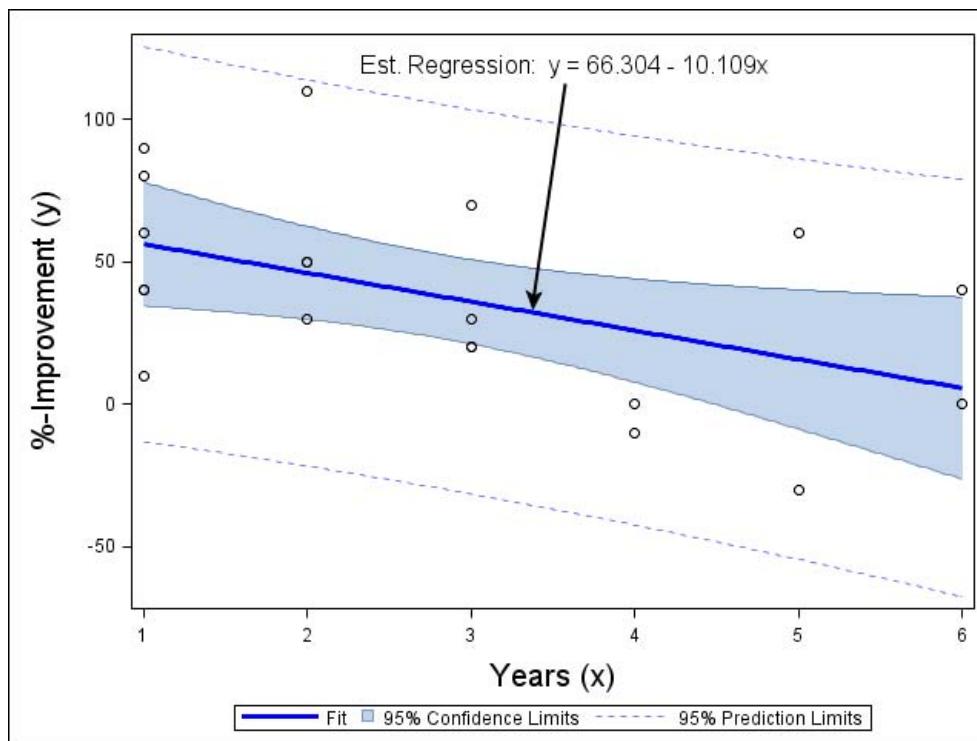
The SS1 and SS2 options in the initial MODEL statement (MODEL1) in Example 10.2 request the SAS Type I and II sums of squares. For Dose Level (DOSE), the Type II SS is 1168.8, so $\text{SSE}(1) = 1168.8 + 3151.2 = 4320.0$ and $r_1^2 = (1168.8 / 4320.0) = 0.271$.

The SAS Type I SS, sometimes called the sequential sum of squares, represent the sum of squares adjusted only for the factors preceding it in the model specification. Consequently, the sequence in which they are specified in the MODEL statement is important.

- **10.4.4** When using PROC GLM for performing a regression analysis, the Types I and III sums of squares are printed by default. In the SAS Code for Example 10.1 only Type I is requested by using SS1 in the MODEL statement because Type III usually pertains to ANOVA rather than regression analysis. In the *simple linear regression* case, SS Types I, II, and III are identical.
- **10.4.5** When estimating a mean response for a specified value of x , say x_0 , the confidence interval will be smallest when x_0 is closest to the mean, \bar{x} , because the confidence interval width is a monotonically increasing function of $(x_0 - \bar{x})^2$. Such intervals can be computed for multiple x_0 values and plotted along with the regression equation to form confidence bands. The shaded area of Figure 10.2 represents the confidence bands for Example 10.1. This plot

was generated using ODS graphics (PLOTS=ALL or PLOTS=FITPLOT) with PROC GLM along with cosmetic enhancements using the ODS Graphics editor.

FIGURE 10.2 95% Confidence Bands for Example 10.1



- **10.4.6** For a particular value of x , say x_p , a 95% ‘prediction interval’ for the predicted response of a future observation is given by

$$\hat{y}_{x_p} \pm t_{0.025,n-2} \cdot s \sqrt{1 + \frac{1}{n} + \frac{(x_p - \bar{x})^2}{S_{xx}}}$$

where $\hat{y}_{x_p} = a + b x_p$

Prediction bands can be established around the prediction equation in a way that is similar to that described for confidence bands in Section 10.4.5. These are shown in Figure 10.2 (dashed lines).

- **10.4.7** The prediction equation can be used to estimate the response, y , for any value of the predictor, x , within the experimental region. The experimental region consists of the range of x -values from the data used to compute the regression estimates. Predicting a response, y , for a value of x outside the experimental region is called ‘extrapolation’. Extrapolation to the point x_e should not be used unless it can be safely assumed that x and y have the same linear relationship outside the experimental region at point x_e . Extrapolation should be avoided if there is any uncertainty about this assumption.

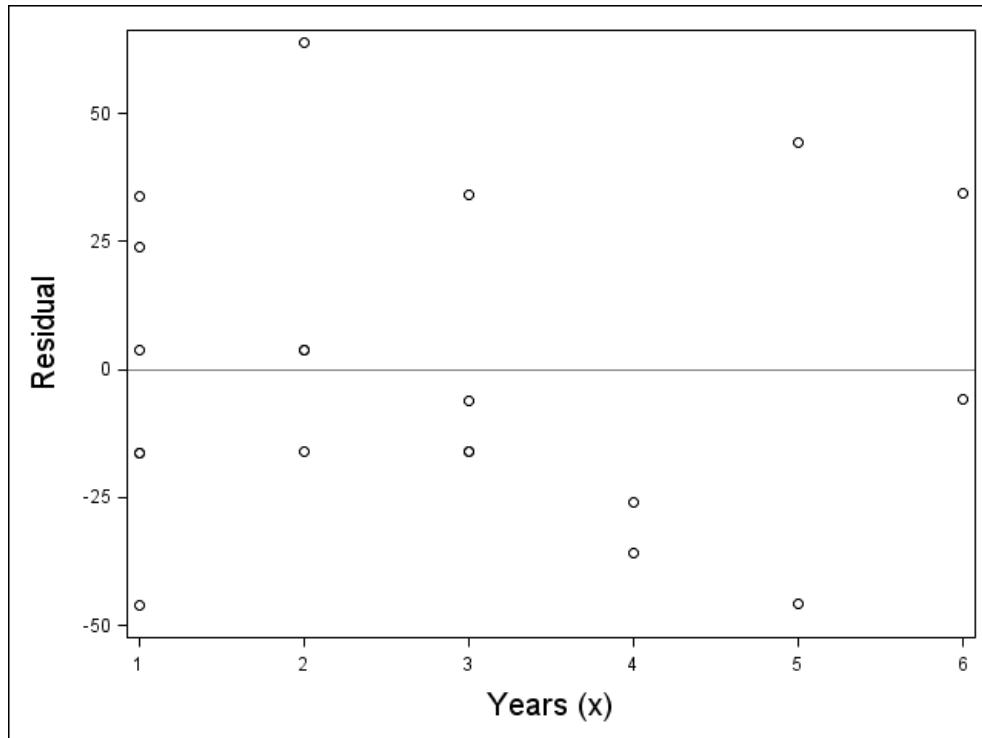
- **10.4.8** The P and CLM options can be used in PROC REG with *multiple linear regression* models to obtain predicted values of the response variable and 95% confidence intervals, in the same way as illustrated in the *simple linear regression* case (Example 10.1).
- **10.4.9** A significant correlation implies a significant linear relationship between x and y but does not imply causality. Conversely, a non-significant correlation does not imply that there is no causal (or other) relationship between x and y. It may be that no relationship is detectable due to low power of the test, or if there is a relationship, it may be concomitant rather than causal.

If you do not find a linear relationship, there might in fact be a quadratic or parabolic relationship, or some other relationship between x and y resulting in the finding of ‘no *linear* correlation’. It is always a good idea to plot the data to obtain a visual impression of any relationship. Using SAS, you can quickly obtain a number of plots which are helpful in diagnosing the adequacy of the fit simply by using the FitPlots and Diagnostics panels in ODS Graphics (see Section 10.4.10). To get these plots, simply include the following code to request all relevant default ODS graphs which include the fit and diagnostic plots:

```
ods graphics on;
proc glm plots = all;
<more SAS statements>
run;
ods graphics off;
```

- **10.4.10** One of the assumptions needed to perform regression analysis is a constant error variance over the experimental region. To investigate the validity of this assumption, you can look for a random pattern in the plot of the residuals (i.e., the difference between the observed and predicted values) against the x-values. A random appearance of the residuals above and below the horizontal zero line is consistent with variance homogeneity, as shown for Example 10.1 in Figure 10.3. This plot, a default plot using the ODS Graphics PLOTS=ALL or PLOTS=RESIDUALS option, shows no obvious pattern in the residuals across the experimental region. If the residuals appear to increase or decrease with x, or show some other non-random pattern, the assumption of variance homogeneity might be violated.

FIGURE 10.3 Residuals Plot for Example 10.1



It is also important to rule out an incorrect model specification. For example, if you try to fit a simple linear regression model to non-linear data, the residuals may be much larger within certain portions of the experimental region. Your graphical regression diagnostics can also include a plot of the residuals or standardized residuals vs. the predicted values of the response as an aid to help check the goodness of fit. Here too, a non-random pattern of residuals would indicate a poor fit. The standardized residual is the residual divided by its standard error, sometimes referred to as the ‘Studentized residual’. It is usually much easier to detect patterns using these plots than a simple plot of the data superimposed over the regression line as in Figure 10.2. These plots are automatically created with the ODS Graphics diagnostics panel (PLOTS=ALL or PLOTS=DIAGNOSTICS) when using SAS modeling procedures, as shown for Example 10.1 in Figure 10.4 (Plots (A) and (B)). Both of these plots show a random pattern of residuals indicating no obvious concerns regarding the model fit.

This same diagnostics panel can help you determine if the residuals are consistent with the assumption of normality. Plot (D) shows the residuals plotted vs. the quantiles from a normal distribution. A pattern of deviations of the residuals from the 45-degree line would indicate a departure from normality. The histogram of the residuals superimposed with a normal curve is also plotted (Plot (G)). Neither of these plots suggests any notable departures from normality in the residuals.

You can also use the diagnostics panel to check for possible outliers using Plots (C) and (F). In Figure 10.4, Plot (C) shows the standardized residuals plotted against a ‘leverage’ factor, a measure of how much influence individual observations have on the analysis. Observations on this plot which have a Studentized residual greater than +2 or less than -2 (above or below the horizontal dotted lines) should be examined as possible outliers. Also, those with a leverage value greater than $2\cdot P/N$, where P =number of parameters and N =number of observations, are designated as ‘high leverage points’. A vertical dotted line is shown at $2\cdot P/N = 0.2$ in this plot since there are 2 parameters with 20 observations. Cook’s Distance, another measure of the influence an observation has on the analysis, is also plotted for each observation in Plot (F). Large values (those above the horizontal dotted line) should be examined as possible outliers.

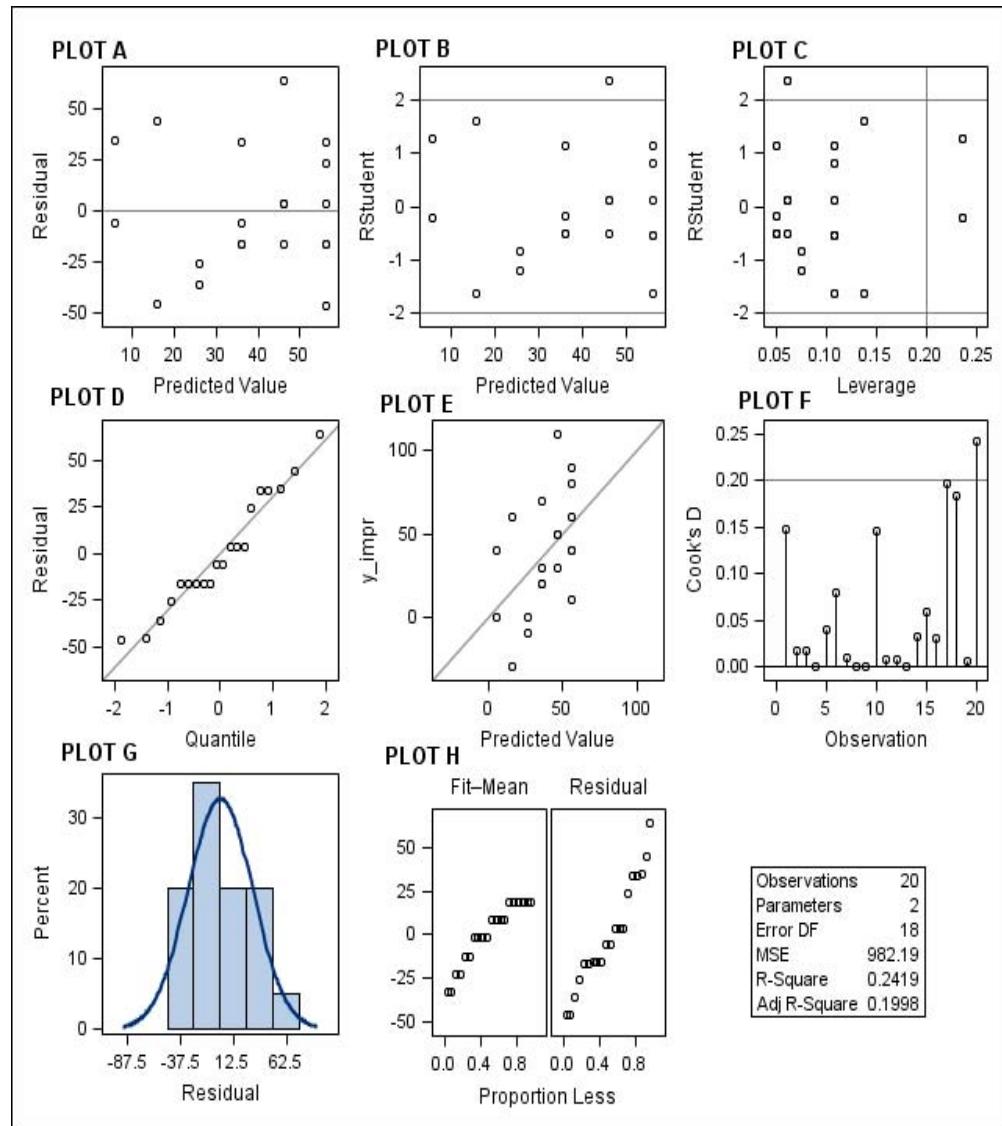
To further investigate the high influence observations identified by the graphics plots in Example 10.1, you can print out their values using the OUTPUT statement in PROC REG or PROC GLM using keywords like STUDENT for the Studentized residuals, COOKD for Cook’s Distance, etc. (see SAS Documentation for the OUTPUT statement). Adding the following code after the MODEL statement in the PROC GLM for Example 10.1 produces Output 10.3.

```
output out = outstat
      p      = Predicted
      r      = Residual
      stdr   = se_resid
      student = RStudent
      h      = Leverage
      cookd  = CooksD ;
```

The resulting output shows the original variables (pat, x_dur and y_impr), along with the predicted value for x_dur (predicted), the residual (y_impr-predicted), the standard error of the predicted value (se_resid), the Studentized residual (RStudent = residual/se_resid), the leverage factor (Leverage) and Cooks Distance (CooksD). You can see in this output that the high leverage points identified in Plot (C) are Observations #10 (RStudent=2.1052), #19 and #20 (Leverage=0.2355). Observation #20 is also flagged in Plot (F). Observations at the ends of the experimental region, such as Observations #19 and 20 ($x_{dur} = 6$), are known to have the greatest influence on the model estimation, and since there are only two at the upper end of the range, they can greatly influence the estimates of the predictor equation. These, however, would not be considered outliers. Observation #10 has a large response value (110) resulting in the highest residual value (63.9). This does not appear to be a gross outlier, however, it might be worth checking if there is any clinical significance to this.

In multiple linear regression, such as Example 10.2, ODS Graphics will show panels of plots of the residuals versus each of the regressors in the model by default. Patterns in these plots would be indications of an inadequate model.

FIGURE 10.4 Fit Diagnostics for y_impr for Example 10.1



OUTPUT 10.3 SAS Output Data Set with Diagnostics for Example 10.1

Obs	pat	x_dur	y_impr	Predicted	Residual	se_resid	RStudent	Leverage	CooksD
1	7	1	10	56.1957	-46.1957	29.5876	-1.5613	0.1087	0.1486
2	1	1	40	56.1957	-16.1957	29.5876	-0.5474	0.1087	0.0183
3	19	1	40	56.1957	-16.1957	29.5876	-0.5474	0.1087	0.0183
4	11	1	60	56.1957	3.8043	29.5876	0.1286	0.1087	0.0010
5	5	1	80	56.1957	23.8043	29.5876	0.8045	0.1087	0.0395
6	2	1	90	56.1957	33.8043	29.5876	1.1425	0.1087	0.0796
7	4	2	30	46.0870	-16.0870	30.3593	-0.5299	0.0616	0.0092
8	9	2	50	46.0870	3.9130	30.3593	0.1289	0.0616	0.0006
9	13	2	50	46.0870	3.9130	30.3593	0.1289	0.0616	0.0006
10	14	2	110	46.0870	63.9130	30.3593	2.1052	0.0616	0.1455
11	15	3	20	35.9783	-15.9783	30.5347	-0.5233	0.0507	0.0073
12	18	3	20	35.9783	-15.9783	30.5347	-0.5233	0.0507	0.0073
13	3	3	30	35.9783	-5.9783	30.5347	-0.1958	0.0507	0.0010
14	16	3	70	35.9783	34.0217	30.5347	1.1142	0.0507	0.0332
15	8	4	-10	25.8696	-35.8696	30.1240	-1.1907	0.0761	0.0584
16	12	4	0	25.8696	-25.8696	30.1240	-0.8588	0.0761	0.0304
17	17	5	-30	15.7609	-45.7609	29.1025	-1.5724	0.1377	0.1974
18	6	5	60	15.7609	44.2391	29.1025	1.5201	0.1377	0.1845
19	20	6	0	5.6522	-5.6522	27.4021	-0.2063	0.2355	0.0066
20	10	6	40	5.6522	34.3478	27.4021	1.2535	0.2355	0.2420

- **10.4.11** If fit diagnostics lead you to believe the regression assumptions may be violated, you might consider using ranked data or a normalizing or variance stabilizing transformation of the data. Popular transformations include the natural logarithm, square root, and arcsine transformation (see Appendix F).

If model fit is the issue, quadratic, cubic, and higher-order regression analyses can be performed quite easily by including the appropriate variables in the MODEL statement when using PROC GLM or PROC REG in SAS. For example, a quadratic model for Example 10.1 can be specified by including the quadratic term, x_dur*x_dur in the MODEL statement:

```
proc glm data = angina;
model y_impr = x_dur x_dur*x_dur;
```

Exponential, logarithmic and other types of non-linear regression models can also be appropriately fit using procedures like PROC NLIN (see SAS/STAT Documentation).

- **10.4.12** Another assumption of the regression procedures is that of independent observations. If x represents time, and the responses, y, represent measurements on the same experimental unit (e.g., patient) at various time points, the assumption of independence is violated. In such cases, use of a *repeated measures ANOVA* might be more appropriate (see Chapter 8).

A correlation over time is referred to as ‘autocorrelation’ in time-series analysis. In SAS, you can use the *Durbin-Watson test* to check for autocorrelation. A value close to 2, such as 1.92 ⑨ in Output 10.1 for Example 10.1, indicates no significant autocorrelation. When using PROC REG, the *Durbin-Watson test* can be requested by using the DW option in the MODEL statement to check for autocorrelation.

- **10.4.13** Diagnostics available for detecting collinearity in *multiple linear regression* models that contain a large number of independent variables include the variance inflation factor (VIF) and eigenvalue analysis of the model factors. These analyses are performed in SAS by using the VIF and COLLINOPT options in the MODEL statement in PROC REG. Very large relative values of VIF might suggest collinearity. The eigenvalue analysis is a method used in a statistical technique called *principal component analysis*, often encountered in the social sciences. The basic idea is that very small values of the eigenvalues might suggest collinearity, especially if the ‘condition index’ (also printed in the SAS output) is large, e.g., >30.

Note: For further reading, an entire chapter is devoted to a discussion of principal component analysis in *A Step-by-Step Approach to Using the SAS System for Univariate and Multivariate Statistics, Second Edition* by O’Rourke, Hatcher, and Stepanski, which was written under the SAS Press program.

In Example 10.2, the VIF (as shown in the Parameter Estimates section in Model 1 of Output 10.2), is 8.16 for both Age and Weight. These are relatively large values compared with the VIF for Dose Level (1.0), consistent with collinearity. The third eigenvalue in the Collinearity Diagnostics output²⁰ is very small, and although the condition index is not close to exceeding 30, you see that weightings for Age and Weight are large (0.967) compared with Dose. This points to evidence of a linear dependence between these two independent variables. These observations support the suspicion of collinearity between Age and Weight, and suggest that the model should avoid including both of these factors simultaneously.

- **10.4.14** PROC REG is very useful for ‘model-building’. The process of fitting various models and checking the R^2 (as shown in Example 10.2) is similar to one of the model building procedures that uses the stepwise option in PROC REG (see SAS/STAT Documentation) to help determine the best fitting model to describe the response. Forward or backwards stepwise regression techniques can be very useful in fitting a model to existing data. Great caution must be used in making inferences, however, whenever the model is data-generated.
- **10.4.15** Regression analysis has limited use in comparative clinical trials because the ‘Treatment Group’ effect is almost always a class variable. When classification variables, such as Treatment effect, are included in the same model as numeric explanatory variables, you use an analytic procedure known

as *analysis of covariance (ANCOVA)*, which is the subject of the next chapter. The concepts of regression analysis are introduced in this chapter to provide the necessary background for applying *ANCOVA* methods, which have greater use in the clinical setting.

PROC REG in SAS is very efficient for performing regression analysis and selecting appropriate models, but it cannot be used for *ANCOVA*. PROC GLM, although lacking some of the regression diagnostics available in PROC REG, can be used for regression, in addition to *ANOVA* and *ANCOVA*.

Note: For more details about regression analysis using PROC REG in SAS, see *SAS System for Regression, Third Edition*, by Freund and Littell, which was written under the SAS Press program.

CHAPTER II

Analysis of Covariance

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II.1 Introduction

Analysis of covariance (ANCOVA) provides a method for comparing response means among two or more groups adjusted for a quantitative concomitant variable, or ‘covariate’, thought to influence the response. The attention here is confined to cases in which the response, y , might be linearly related to the covariate, x . *ANCOVA* combines regression and *ANOVA* methods by fitting simple linear regression models within each group and comparing regressions among groups.

ANCOVA methods represent one of the most widely used statistical methods in clinical trials. Examples where *ANCOVA* might be applied include:

- comparing cholesterol levels (y) between a treated group and a reference group adjusted for age (x , in years)
- comparing scar healing (y) between conventional and laser surgery adjusted for excision size (x , in mm)
- comparing exercise tolerance (y) in 3 dose levels of a treatment used for angina patients adjusted for smoking habits (x , in cigarettes/day).

ANCOVA can often increase the precision of comparisons of the group means by including the covariate, x , as a source of variation, thereby decreasing the estimated error variance. *ANCOVA* is especially useful when the values of the covariate differ among the groups. When this occurs and the response is linearly related to the covariate, covariance adjustments can lead to markedly different conclusions than those obtained using the unadjusted *ANOVA* methods.

11.2 Synopsis

Suppose you have k groups with n_i independent x - y points in Group i ($i = 1, 2, \dots, k$), as shown in Table 11.1.

TABLE 11.1 ANCOVA Layout

GROUP 1		GROUP 2		...	GROUP k	
X	y	x	y		x	y
X_{11}	y_{11}	X_{21}	y_{21}	...	X_{k1}	y_{k1}
X_{12}	y_{12}	X_{22}	y_{22}	...	X_{k2}	y_{k2}
X_{13}	y_{13}	X_{23}	y_{23}	...	X_{k3}	y_{k3}
...
X_{1n_1}	y_{1n_1}	X_{2n_2}	y_{2n_2}	...	X_{kn_k}	y_{kn_k}

Using the linear regression methods discussed in Chapter 10, you can model the response, y , as a linear function of the covariate, x , with slope β for each of the k groups. We will assume that the regression slopes are the same for each group, i.e., $\beta_1 = \beta_2 = \dots = \beta_k = \beta$. As with *ANOVA*, *ANCOVA* assumes independent groups with a normally distributed response measure (y) and variance homogeneity. The mean response within each group depends on the covariate, which results in a model for the i^{th} group mean as

$$\mu_i = \alpha_i + \beta x$$

For each group, you can compute \bar{x} , \bar{y} , S_{xx} , S_{yy} , and S_{xy} using the formulas given in Chapter 10. Let \bar{x}_i , \bar{y}_i , $S_{xx(i)}$, $S_{yy(i)}$, and $S_{xy(i)}$ represent these quantities, respectively, for Group i ($i = 1, 2, \dots, k$). Also, compute S_{xx} , S_{yy} , and S_{xy} for all groups combined (ignoring group). The estimated regression line for Group i is

$$\hat{y} = a_i + b x$$

where a_i and b are the Least Squares estimates computed as

$$b = \frac{\sum_i S_{xy(i)}}{\sum_i S_{xx(i)}}$$

and

$$a_i = \bar{y}_i - b \bar{x}$$

With $N = n_1 + n_2 + \dots + n_k$, the ANOVA table summarizing the significance of the sources of variation is shown in Table 11.2.

TABLE 11.2 ANOVA Summary Table for a Simple ANCOVA Model

SOURCE	df	SS	MS	F
GROUP	$k-1$	SSG	MSG	$F_G = MSG/MSE$
X (covariate)	1	SSX	MSX	$F_X = MSX/MSE$
Error	$N-k-1$	SSE	MSE	
Total	$N-1$	TOT(SS)		

The sums of squares (SS) can be found from the following computing formulas:

$$TOT(SS) = S_{yy}$$

$$SSE = \frac{\left(\sum_i S_{xx(i)} \right) \cdot \left(\sum_i S_{yy(i)} \right) - \left(\sum_i S_{xy(i)} \right)^2}{\left(\sum_i S_{xx(i)} \right)}$$

$$SSG = \frac{S_{xx} \cdot S_{yy} - S_{xy}^2}{S_{xx}} - SSE$$

$$SSX = \sum_i S_{yy(i)} - SSE$$

Notice that when $k=1$ (one group), $SSG = 0$ and the computing formulas are identical to those used for *linear regression* in Chapter 10.

The mean squares (MS) are the sum of squares (SS) divided by the degrees of freedom (df). Of primary interest is the comparison of the group means adjusted to a common value of the covariate, e.g., x_0 . Letting μ_{i0} represent the mean of Group i for $x = x_0$, the test can be summarized as follows:

null hypothesis:	$H_0: \mu_{10} = \mu_{20} = \dots = \mu_{k0}$
alt. hypothesis:	$H_A: \text{NOT } H_0$
test statistic:	$F_G = MSG / MSE$
rejection region:	reject H_0 if $F_G > F_{N-k-1}^{k-1}(\alpha)$

This hypothesis is equivalent to the equality of intercepts among groups,

$$H_0: \alpha_1 = \alpha_2 = \dots = \alpha_k$$

and the test does not depend on the value of x_0 .

This test might result in improved precision for the group means comparisons over *one-way ANOVA* methods if the slope, β , differs from 0. To determine whether the covariate has a significant effect on the response, use the F_X ratio to test for 0 regression slope using the following test summary:

null hypothesis:	$H_0: \beta = 0$
alt. hypothesis:	$H_A: \beta \neq 0$
test statistic:	$F_X = MSX / MSE$
rejection region:	reject H_0 if $F_X > F_{N-k-1}^1(\alpha)$

11.3 Examples

Example 11.1—Triglyceride Changes Adjusted for Glycemic Control

The new cholesterol-lowering supplement, Fibralo, was studied in a double-blind study against the marketed reference supplement, Gemfibrozil, in 34 non-insulin dependent diabetic (NIDDM) patients. One of the study's objectives was to compare the mean decrease in triglyceride levels between groups. The degree of glycemic control, measured by hemoglobin A_{1c} levels (HbA_{1c}), was thought to be an important factor in response to the treatment. This covariate was measured at the start of the study and is shown in Table 11.3, with the percent changes in triglycerides from pre-treatment to the end of the 10-week trial. Is there a difference in mean responses between supplements?

TABLE 11.3 Raw Data for Example 11.1

FIBRALO GROUP			GEMFIBROZIL GROUP		
Patient Number	HbA _{1c} ng/ml (X)	Triglyceride % Change (Y)	Patient Number	HbA _{1c} ng/ml (X)	Triglyceride % Change (Y)
2	7.0	5	1	5.1	10
4	6.0	10	3	6.0	15
7	7.1	-5	5	7.2	-15
8	8.6	-20	6	6.4	5
11	6.3	0	9	5.5	10
13	7.5	-15	10	6.0	-15
16	6.6	10	12	5.6	-5
17	7.4	-10	14	5.5	-10
19	5.3	20	15	6.7	-20
21	6.5	-15	18	8.6	-40
23	6.2	5	20	6.4	-5
24	7.8	0	22	6.0	-10
27	8.5	-40	25	9.3	-40
28	9.2	-25	26	8.5	-20
30	5.0	25	29	7.9	-35
33	7.0	-10	31	7.4	0
			32	5.0	0
			34	6.5	-10

Solution

The goal is to compare mean triglyceride changes between groups adjusted for HbA_{1c}. To apply ANCOVA using HbA_{1c} as a covariate, first obtain some summary results from the data as shown in Table 11.4.

TABLE 11.4 Summary Results for Example 11.1

	<i>Fibralo</i> (Group 1)	<i>Gemfibrozil</i> (Group 2)	Combined
Σx	112.00	119.60	231.60
Σx^2	804.14	821.64	1625.78
Σy	-65.00	-185.00	-250.00
Σy^2	4575.00	6475.00	11050.00
Σxy	-708.50	-1506.50	-2215.00
\bar{x}	7.0000	6.6444	6.8118
\bar{y}	-4.0625	-10.2778	-7.3529
N	16	18	34

Using the formulas in Chapter 10, you compute for the *Fibralo* group (i=1):

$$S_{xx(1)} = 804.14 - (112)^2 / 16 = 20.140$$

$$S_{yy(1)} = 4575.00 - (-65)^2 / 16 = 4310.938$$

$$S_{xy(1)} = -708.50 - (112)(-65) / 16 = -253.500$$

Similarly, computing for the *Gemfibrozil* group (i=2), you obtain:

$$S_{xx(2)} = 26.964$$

$$S_{yy(2)} = 4573.611$$

$$S_{xy(2)} = -277.278$$

Finally, for the combined data (ignoring groups), compute

$$S_{xx} = 48.175$$

$$S_{yy} = 9211.765$$

$$S_{xy} = -512.059$$

The sums of squares can now be obtained as

$$TOT(SS) = 9211.8$$

$$SSE = \frac{(20.140 + 26.964)(4310.938 + 4573.611) - (-253.500 - 277.278)^2}{(20.140 + 26.964)} \\ = 2903.6$$

$$SSG = \frac{(48.175)(9211.765) - (-512.059)^2}{48.175} - 2903.6 = 865.4$$

$$SSX = (4310.938 + 4573.611) - 2903.6 = 5980.9.$$

and the ANOVA summary table can be completed as shown in Table 11.5.

TABLE 11.5 ANCOVA Summary for Example 11.1

SOURCE	df	SS	MS	F
Treatment	1	865.4	865.4	9.2 *
X (HbA _{1c})	1	5980.9	5980.9	63.8 *
Error	31	2903.6	93.7	
Total	33	9211.8		

* Significant ($p < 0.05$); critical F-value = 4.16

The F-statistics are formed as the ratios of effect mean squares (MS) to the MSE (93.7). Each F-statistic is compared with the critical F-value with 1 upper and 31 lower degrees of freedom. The critical F-value for $\alpha = 0.05$ is 4.16.

The significant covariate effect ($F=63.8$) indicates that the triglyceride response has a significant linear relationship with HbA_{1c}. The significant F-value for Treatment indicates that the mean triglyceride response adjusted for glycemic control differs between treatment groups.

Suppose you ignored the covariate and compare treatment group means using the *one-way ANOVA* (Chapter 6). You would get the following ANOVA table:

TABLE 11.6 ANOVA for Example 11.1 Ignoring Covariate

SOURCE	df	SS	MS	F
Treatment	1	327.22	327.22	1.18
Error	32	8884.55	277.64	
Total	33	9211.76		

The rejection region for the Treatment effect based on a significance level of $\alpha=0.05$ includes F-values greater than 4.15, the critical F-value with 1 upper and 32 lower degrees of freedom. With an F-value of only 1.18, you cannot reject the hypothesis of equal means. Therefore, you must conclude that no difference in mean response between treatment groups is evident based on this test.

One reason the *ANCOVA* produces a significant treatment group difference but the *ANOVA* does not is a large reduction in MSE, (from 277.6 to 93.7). This results in greater precision of the treatment group comparison.

Another reason for this difference is the increase in the difference between means by using the adjusted values, as shown below.

You estimate the common slope, β , as

$$\begin{aligned} b &= ((-253.500) + (-277.278)) / (20.140 + 26.964) \\ &= -11.268 \end{aligned}$$

and the intercepts (a_i 's) as

$$a_1 = (-4.0625) - (-11.268)(7.0000) = 74.81$$

$$a_2 = (-10.2778) - (-11.268)(6.6444) = 64.59$$

which yield the estimated regression equations:

$$\text{Fibralo Group: } \hat{y} = 74.81 - 11.268 x$$

$$\text{Gemfibrozil: } \hat{y} = 64.59 - 11.268 x$$

The adjusted mean responses for each group are the values of the estimated regression equations evaluated at the overall mean of the covariate, $\bar{x} = 6.8118$, as follows:

$$\text{Fibralo: } 74.81 - 11.268 (6.8118) = -1.9$$

$$\text{Gemfibrozil: } 64.59 - 11.268 (6.8118) = -12.2$$

These means differ considerably from the unadjusted means already computed (Table 11.4). Table 11.7 summarizes the unadjusted and adjusted means by treatment groups.

TABLE 11.7 Means by Treatment Group for Example 11.1

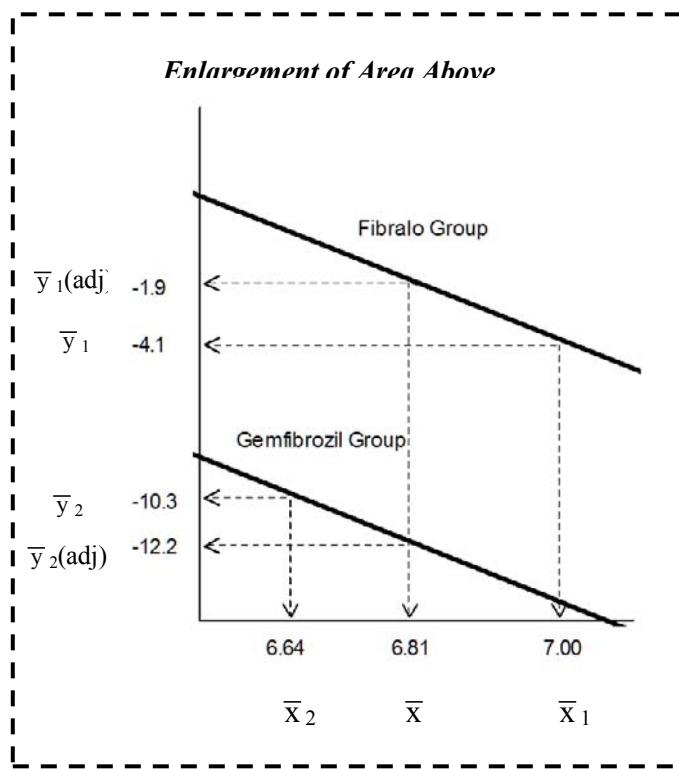
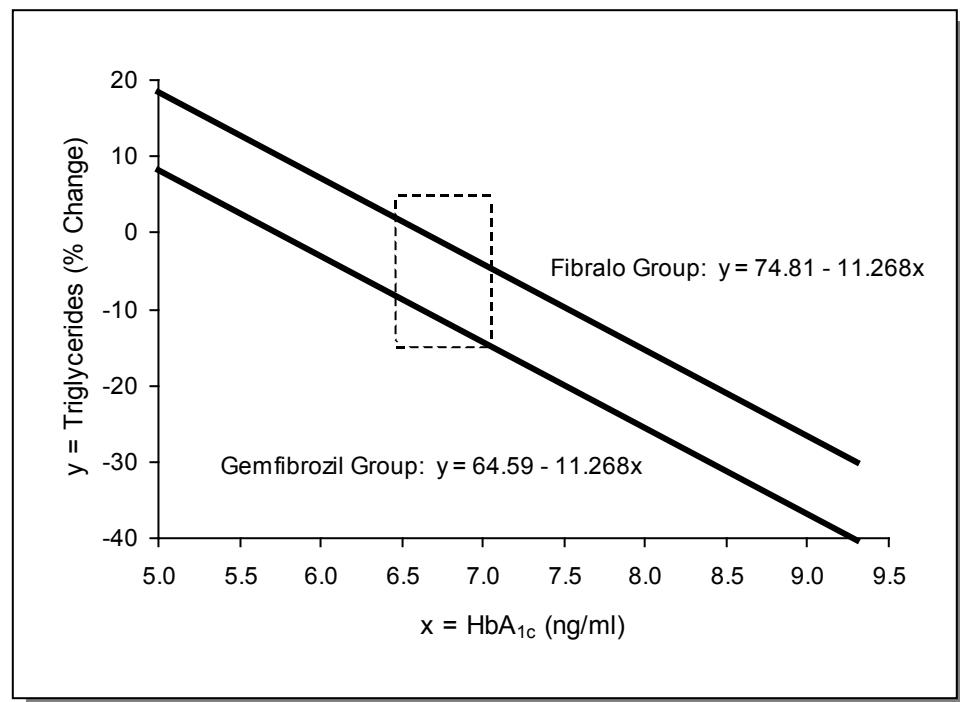
Mean Response	Fibralo Group	Gemfibrozil Group	p-value
Unadjusted	-4.1%	-10.3%	0.2858
Adjusted	-1.9%	-12.2%	0.0048 *

* Significant ($p < 0.05$)

(p-values from SAS output)

The estimated slope of $b = -11.268$ means the triglycerides decrease by an average of 11.27% for every 1 ng/ml increase in HbA_{1c}, and this rate of change is seen to be significant. Notice that (Table 11.4) the mean HbA_{1c} level is slightly higher in the *Fibralo* group compared with the *Gemfibrozil* group (7.00 v. 6.64). The unadjusted mean responses (-4.1 and -10.3) are those that lie on the regression lines that correspond to the mean of the covariate for each group, as shown in Figure 11.1. Comparing the mean responses of the two groups at the same value of the covariate ($x = 6.81$) requires using a smaller x value for the *Fibralo* group (which results in a smaller triglyceride decrease), and a larger x value for the *Gemfibrozil* group (which results in a larger triglyceride decrease). The end result is a larger difference in response means based on the adjusted values compared with the unadjusted values.

FIGURE 11.1 ANCOVA-Adjusted Means for Example 11.1



SAS Analysis of Example 11.1

The SAS code for performing this analysis is shown below followed by the SAS output (Output 11.1). The PRINT and MEANS procedures are used to provide a data listing ❶ and basic summary statistics ❷ respectively. ODS Graphics is used with the PLOTS option of PROC GLM to create a scatterplot of the data including regression lines for each treatment. ❸

PROC GLM is used to conduct the *ANCOVA*, using the Treatment (trt) effect as a class variable and hba1c as a numeric covariate. The sums of squares, mean squares, and *F-tests* ❹ all corroborate the manual calculations.

The SOLUTION option in the MODEL statement ❺ in PROC GLM obtains the estimates for the regression equations. The slope estimate, b , is -11.268 ❻. The intercept estimates are found by adding each treatment group effect to the intercept estimate:

$$a_1 = 64.59 + 10.22 = 74.81$$

and

$$a_2 = 64.59 + 0.00 = 64.59 \quad ❼$$

The LSMEANS statement instructs SAS to print out the adjusted mean responses ❽ at the common covariate mean, \bar{x} , as demonstrated in the manual calculations.

The data set is also analyzed using the *one-way ANOVA*, ignoring the covariate ❾. These results, as discussed previously, show a non-significant treatment effect ($p = 0.2858$).

SAS Code for Example 11.1

```
data tri;
  input trt $ pat hgbalc trichg @@;
  datalines;
FIB 2 7.0 5   FIB 4 6.0 10   FIB 7 7.1 -5
FIB 8 8.6 -20  FIB 11 6.3 0   FIB 13 7.5 -15
FIB 16 6.6 10  FIB 17 7.4 -10  FIB 19 5.3 20
FIB 21 6.5 -15 FIB 23 6.2 5   FIB 24 7.8 0
FIB 27 8.5 -40 FIB 28 9.2 -25  FIB 30 5.0 25
FIB 33 7.0 -10 GEM 1 5.1 10   GEM 3 6.0 15
GEM 5 7.2 -15 GEM 6 6.4 5   GEM 9 5.5 10
GEM 10 6.0 -15 GEM 12 5.6 -5  GEM 14 5.5 -10
GEM 15 6.7 -20 GEM 18 8.6 -40 GEM 20 6.4 -5
GEM 22 6.0 -10 GEM 25 9.3 -40 GEM 26 8.5 -20
GEM 29 7.9 -35 GEM 31 7.4 0   GEM 32 5.0 0
GEM 34 6.5 -10
;

proc sort data = tri;
  by trt hgbalc trichg;

/* Print data set */
proc print data = tri;
  var trt pat hgbalc trichg;
```

```

title1 'Analysis of Covariance';
title2 'Example 11.1: Triglyceride Changes Adjusted for
Glycemic Control';
run;

/* Obtain summary statistics for each group */
proc means mean std n data = tri; ❷
  by trt;
  var hgbalc trichg;
run;

/* Use glycemic control as covariate */
ods graphics on;
proc glm data = tri plots = ancovaplot; ❸
  class trt;
  model trichg = trt hgbalc / solution; ❹
    lsmeans trt/pdiff stderr cl; ❻
run;
quit;
ods graphics off;

/* Compare groups with ANOVA, ignoring the covariate */
proc glm data = tri; ❼
  class trt;
  model trichg = trt / ss3;
run;
quit;

```

OUTPUT 11.1 SAS Output for Example 11.1

Analysis of Covariance				
Example 11.1: Triglyceride Changes Adjusted for Glycemic Control				
Obs	trt	pat	hgbalc	trichg
1	FIB	30	5.0	25
2	FIB	19	5.3	20
3	FIB	4	6.0	10
4	FIB	23	6.2	5
5	FIB	11	6.3	0
6	FIB	21	6.5	-15
7	FIB	16	6.6	10
8	FIB	33	7.0	-10
9	FIB	2	7.0	5
10	FIB	7	7.1	-5
11	FIB	17	7.4	-10
12	FIB	13	7.5	-15
13	FIB	24	7.8	0
14	FIB	27	8.5	-40 ❶
15	FIB	8	8.6	-20
16	FIB	28	9.2	-25
17	GEM	32	5.0	0
18	GEM	1	5.1	10
19	GEM	14	5.5	-10
20	GEM	9	5.5	10
21	GEM	12	5.6	-5
22	GEM	10	6.0	-15
23	GEM	22	6.0	-10
24	GEM	3	6.0	15
25	GEM	20	6.4	-5

OUTPUT 11.1 SAS Output for Example 11.1 (continued)

26	GEM	6	6.4	5
27	GEM	34	6.5	-10
28	GEM	15	6.7	-20
29	GEM	5	7.2	-15
30	GEM	31	7.4	0
31	GEM	29	7.9	-35
32	GEM	26	8.5	-20
33	GEM	18	8.6	-40
34	GEM	25	9.3	-40

The MEANS Procedure

----- trt=FIB -----

Variable	Mean	Std Dev	N
hgbalc	7.0000000	1.1587349	16
trichg	-4.0625000	16.9527530	16

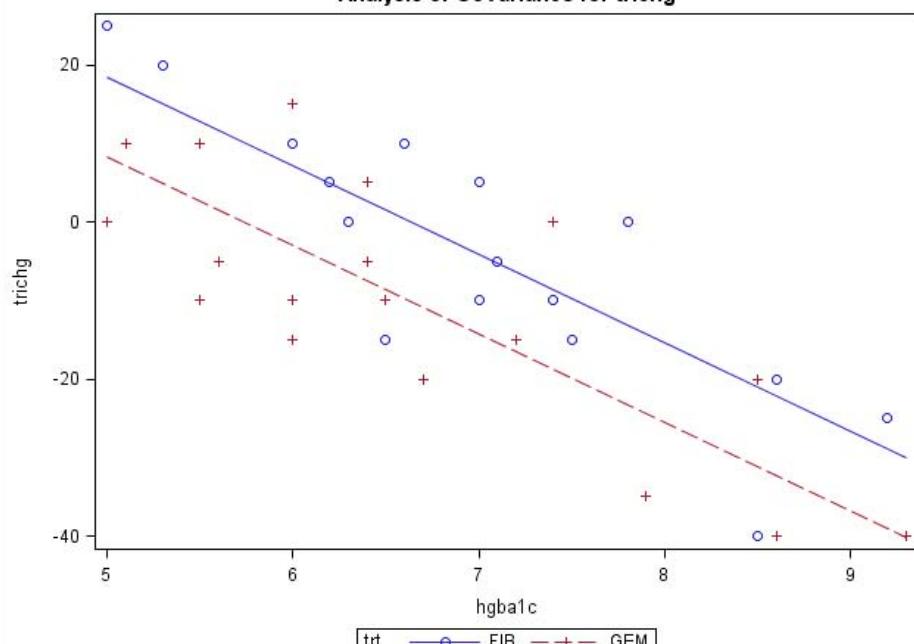
②

----- trt=GEM -----

Variable	Mean	Std Dev	N
hgbalc	6.6444444	1.2594220	18
trichg	-10.2777778	16.4023153	18

③

Analysis of Covariance for trichg



OUTPUT 11.1 SAS Output for Example 11.1 (continued)

Analysis of Covariance																																			
Example 11.1: Triglyceride Changes Adjusted for Glycemic Control																																			
The GLM Procedure																																			
Class Level Information																																			
<table> <thead> <tr> <th>Class</th> <th>Levels</th> <th>Values</th> </tr> </thead> <tbody> <tr> <td>trt</td> <td>2</td> <td>FIB GEM</td> </tr> </tbody> </table>						Class	Levels	Values	trt	2	FIB GEM																								
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NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.																																			
<table> <thead> <tr> <th colspan="6">Least Squares Means</th> </tr> <tr> <th></th> <th>trichg</th> <th>Standard Error</th> <th>H0:LSMEAN=0</th> <th colspan="2">H0:LSMean1=LSMean2</th> </tr> <tr> <th>trt</th> <th>LSMEAN</th> <th></th> <th>Pr > t </th> <th colspan="2">Pr > t </th> </tr> </thead> <tbody> <tr> <td>FIB</td> <td>-1.9414451</td> <td>2.4340646</td> <td>⑪ 0.4312</td> <td colspan="2">0.0048</td> </tr> <tr> <td>GEM</td> <td>-12.1631599</td> <td>⑧ 2.2933414</td> <td><.0001</td> <td colspan="2"></td> </tr> </tbody> </table>						Least Squares Means							trichg	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2		trt	LSMEAN		Pr > t	Pr > t		FIB	-1.9414451	2.4340646	⑪ 0.4312	0.0048		GEM	-12.1631599	⑧ 2.2933414	<.0001		
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OUTPUT 11.1 SAS Output for Example 11.1 (continued)

```
Analysis of Covariance
Example 11.1: Triglyceride Changes Adjusted for Glycemic Control

The GLM Procedure

          trichg
trt      LSMEAN      95% Confidence Limits
FIB      -1.941445     -6.905753     3.022862
GEM      -12.163160    -16.840461    -7.485859

Least Squares Means for Effect trt

Difference
Between      95% Confidence Limits for
i       j      Means      LSMean(i)-LSMean(j)
1       2      10.221715      3.362960      17.080469

Class Level Information

      Class      Levels      Values
trt          2      FIB GEM

      Number of Observations Read      34
      Number of Observations Used      34

Dependent Variable: trichg
      Sum of
Source      DF      Squares      Mean Square      F Value      Pr > F
Model        1      327.216095     327.216095      1.18      0.2858
Error       32      8884.548611    277.642144
Corrected Tot 33      9211.764706

R-Square      Coeff Var      Root MSE      trichg Mean
0.035522      -226.6113      16.66260      -7.352941

Source      DF      Type III SS      Mean Square      F Value      Pr > F
trt         1      327.2160948     327.2160948      1.18      0.2858
```

9

For Example 11.1, you could use PROC MIXED and get the identical results as those shown for PROC GLM. The next example illustrates PROC MIXED for ANCOVA using multiple numeric covariates with multiple classification factors.

Example 11.2—ANCOVA in Multi-Center Hypertension Study

The experimental anti-hypertensive agent, GB2995, was studied in 4 study centers to compare 12 weeks of treatment using GB2995 as the sole therapy vs. using GB2995 in combination with a stable dose of a marketed calcium-channel blocker when given to patients with moderate to severe hypertension. The primary analysis focuses on a comparison of the mean decreases in diastolic blood pressure between the two treatment groups, adjusted for the patient's age and severity of hypertension at study entry. (Baseline severity is the average diastolic blood pressure obtained on three pre-study visits). Do the data in Table 11.8 show any difference in response between the two treatment groups?

TABLE 11.8 Raw Data for Example 11.2

Sole Therapy				Combination Therapy			
Patient Number*	AGE (yrs.)	Baseline DIA BP (mm Hg)	DIA BP Change (mm Hg)	Patient Number*	AGE (yrs.)	Baseline DIA BP (mm Hg)	DIA BP Change (mm Hg)
101	55	102	-6	102	68	112	-10
103	68	115	-10	105	64	105	-4
104	45	97	-2	107	48	107	-9
106	69	107	-5	108	60	107	-5
109	54	115	-7	201	44	109	-7
202	58	99	-4	205	53	99	0
203	62	119	-11	207	48	107	-9
204	51	107	-9	209	65	106	-3
206	47	96	0	210	79	108	-6
208	61	98	-6	213	45	110	-9
211	40	110	5	215	44	93	0
212	36	103	-3	218	62	99	-5
214	58	109	10	220	59	119	-14
216	64	119	-12	221	50	104	-6
217	55	104	4	222	61	107	-14
219	54	97	-5	224	36	95	3
223	39	95	-8	225	34	95	-9
301	49	115	-3	303	49	115	-8
302	46	105	4	305	57	115	7
304	59	116	-10	306	58	116	-20
307	42	108	-5	309	43	108	-7
308	65	101	-7	310	66	101	-5
311	68	102	-5	312	55	102	-9
313	57	110	-8	314	70	110	-16
402	73	119	-12	315	52	104	-8
404	48	99	-7	401	42	119	-15
406	53	117	0	403	49	109	-6
407	46	96	4	405	53	117	4

*First digit indicates study center

Sole Therapy				Combination Therapy			
Patient Number*	AGE (yrs.)	Baseline DIA BP (mm Hg)	DIA BP Change (mm Hg)	Patient Number*	AGE (yrs.)	Baseline DIA BP (mm Hg)	DIA BP Change (mm Hg)
311	68	102	-5	312	55	102	-9
313	57	110	-8	314	70	110	-16
402	73	119	-12	315	52	104	-8
404	48	99	-7	401	42	119	-15
406	53	117	0	403	49	109	-6
407	46	96	4	405	53	117	4
409	60	118	-15	408	55	96	6
412	66	104	-3	410	65	98	-8
414	59	115	-4	411	75	108	-7
415	41	109	3	413	59	104	-10
418	53	116	-10	416	60	115	-16
421	57	100	-8	417	53	109	3
424	52	103	0	419	43	96	-14
425	41	95	9	420	77	100	-5

*First digit indicates study center

SAS Analysis of Example 11.2

The SAS code and output for this example are shown on the following pages. The PRINT and MEANS procedures provide a (partial) data listing ⑬ and summary statistics by treatment group ⑭. Combination therapy (COMB) shows an unadjusted mean decrease in diastolic blood pressure of 6.82 mm Hg, compared with a decrease of 4.06 mm Hg for sole therapy (SOLE) (see Output 11.2 for “The MEANS Procedure”).

PROC MIXED is used to conduct an *ANCOVA* using Treatment Group (trt) and Study Center (center) as variables in the CLASS statement ⑮. Age (age) and baseline diastolic blood pressure (bpdia0) are used as numeric covariates by including them in the MODEL statement but omitting them from the CLASS statement. The MODEL statement corresponds to a main effects model that ignores any interactions. For now, both Treatment and Center are considered fixed effects. The linear model used in this analysis can be written as

$$y_{ij} = \alpha + \tau_i + \beta_j + \gamma_1 x_1 + \gamma_2 x_2 + \varepsilon$$

where y_{ij} is the response measurement (change in diastolic blood pressure) for Treatment i ($i=1$ or 2) and Center j ($j=1, 2, 3$, or 4), α is the overall mean response, τ_i is the effect of Treatment i , β_j is the effect of Center j , x_1 is age (in years), x_2 is baseline diastolic blood pressure (in mm Hg), γ_k is the increase in response associated with a one-unit change in x_k ($k=1$ or 2), and ε represents the random error component.

This analysis assumes that if the response is linearly related to the covariates, then that same linear relationship exists within each subgroup formed by combinations of levels of the class variables. This assumption can be tested by including interactions such as trt*age or trt*bpdia0 in the model (see Section 11.4.7). A preliminary test for a Treatment-by-Center interaction can also be made before proceeding with this model.

SAS Code for Example 11.2

```

data bp;
    input trt $ pat age bpdia0 bpdiaach @@;
    center = int(pat/100);
    datalines;
SOLE 101 55 102 -6    SOLE 103 68 115 -10
SOLE 104 45 97 -2    SOLE 106 69 107 -5
SOLE 109 54 115 -7    SOLE 202 58 99 -4

    ... (more data lines) ...

COMB 422 55 103 -8    COMB 423 66 115 -14
COMB 426 38 105 -3
;

proc print data = bp; 13
    title1 'Analysis of Covariance';
    title2 'Example 11.2: ANCOVA in Multi-Center Hypertension
Study';
run;

proc sort data = bp;
    by trt center;

proc means mean std n min max data = bp; 14
    by trt;
    var age bpdia0 bpdiaach;
run;

proc mixed data = bp; 15
    classes trt center;
    model bpdiaach = trt center age bpdia0 / solution;
    lsmeans trt / diff;
run;

```

OUTPUT 11.2 SAS Output for Example 11.2

Analysis of Covariance Example 11.2: ANCOVA in Multi-Center Hypertension Study						
Obs	trt	pat	age	bpdia0	bpdiach	center
1	SOLE	101	55	102	-6	1
2	SOLE	103	68	115	-10	1
3	SOLE	104	45	97	-2	1
4	SOLE	106	69	107	-5	1
5	SOLE	109	54	115	-7	1
6	SOLE	202	58	99	-4	2
7	SOLE	203	62	119	-11	2
8	SOLE	204	51	107	-9	2
9	SOLE	206	47	96	0	2
10	SOLE	208	61	98	-6	2
11	SOLE	211	40	110	5	2
12	SOLE	212	36	103	-3	2
13	SOLE	214	58	109	10	2
14	SOLE	216	64	119	-12	2
15	SOLE	217	55	104	4	2
.						
.						
.						
70	COMB	417	53	109	3	4
71	COMB	419	43	96	-14	4
72	COMB	420	77	100	-5	4
73	COMB	422	55	103	-8	4
74	COMB	423	66	115	-14	4
75	COMB	426	38	105	-3	4

⑬

Analysis of Covariance Example 11.2: ANCOVA in Multi-Center Hypertension Study						
The MEANS Procedure						
----- trt=COMB -----						
Variable	Mean	Std Dev	N	Minimum	Maximum	
age	55.3846154	11.0706235	39	34.0000000	79.0000000	
bpdia0	106.3333333	7.0012530	39	93.0000000	119.0000000	
bpdiach	-6.8205128	6.2318021	39	-20.0000000	7.0000000	
----- trt=SOLE -----						
Variable	Mean	Std Dev	N	Minimum	Maximum	
age	54.1944444	9.4923110	36	36.0000000	73.0000000	
bpdia0	106.6666667	7.9677923	36	95.0000000	119.0000000	
bpdiach	-4.0555556	5.9997354	36	-15.0000000	10.0000000	

⑭

OUTPUT 11.2 SAS Output for Example 11.2 (continued)

Analysis of Covariance
Example 11.2: ANCOVA in Multi-Center Hypertension Study

The Mixed Procedure

Model Information

Data Set	WORK.BP
Dependent Variable	bpdiach
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information

Class	Levels	Values
trt	2	COMB SOLE
center	4	1 2 3 4

Dimensions

Covariance Parameters	1
Columns in X	9
Columns in Z	0
Subjects	1
Max Obs Per Subject	75

Number of Observations

Number of Observations Read	75
Number of Observations Used	75
Number of Observations Not Used	0

Covariance Parameter
Estimates

Cov Parm	Estimate
Residual	32.8994

16

Fit Statistics

-2 Res Log Likelihood	462.0
AIC (smaller is better)	464.0
AICC (smaller is better)	464.0
BIC (smaller is better)	466.2

OUTPUT 11.2 SAS Output for Example 11.2 (continued)

Analysis of Covariance
Example 11.2: ANCOVA in Multi-Center Hypertension Study

Solution for Fixed Effects

Effect	trt	center	Estimate	Standard			
				Error	DF	t Value	Pr > t
Intercept			30.8866	10.0444	68	3.08	0.0030
trt	COMB		-2.7401	1.3319	68	-2.06	0.0435
trt	SOLE		0
center		1	-0.9642	2.2362	68	-0.43	0.6677
center		2	-0.6501	1.6347	68	-0.40	0.6921
center		3	-1.0311	1.8643	68	-0.55	0.5820
center		4	0
age			-0.1150	0.06853	68	-1.68	0.0979
bpdia0			-0.2640	0.09498	68	-2.78	0.0070

Type 3 Tests of Fixed Effects

Effect	Num	Den	F Value	Pr > F
	DF	DF		
trt	1	68	4.23	0.0435
center	3	68	0.13	0.9403
age	1	68	2.82	0.0979
bpdia0	1	68	7.72	0.0070

Least Squares Means

Effect	trt	Estimate	Standard			
			Error	DF	t Value	Pr > t
trt	COMB	-6.9313	0.9801	68	-7.07	<.0001
trt	SOLE	-4.1912	0.9931	68	-4.22	<.0001

Differences of Least Squares Means

Effect	trt	_trt	Estimate	Standard			
				Error	DF	t Value	Pr > t
trt	COMB	SOLE	-2.7401	1.3319	68	-2.06	0.0435

The output shows an error variation of 32.9 ⑯. This is associated with 68 degrees of freedom, as shown under the tests of fixed effects. As indicated, there is a significant treatment (trt) effect ($p=0.0435$) ⑰. No evidence of differences is seen among Centers ($p=0.9403$), while the baseline severity (bpdia0) is a significant covariate ($p=0.0070$). The age covariate is not significant ($p=0.0979$) when included in this model (see Section 11.4.7 for other models).

The adjusted mean decreases in diastolic blood pressure (Least Squares Means), 6.93 mm Hg for combination treatment and 4.19 mm Hg for sole therapy ⑱, are slightly greater than the unadjusted means, 6.82 and 4.06, respectively.

The SOLUTION option used in the MODEL statement estimates the model parameters for each of the fixed effects, as shown in the Estimate column in Output 11.2 ⑲. For example, the estimated response for combination therapy in Center 1 can be found by using the equation

$$\hat{y} = 30.8866 - 2.7401 - 0.9642 - (0.1150 * \text{age}) - (0.2640 * \text{bpdia0})$$

or

$$\hat{y} = 27.1823 - 0.115 * \text{age} - 0.264 * \text{bpdia0}$$

An estimate of the mean response for patients who receive combination therapy in Center 1 can be found by substituting the overall mean age (54.8133 yrs.) and the overall mean baseline diastolic blood pressure (106.4933 mm Hg) into this equation, as follows:

$$\bar{y} = 27.1823 - 0.115 * (54.8133) - 0.264 * (106.4933) = -7.23$$

Similarly, estimated response equations and means for the other combinations of Treatment and Center are shown in Table 11.9.

The adjusted treatment means (LS-means) ⑲ can be verified by averaging the estimates of the within-center means over all centers for that treatment:

$$\bar{y}_{\text{comb}} = (-7.234 - 6.920 - 7.301 - 6.270) / 4 = -6.93$$

$$\bar{y}_{\text{sole}} = (-4.494 - 4.180 - 4.561 - 3.520) / 4 = -4.19$$

**TABLE 11.9 Adjusted Mean Responses by Treatment Group and Center
for Example 11.2**

TRT	CENTER	Estimated Response Equation	Mean Response at Average Value of Covariates
COMB	1	$\hat{y} = 27.1823 - 0.115\text{age} - 0.264\text{bpdia0}$	-7.23
	2	$\hat{y} = 27.4964 - 0.115\text{age} - 0.264\text{bpdia0}$	-6.92
	3	$\hat{y} = 27.1154 - 0.115\text{age} - 0.264\text{bpdia0}$	-7.30
	4	$\hat{y} = 28.1465 - 0.115\text{age} - 0.264\text{bpdia0}$	-6.27
SOLE	1	$\hat{y} = 29.9224 - 0.115\text{age} - 0.264\text{bpdia0}$	-4.49
	2	$\hat{y} = 30.2365 - 0.115\text{age} - 0.264\text{bpdia0}$	-4.18
	3	$\hat{y} = 29.8555 - 0.115\text{age} - 0.264\text{bpdia0}$	-4.56
	4	$\hat{y} = 30.8866 - 0.115\text{age} - 0.264\text{bpdia0}$	-3.53

As an alternative, you may wish to treat Study Center as a random effect, as discussed in Chapter 7. In that case, you would include center in the RANDOM statement instead of in the MODEL statement as follows:

```
proc mixed data = bp;
  classes trt center;
  model bpdiaach = trt age bpdia0 / solution;
  random center;
  lsmeans trt / diff;
run;
```

The corresponding output is shown in Output 11.3. Similar results as in the fixed case are seen regarding treatment inferences and the significance of the covariates ²⁰. In this case, the predicted response equations, obtained from the estimates ²¹, are the same within each Center as follows:

For Treatment = COMB,

$$\begin{aligned}\hat{y} &= 30.4456 - 2.7151 - 0.1160\text{age} - 0.2645\text{bpdia0} \\ &= 27.7305 - 0.1160\text{age} - 0.2645\text{bpdia0}\end{aligned}$$

And for Treatment = SOLE,

$$\hat{y} = 30.4456 - 0.1160\text{age} - 0.2645\text{bpdia0}.$$

Plugging the overall means for age and bpdia0 into these equations yield the LSMEANS as follows, and as shown in the SAS output ㉒:

$$\bar{y}_{\text{comb}} = 27.7305 - 0.1160*54.8133 - 0.2645*106.4933 = -6.80$$

$$\bar{y}_{\text{sole}} = (30.4456 - 0.1160*54.8133 - 0.2645*106.4933) = -4.08$$

OUTPUT 11.3 SAS Output for Example 11.2—Random ‘Center’

```
Analysis of Covariance
Example 11.2: ANCOVA in Multi-Center Hypertension Study
```

```
The Mixed Procedure
```

```
Model Information
```

Data Set	WORK.BP
Dependent Variable	bpdiach
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

```
Class Level Information
```

Class	Levels	Values
trt	2	COMB SOLE
center	4	1 2 3 4

```
Dimensions
```

Covariance Parameters	2
Columns in X	5
Columns in Z	4
Subjects	1
Max Obs Per Subject	75

```
Number of Observations
```

Number of Observations Read	75
Number of Observations Used	75
Number of Observations Not Used	0

```
Iteration History
```

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	471.32211645	
1	1	471.32211645	0.00000000

```
Convergence criteria met.
```

OUTPUT 11.3 SAS Output for Example 11.2—Random ‘Center’ (continued)

Analysis of Covariance Example 11.2: ANCOVA in Multi-Center Hypertension Study							
Covariance Parameter Estimates							
Fit Statistics							
-2 Res Log Likelihood 471.3 AIC (smaller is better) 473.3 AICC (smaller is better) 473.4 BIC (smaller is better) 472.7							
Solution for Fixed Effects							
Effect	trt	Estimate	Standard Error	DF	t Value	Pr > t	
Intercept		30.4456	9.4880	3	3.21	0.0490	
trt	COMB	-2.7151	1.3044	68	-2.08	0.0412	
trt	SOLE	0	
age		-0.1160	0.06621	68	-1.75	0.0842	
bpdia0		-0.2645	0.09155	68	-2.89	0.0052	
Type 3 Tests of Fixed Effects							
Effect		Num DF	Den DF	F Value	Pr > F		
trt		1	68	4.33	0.0412	20	
age		1	68	3.07	0.0842		
bpdia0		1	68	8.35	0.0052		
Least Squares Mean							
Effect	trt	Estimate	Standar Error	DF	t Value	Pr > t	
trt	COMB	-6.7966	0.9026	68	-7.53	<.0001	
trt	SOLE	-4.0815	0.9395	68	-4.34	<.000	
Differences of Least Squares Mean							
Effect	trt	_trt	Estimate	Standar Error	DF	t Value	Pr > t
trt	COMB	SOLE	-2.7151	1.3044	68	-2.08	0.0412

11.4 Details & Notes

- **11.4.1** The standard error of the slope estimate, b , is given by

$$s_b = \sqrt{\frac{MSE}{\sum_i S_{xx(i)}}}$$

Because "*a significant covariate effect*" means the same as "*a non-zero slope*", an alternate but equivalent test to the *F-test* for the covariate effect is the *t-test* as follows:

null hypothesis:	$H_0: \beta = 0$
alt. hypothesis:	$H_A: \beta \neq 0$

test statistic: $t = \frac{b}{s_b}$

decision rule: reject H_0 if $|t| > t_{\alpha/2}$

In Example 11.1, you have $MSE = 93.67$, so that

$$s_b = \sqrt{\frac{93.67}{20.140 + 26.964}} = 1.410$$

The t-statistic is computed as

$$t = (-11.27) / 1.41 = -7.99$$

based on 31 degrees of freedom, as shown in Output 11.1 **10**. Notice that the square of the t-statistic is the F-statistic, $(-7.99)^2 = 63.8$, based on 1 upper and 31 lower degrees of freedom **4**.

- **11.4.2** Differences in adjusted response means are the same regardless of the value of the covariate, x , because the slopes are assumed to be equal among groups. In Example 11.1, the estimated difference in adjusted response means at x_0 is

$$(74.81 - 11.27x_0) - (64.59 - 11.27x_0) = 10.22$$

regardless of what value x_0 takes. This is not the case if the slopes differ among groups.

If the assumption of equal slopes within each group is not valid, the *ANCOVA* methods described in this chapter cannot be used. One method of testing for equal slopes is to use an ‘interaction’ factor between GROUP and X in the MODEL statement in SAS, e.g.,

```
model y = group x group*x;
```

A significant interaction effect ($group*x$) implies that the differences among group levels change for different x values, i.e., different slopes. *ANCOVA* should not be used if a preliminary test results in significantly different slopes among groups. In Example 11.1, you could perform a preliminary test for equal slopes using the SAS statements:

```
proc glm; class trt;
  model trichg = trt hgbalc trt*hgbalc;
```

This analysis would produce a p-value of 0.428 for the interaction term, $trt*hgbalc$, indicating no evidence to contradict the assumption of equal slopes.

- **11.4.3** A 95% confidence interval for the adjusted mean response in Group i ($i = 1, 2, \dots, k$) is given by

$$(a_i + b \bar{x}) \pm t_{0.025, N-k-1} \cdot s \cdot \sqrt{\frac{1}{n_i} + \frac{(\bar{x}_i - \bar{x})^2}{S_{xx}}}$$

where $s = \sqrt{MSE}$ from the ANOVA table.

In Example 11.1, 95% confidence intervals for the adjusted mean responses for each treatment group are

Fibralo Group

$$(74.81 - (11.268 \cdot 6.8118)) \pm 2.04 \cdot \sqrt{93.67} \cdot \sqrt{\frac{1}{16} + \frac{(7.0000 - 6.8118)^2}{48.175}} = \\ -1.95 \pm 4.96 \text{ or } (-6.91 \text{ to } 3.01)$$

Gemfibrozil Group:

$$(64.59 - (11.268 \cdot 6.8118)) \pm 2.04 \cdot \sqrt{93.67} \cdot \sqrt{\frac{1}{18} + \frac{(6.6444 - 6.8118)^2}{48.175}} = \\ -12.17 \pm 4.68 \text{ or } (-16.85 \text{ to } -7.49)$$

Notice that the confidence interval half-widths (4.96 and 4.68) can be easily computed as $t \times \text{SEM}$ where t is the critical t-value, 2.04, and SEM is the standard error of the LS-mean shown in Output 11.1 ⑪

- **11.4.4** A 95% confidence interval for the difference in adjusted mean responses between two groups, say Group u and Group v , is given by

$$(a_u - a_v) \pm t_{0.025, N-k-1} \cdot s \cdot \sqrt{\frac{1}{n_u} + \frac{1}{n_v} + \frac{(\bar{x}_u - \bar{x}_v)^2}{S_{xx}}}$$

In Example 11.1, a 95% confidence interval for the difference in adjusted means between the *Fibralo* and *Gemfibrozil* groups is

$$(74.81 - 64.59) \pm 2.04 \cdot \sqrt{93.7} \cdot \sqrt{\frac{1}{16} + \frac{1}{18} + \frac{(7.0000 - 6.6444)^2}{48.175}} = \\ 10.22 \pm 2.04 \cdot (3.3629) = 10.22 \pm 6.86 \text{ or } (3.36 \text{ to } 17.08)$$

The standard error (3.3629) used in this calculation can be obtained from the SAS output for TRT effect (see Output 11.1 ⑫). The 95% confidence interval for the difference in adjusted means is verified by the confidence limits shown in Output 11.1 under “95% Confidence Limits for LSMean(i)-LSMean(j), as produced by the CL option in the LSMEANS statement ⑬.

- **11.4.5** The standard errors of the adjusted group means and mean differences are smallest when $\bar{x}_i = \bar{x}$ for all $i (= 1, 2, \dots, k)$. In this case, the standard error is the same as that of the unadjusted case (see Chapter 6).

- **11.4.6** As shown in Output 11.1, the adjusted *F*-tests in SAS are found with the Type III sums of squares. SAS prints these by default, in addition to the Type I sums of squares. The Type I results depend on the order in which the factors are specified in the MODEL statement (see Appendix D). When the Treatment effect is specified before the covariate in the MODEL statement in PROC GLM, the Type I sum of squares represents the unadjusted sum of squares for Treatment (ignoring the covariate), which could alternatively be found by using the *one-way ANOVA* with Treatment as the only effect.

Notice that the Type I SS for TRT in Output 11.1 (327.2), using the *ANCOVA* model is the same as the SS for TRT in the *one-way ANOVA* results. While the same sum of squares are obtained, the corresponding *F-test* is not the same *F-test* because the covariate-adjusted MSE is used as a divisor.

- **11.4.7** In Example 11.2, various other models could have been selected for the analysis. Output 11.4 shows the *ANCOVA* results produced by models which correspond to the following MODEL statements in seven separate PROC MIXED calls.

```
(1)      model bpdiaach = trt;
(2)      model bpdiaach = trt center;
(3)      model bpdiaach = trt bpdia0;
(4)      model bpdiaach = trt age;
(5)      model bpdiaach = trt age bpdia0;
(6)      model bpdiaach = trt center bpdia0;
(7)      model bpdiaach = trt center age;
```

The SAS results for each model factor are produced in the output (Output 11.4) for each of the above models by using the `ods select Tests3;` statement before each PROC MIXED statement.

Using a *one-way ANOVA* (Chapter 6) or, equivalently, the *two-sample t-test* (Chapter 5) to compare treatment groups, ignoring all other study factors, results in only marginal significance ($p=0.0545$) of the Treatment effect (Model (1) above). Using a *two-way ANOVA* (Chapter 7) by including Treatment Group and Center without the covariates results in a similar p-value for Treatment ($p=0.0547$) (Model (2) above).

You see that Age is a significant covariate when used as a sole numeric covariate (Models (4) and (7) above), but Age is no longer significant when baseline diastolic blood pressure is also included (Model (5)). There might be some collinearity issues causing this outcome, which can be investigated using the methods discussed in Example 10.2 (Chapter 10). The significance of the Treatment Group effect increases when BPDIA0 is used as a covariate without Age, with p-values of 0.0331–0.0343 (Models (3) and (6) above), as shown in Output 11.4.

Output 11.4 SAS Output for Example 11.2 Using Alternative Models

(1) Effect	Num DF	Den DF	F Value	Pr > F
trt	1	73	3.82	0.0545
<hr/>				
(2) Effect	Num DF	Den DF	F Value	Pr > F
trt	1	70	3.82	0.0547
center	3	70	0.43	0.7352
<hr/>				
(3) Effect	Num DF	Den DF	F Value	Pr > F
trt	1	72	4.72	0.0331
bpdia0	1	72	11.90	0.0009
<hr/>				
(4) Effect	Num DF	Den DF	F Value	Pr > F
trt	1	72	3.51	0.0649
age	1	72	6.32	0.0142
<hr/>				
(5)	Num	Den		
Effect	DF	DF	F Value	Pr > F
trt	1	71	4.33	0.0410
age	1	71	3.07	0.0840
bpdia0	1	71	8.35	0.0051
<hr/>				
(6) Effect	Num DF	Den DF	F Value	Pr > F
trt	1	69	4.66	0.0343
center	3	69	0.18	0.9128
bpdia0	1	69	10.57	0.0018
<hr/>				
(7) Effect	Num DF	Den DF	F Value	Pr > F
trt	1	69	3.45	0.0676
center	3	69	0.22	0.8842
age	1	69	5.41	0.0230

[center is considered a fixed effect for Models (2), (6) and (7)]

These analyses indicate the importance of giving adequate consideration when establishing an appropriate statistical model at the design stage. As discussed in Chapter 1, bias can be introduced at the analysis stage if statistical methods are not pre-planned. This includes specification of appropriate statistical models. In a pivotal study, appropriate covariates should be specified in the primary model only if indicated, such as by earlier phase studies. Inclusion of pertinent covariates provides an automatic adjustment for differences in covariate means between treatment groups and can markedly increase the power for detecting treatment differences. However, inclusion of inappropriate covariates might lead to factor dilution, collinearity problems, and unnecessary reduction in error degrees of freedom, which, in turn, could result in loss of power or even inappropriate p-values.

- **11.4.8** When *ANCOVA* is used for model-building or exploratory analysis rather than as a primary analysis, the coefficient of determination (R-square) can be used as a relative measure of goodness-of-fit when using PROC GLM, as illustrated in Example 10.2. Some stepwise procedures rely on R^2 values for inclusion or exclusion of covariates (see SAS/STAT Documentation for PROC GLM). With PROC MIXED, you can compare the Fit Statistics to get an idea of which models fit the best. Graphical diagnostics available with ODS Graphics, as discussed in Chapter 10, are also helpful in selecting an appropriate model.

Although not included in the SAS output above, the Fit Statistics (AIC, AICC, BIC) for the main model used in Example 11.2 and each of the competing models (Section 11.4.7) are shown below in Table 11.10. A better fit is indicated by smaller values of these statistics. Based on these results, it appears Model (6) could be adequate to use as a final model.

TABLE 11.10 Fit Statistics for the Models Used in Example 11.2

Model	Effects Included	AIC	AICC	BIC
main	trt, center, age, bpdia0	464.0	464.0	466.2
(1)	Trt	480.9	481.0	483.2
(2)	trt, center	470.3	470.3	472.5
(3)	trt, bpdia0	472.8	472.8	475.0
(4)	trt, age	478.4	478.4	480.7
(5)	trt, age, bpdia0	473.3	473.4	475.6
(6)	trt, center, bpdia0	463.2	463.3	465.5
(7)	trt, center, age	468.5	468.6	470.7

CHAPTER 12

The Wilcoxon Signed-Rank Test

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12.1 Introduction

The *Wilcoxon signed-rank test* is a non-parametric analog of the *one-sample t-test* (Chapter 4). The *signed-rank test* can be used to make inferences about a population mean or median without requiring the assumption of normally distributed data. As its name implies, the *Wilcoxon signed-rank test* is based on the ranks of the data.

One of the most common applications of the *signed-rank test* in clinical trials is to compare responses between correlated or paired data, such as testing for significant pre- to post-study changes of a non-normally distributed evaluation measurement. The layout is the same as that for the *paired-difference t-test* (Chapter 4).

12.2 Synopsis

Given a sample, y_1, y_2, \dots, y_n , of n non-zero differences or changes (zeroes are ignored), let R_i represent the rank of $|y_i|$ (when ranked lowest to highest). Let $R^{(+)}$ represent the sum of the ranks associated with the positive values of the y_i 's, and let $R^{(-)}$ represent the sum of the ranks associated with the negative values of the y_i 's. The test statistic is based on the smaller of $R^{(+)}$ and $R^{(-)}$. The hypothesis of 'no mean difference' is rejected if this statistic is larger than the critical value found in a special set of tables.

To avoid the requirement of special table values, you can use an approximation to this test based on the t-distribution, as shown next.

Let

$$S = (R^{(+)} - R^{(-)}) / 2$$

and

$$V = (n(n + 1)(2n + 1)) / 24$$

The approximate test can be summarized as

null hypothesis:	$H_0: \theta = 0$
alt. hypothesis:	$H_A: \theta \neq 0$
test statistic:	$T = \frac{S \cdot \sqrt{n-1}}{\sqrt{n \cdot V - S^2}}$
decision rule:	reject H_0 if $ T > t_{\alpha/2, n-1}$

θ represents the unknown population mean, median, or other location parameter. When H_0 is true, T has an approximate Student-t distribution with $n-1$ degrees of freedom. Widely available t-tables can be used to determine the rejection region.

When there are tied data values, the averaged rank is assigned to the corresponding R_i values. A small adjustment to V for tied data values is appropriate, as follows: suppose there are g groups of tied data values. For the j^{th} group, compute $c_j = m(m-1)(m+1)$, where m is the number of tied data values for that group. The correction factor of $C = c_1 + c_2 + \dots + c_g$ is used to adjust V , as follows:

$$V = (n(n + 1)(2n + 1) - C) / 24$$

12.3 Examples

Example 12.1—Rose-Bengal Staining in KCS

A study enrolling 24 patients diagnosed with keratitis sicca (KCS) was conducted to determine the effect of a new ocular wetting agent, Oker-Rinse, for improving ocular dryness based on Rose-Bengal staining scores. Patients were instructed to use Oker-Rinse in one eye and Hypotears®, as a control, in the other eye 4 times a day for 3 weeks. Rose-Bengal staining scores were measured as 0, 1, 2, 3, or 4 in each of four areas of the eye: cornea, limbus, lateral conjunctiva, and medial conjunctiva. Higher scores represent a greater number of devitalized cells, a condition associated with KCS. The overall scores for each eye (see Table 12.1) were obtained by adding the scores over the four areas. Is there evidence of a difference between the two test preparations in the overall Rose-Bengal staining scores?

TABLE 12.1 Raw Data for Example 12.1

Patient Number	Hypotears	Oker-Rinse	Patient Number	Hypotears	Oker-Rinse
1	15	8	13	8	10
2	10	3	14	10	2
3	6	7	15	11	4
4	5	13	16	13	7
5	10	2	17	6	1
6	15	12	18	6	11
7	7	14	19	9	3
8	5	8	20	5	5
9	8	13	21	10	2
10	12	3	22	9	8
11	4	9	23	11	5
12	13	3	24	8	8

Solution

Because each patient receives both treatments, you have a matched-pairs setup. The analysis is performed on the differences in Rose-Bengal scores between the treatment groups. The data can be plotted to reveal that the differences have a bimodal distribution (two peaks), which is not consistent with an assumption of normality. Indeed, a formal test for normality (see SAS results later) indicates a significant departure from this assumption. Therefore, use of the *Wilcoxon signed-rank test* is preferable to using the *paired-difference t-test*.

You want to test the hypothesis that θ , the ‘average’ difference in Rose-Bengal scores between the treatment groups, is 0. The differences in scores and ranks of their absolute values are shown in Table 12.2.

The average rankings for tied differences are used. For example, a difference of ± 1 occurs twice (Patients 3 and 22) so the average of the ranks 1 and 2 ($= 1.5$) is used. Notice that the 0 differences are omitted from the rankings, leaving $n = 22$, not 24.

TABLE 12.2 Ranks of the Data for Example 12.1

Patient Number	Difference	Rank	Patient Number	Difference	Rank
1	7	14.5	13	-2	3
2	7	14.5	14	8	18.5
3	-1	1.5	15	7	14.5
4	-8	18.5	16	6	11
5	8	18.5	17	5	7.5
6	3	4.5	18	-5	7.5
7	-7	14.5	19	6	11
8	-3	4.5	20	0	--
9	-5	7.5	21	8	18.5
10	9	21	22	1	1.5
11	-5	7.5	23	6	11
12	10	22	24	0	--

Compute

$$R^{(+)} = (14.5 + 14.5 + 18.5 + 4.5 + 21 + 22 + 18.5 + \\ 14.5 + 11 + 7.5 + 11 + 18.5 + 1.5 + 11) \\ = 188.5$$

and

$$R^{(-)} = (1.5 + 18.5 + 14.5 + 4.5 + 7.5 + 7.5 + 3 + 7.5) \\ = 64.5$$

so that

$$S = (188.5 - 64.5) / 2 = 62.0$$

To apply the correction factor for ties, you record 6 groups of ties with the frequencies (m's): 2, 2, 4, 3, 4, and 4, (see Table 12.3). Thus, $c_1 = 2(1)(3) = 6$, $c_2 = 6$, $c_3 = 4(3)(5) = 60$, $c_4 = 3(2)(4) = 24$, $c_5 = 60$, and $c_6 = 60$, yielding $C = 216$.

TABLE 12.3 Calculation of Correction for Ties for Example 12.1

Tie Group (i)	Difference	Number of Ties (m)	$c_i = m(m-1)(m+1)$
1	1	2	6
2	3	2	6
3	5	4	60
4	6	3	24
5	7	4	60
6	8	4	60
			C=216

Finally,

$$\begin{aligned} V &= (n(n+1)(2n+1) - C)/24 \\ &= (22(23)(45) - 108)/24 = 944.25 \end{aligned}$$

The test is summarized as follows:

null hypothesis:	$H_0: \theta = 0$
alt. hypothesis:	$H_A: \theta \neq 0$
test statistic:	$T = \frac{S \cdot \sqrt{n-1}}{\sqrt{n \cdot V - S^2}}$ $= \frac{62 \cdot \sqrt{21}}{\sqrt{(22 \cdot 944.25) - 62^2}} = 2.184$
decision rule:	reject H_0 if $ T > t_{0.025, 21} = 2.080$
conclusion:	Because $2.184 > 2.080$, reject H_0 , and conclude that there is a significant difference in Rose-Bengal scores between treatments.

SAS Analysis of Example 12.1

In the test summary just presented, the SAS function PROBT can be used to obtain the p-value associated with a t-statistic of 2.184 based on 21 degrees of freedom, namely, $p = 0.0405$ (found by using the SAS expression $2*(1-PROBT(2.184, 21))$).

The SAS code and output for executing this analysis are shown on the next pages. The *Wilcoxon signed-rank test* can be performed in SAS using PROC UNIVARIATE. The NORMAL option ❶ is used to provide a test for normality based on the *Shapiro-Wilk test*. This test is rejected with a p-value of 0.0322 ❷, which indicates that the data cannot be assumed to have come from a normal distribution. This result makes the paired-difference *one-sample t-test* (Chapter 4) inappropriate for this analysis.

In Output 12.1, the signed-rank statistic is confirmed to be 62 ❸ with a p-value of 0.0405 ❹.

SAS uses the t-approximation to the *Wilcoxon signed-rank test* if n is greater than 20. For n ≤ 20, SAS computes the exact probability.

SAS Code for Example 12.1

```
data kcs;
    input pat hypotear okerinse @@;
    diff = hypotear - okerinse;
    datalines;
1 15 8      2 10 3      3 6 7      4 5 13
5 10 2      6 15 12     7 7 14     8 5 8
9 8 13     10 12 3     11 4 9     12 13 3
13 8 10    14 10 2     15 11 4     16 13 7
17 6 1     18 6 11     19 9 3     20 5 5
21 10 2    22 9 8     23 11 5    24 8 8
;

proc univariate normal data = kcs;           ①
    var diff;
    title1 'The Wilcoxon-Signed-Rank Test';
    title2 'Example 12.1: Rose Bengal Staining in KCS';
run;
```

OUTPUT 12.1 SAS Output for Example 12.1

The Wilcoxon-Signed-Rank Test			
Example 12.1: Rose Bengal Staining in KCS			
The UNIVARIATE Procedure			
Variable: diff			
Moments			
N	24	Sum Weights	24
Mean	2.2916667	Sum Observations	55
Std Deviation	5.66821164	Variance	32.1286232
Skewness	-0.4011393	Kurtosis	-1.2859334
Uncorrected SS	865	Corrected SS	738.958333
Coeff Variation	247.340144	Std Error Mean	1.15701886
Basic Statistical Measures			
Location		Variability	
Mean	2.29167	Std Deviation	5.66821
Median	4.00000	Variance	32.12862
Mode	-5.00000	Range	18.00000
		Interquartile Range	9.50000
NOTE: The mode displayed is the smallest of 4 modes with a count of 3.			

OUTPUT 12.1 SAS Output for Example 12.1 (continued)

The Wilcoxon-Signed-Rank Test
Example 12.1: Rose Bengal Staining in KCS

The UNIVARIATE Procedure
Variable: diff

Tests for Location: Mu0=0

Test	-Statistic-	-----p Value-----
Student's t	t 1.980665	Pr > t 0.0597
Sign	M 3	Pr >= M 0.2863
Signed Rank	S 62	Pr >= S 0.0405 ③ ④

Tests for Normality

Test	--Statistic---	-----p Value-----
Shapiro-Wilk	W 0.908189	Pr < W 0.0322
Kolmogorov-Smirnov	D 0.201853	Pr > D 0.0125
Cramer-von Mises	W-Sq 0.145333	Pr > W-Sq 0.0248
Anderson-Darling	A-Sq 0.859468	Pr > A-Sq 0.0235

Quantiles (Definition 5)

Quantile	Estimate
100% Max	10.0
99%	10.0
95%	9.0
90%	8.0
75% Q3	7.0
50% Median	4.0
25% Q1	-2.5
10%	-5.0
5%	-7.0
1%	-8.0
0% Min	-8.0

Extreme Observations

----Lowest----		----Highest---	
Value	Obs	Value	Obs
-8	4	8	5
-7	7	8	14
-5	18	8	21
-5	11	9	10
-5	9	10	12

12.4 Details & Notes

- **12.4.1** The *Wilcoxon signed-rank test* is considered a non-parametric test because it makes no assumptions regarding the distribution of the population from which the data are collected. This appears to be a tremendous advantage over the *t-test*, so why not use the *signed-rank test* in all situations?

First, the *t-test* has been shown to be a more powerful test in detecting true differences when the data *are* normally distributed. Because the normal distribution occurs quite often in nature, the *t-test* is the method of choice for a wide range of applications. Secondly, analysts often feel more comfortable reporting a *t-test* whenever it is appropriate, especially to a non-statistician, because many clinical research professionals have become familiar with the terminology. One reason the *t-test* has enjoyed popular usage is its robustness under deviations to the underlying assumptions. Finally, the *Wilcoxon signed-rank test* does require the assumption of a symmetrical underlying distribution. When the data are highly skewed or otherwise non-symmetrical, an alternative to the *signed-rank test*, such as the *sign test* (discussed in Chapter 15), can be used.

- **12.4.2** Let $W_i = R_i$ if $y_i > 0$ and $W_i = -R_i$ if $y_i < 0$. It can be shown that the T statistic discussed is equivalent to performing a *one-sample t-test* (Chapter 4) on the W_i 's, that is, the ranked data adjusted by the appropriate sign. This is sometimes referred to as performing a *t-test* on the 'rank-transformed' data.
- **12.4.3** An alternative approximation to the *signed-rank test* for large samples is the *Z-test*. The test statistic is taken to be $S = \text{smaller of } (R^{(+)}, R^{(-)})$. S' has mean and variance:

$$\mu_{S'} = \frac{n \cdot (n+1)}{4}$$

and

$$\sigma_{S'}^2 = \frac{n \cdot (n+1) \cdot (2n+1)}{24}$$

Under H_0 ,

$$Z = \frac{S' - \mu_{S'}}{\sigma_{S'}}$$

has an approximate normal distribution with mean 0 and variance 1. The *Z-test* statistic is compared with the tabled value of $Z_{\alpha/2}$ for a *two-tailed test* ($= 1.96$ for $\alpha = 0.05$).

To illustrate this approximation for Example 12.1, compute

$$S' = 64.5$$

$$\mu_{S'} = \frac{22 + 23}{4} = 126.5$$

and

$$\sigma_{S'} = \sqrt{\frac{22 \cdot 23 \cdot 45}{24}} = 30.8$$

so that

$$Z = (64.5 - 126.5) / 30.8 = -2.013.$$

At $p = 0.05$, you reject H_0 because $2.013 > 1.96$. The SAS function PROBNORM can be used to obtain the actual p-value of 0.044 (i.e., $0.44=2*\text{probnorm}(-2.013)$).

- **12.4.4** The *one-sample t-test* is often used with larger samples, regardless of the distribution of the data, because the *Central Limit Theorem* states that the sample mean \bar{y} is normally distributed for large n , no matter what distribution the underlying data have (see Chapter 1). Usually, for $n > 30$, we can safely use the *one-sample t-test* (Chapter 4), and ignore distributional assumptions, providing the distribution is symmetric. The *Wilcoxon signed-rank test* and the *t-test* become closer as n gets larger. For non-symmetrical distributions, the mean, median, and other measures of central tendency might have widely disparate values. The *t-test* should be used only if the mean is the appropriate measure of central tendency for the population being studied.
- **12.4.5** The method shown here is for a *two-tailed test*. For a *one-tailed test*, the same methods that are shown in Chapter 4 for the *one-sample t-test* can be used. If SAS is used, the p-value associated with the *signed-rank test* can be halved for an approximate *one-tailed test*.

CHAPTER 13

The Wilcoxon Rank-Sum Test

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13.1 Introduction

The *Wilcoxon rank-sum test* is a non-parametric analog of the *two-sample t-test* (Chapter 5). It is based on ranks of the data, and is used to compare location parameters, such as the mean or median, between two independent populations without the assumption of normally distributed data.

Although the *Wilcoxon rank-sum test* was developed for use with continuous numeric data, the test is also applied to the analysis of ordered categorical data. In clinical data analysis, this test is often useful, for example, for comparing patient global assessments or disease severity ratings between two treatment groups.

13.2 Synopsis

The data are collected as two independent samples of size n_1 and n_2 , denoted by $y_{11}, y_{12}, \dots, y_{1n_1}$ and $y_{21}, y_{22}, \dots, y_{2n_2}$. The data are ranked, from lowest to highest, over the combined samples, and the test statistic is based on the sum of ranks for the first group.

Let $r_{1j} = \text{rank of } y_{1j}$ ($j = 1, 2, \dots, n_1$) and $r_{2j} = \text{rank of } y_{2j}$ ($j = 1, 2, \dots, n_2$), and compute

$$R_1 = \sum_{j=1}^{n_1} r_{1j}$$

and

$$R_2 = \sum_{j=1}^{n_2} r_{2j}$$

The hypothesis of equal means would be supported by similar average ranks between the two groups, i.e., if R_1/n_1 is close to R_2/n_2 . R_1 is compared to a critical value obtained from a special set of tables based on *Wilcoxon rank-sum* exact probabilities to determine the appropriate rejection region. Such tables can be found in many intermediate statistical books or non-parametric statistical references. Exact tests are also available in SAS (see Section 13.4.8) and other statistical software.

For larger samples (typically at least 10 per group), a normal approximation to the *Wilcoxon rank sum test* can be used, as described below. It has been shown that this approximation is excellent for samples as small as 8 per group in some cases.

With $N = n_1 + n_2$, the sum of the ranks ($1 + 2 + \dots + N$) can be expressed as $N \cdot (N+1)/2$. When the hypothesis of equality is true (H_0), you would expect the proportion of this sum from Sample 1 to be about n_1/N and the proportion from Sample 2 to be about n_2/N . That is, the expected value of R_1 under H_0 is

$$\mu_{R_1} = \left(\frac{n_1}{N} \right) \cdot \left(\frac{N(N+1)}{2} \right) = \frac{n_1 \cdot (N+1)}{2}$$

Additionally, the variance of R_1 can be computed as

$$\sigma^2_{R_1} = \frac{n_1 \cdot n_2}{12} (N+1)$$

If there are tied data values, the average rank is assigned to the corresponding r_{ij} values. Suppose there are g groups of tied data values. For the k^{th} group, compute $c_k = m(m^2-1)$, where m is the number of tied values for that group. You can make a small adjustment to the variance by using the correction factor $C = c_1 + c_2 + \dots + c_g$, as follows:

$$\sigma^2_{R_1} = \frac{n_1 \cdot n_2}{12} \left(N+1 - \frac{C}{N(N-1)} \right)$$

The test statistic, using a 0.5 continuity correction, is based on an approximate normal distribution, summarized as follows:

null hypothesis:	$H_0: \theta_1 = \theta_2$
alt. hypothesis:	$H_A: \theta_1 \neq \theta_2$
test statistic:	$Z = \frac{ R_1 - \mu_{R_1} - 0.5}{\sigma_{R_1}}$
decision rule:	reject H_0 if $ Z > Z_{\alpha/2}$

where θ_1 and θ_2 represent the mean, median, or other location parameters for the two populations.

13.3 Examples

Example 13.1—Global Evaluations of Seroxatene in Back Pain

In previous studies of the new anti-depressant, Seroxatene, researchers noticed that patients with low back pain experienced a decrease in radicular pain after 6 to 8 weeks of daily treatment. A new study was conducted in 28 patients to determine whether this phenomenon is a drug-related response or coincidental. Patients with MRI-confirmed disk herniation and symptomatic leg pain were enrolled and randomly assigned to receive Seroxatene or a placebo for 8 weeks. At the end of the study, patients were asked to provide a global rating of their pain, relative to baseline, on a coded improvement scale as follows:

Deterioration				Improvement		
Marked	Moderate	Slight	No Change	Slight	Moderate	Marked
-3	-2	-1	0	+1	+2	+3

The data are shown in Table 13.1. Is there evidence that Seroxatene has any effect on radicular back pain?

TABLE 13.1 Raw Data for Example 13.1

----- Seroxatene Group -----				----- Placebo Group -----			
Patient Number	Score	Patient Number	Score	Patient Number	Score	Patient Number	Score
2	0	16	-1	1	3	15	0
3	2	17	2	4	-1	18	-1
5	3	20	-3	7	2	19	-3
6	3	21	3	9	3	23	-2
8	-2	22	3	11	-2	25	1
10	1	24	0	13	1	28	0
12	3	26	2				
14	3	27	-1				

Solution

With a situation involving a two-sample comparison of means, you might first consider the *two-sample t-test* or the *Wilcoxon rank-sum test*. Because the data consist of ordered categorical responses, the assumption of normality is dubious, so you can apply the *rank-sum test*.

Tied data values are identified as shown in Table 13.2.

TABLE 13.2 Calculation of Correction for Ties in Example 13.1

Response (y)	Number of Ties (m)	Ranks	Average Rank	$c_k = m(m^2 - 1)$
-3	2	1, 2	1.5	6
-2	3	3, 4, 5	4	24
-1	4	6, 7, 8, 9	7.5	60
0	4	10, 11, 12, 13	11.5	60
+1	3	14, 15, 16	15	24
+2	4	17, 18, 19, 20	18.5	60
+3	8	21, 22, 23, 24, 25, 26, 27, 28	24.5	504
				C = 738

With $n_1 = 16$, $n_2 = 12$, and $N = 28$, the ranked values (r 's) of the responses (y 's) are shown in Table 13.3.

TABLE 13.3 Ranks of the Data for Example 13.1

----- Seroxatene Group -----				----- Placebo Group -----			
Patient Number	Score Rank	Patient Number	Score Rank	Patient Number	Score Rank	Patient Number	Score Rank
2	11.5	16	7.5	1	24.5	15	11.5
3	18.5	17	18.5	4	7.5	18	7.5
5	24.5	20	1.5	7	18.5	19	1.5
6	24.5	21	24.5	9	24.5	23	4
8	4	22	24.5	11	4	25	15
10	15	24	11.5	13	15	28	11.5
12	24.5	26	18.5				
14	24.5	27	7.5				

Compute

$$R_1 = 11.5 + 18.5 + 24.5 + \dots + 7.5 = 261$$

and

$$R_2 = 24.5 + 7.5 + 18.5 + \dots + 11.5 = 145$$

As a check, note that $R_1 + R_2 = N(N+1)/2 = 406$. You further compute

$$\mu_{R_1} = \frac{16 \cdot (29)}{2} = 232$$

and

$$\sigma^2_{R_1} = \frac{(16)(12)}{12} \cdot \left(29 - \frac{738}{(28)(27)} \right) = 448.38$$

The test summary, based on a normal approximation at a significance level of $\alpha=0.05$, becomes

null hypothesis:	$H_0: \theta_1 = \theta_2$
alt. hypothesis:	$H_A: \theta_1 \neq \theta_2$
test statistic:	$Z = \frac{ R_1 - \mu_{R1} - 0.5}{\sigma_{R1}}$ $= \frac{(261 - 232) - 0.5}{\sqrt{448.38}} = 1.346$
decision rule:	reject H_0 if $ Z > 1.96$
conclusion:	Because 1.346 is not > 1.96 , you do not reject H_0 , and conclude that there is insufficient evidence of a difference between <i>Seroxatene</i> and placebo in global back pain evaluations.

Interpolation of normal probabilities tabulated in Appendix A.1 results in a two-tailed p-value of 0.178 for the Z-statistic of 1.346. The p-value can also be found by using the SAS function PROBNORM. In SAS, the two-tailed p-value for a Z-value of 1.346 is (1-PROBNORM(1.346)) = 0.1783.

SAS Analysis of Example 13.1

The *Wilcoxon rank-sum test* is performed in SAS by using PROC NPAR1WAY with the WILCOXON option, as shown in the SAS code on the next page. The sum of ranks for either group can be used to compute the test statistic. The manual calculations use $R_1 = 261$, while SAS uses $R_2 = 145$ ❶. When the smaller value is used, the result is a negative Z-value ❷. In either case, for a *two-tailed test*, $|Z| = 1.346$ with a p-value of 0.1783 ❸, which confirms the manual calculations.

The output from PROC NPAR1WAY also gives the results of the analysis using the *Kruskal-Wallis* chi-square approximation ❹ (Chapter 14).

SAS Code for Example 13.1

```
data rnksm;
    input trt $ pat score @@;
    datalines;
SER 2 0 SER 3 2 SER 5 3 SER 6 3
SER 8 -2 SER 10 1 SER 12 3 SER 14 3
SER 16 -1 SER 17 2 SER 20 -3 SER 21 3
SER 22 3 SER 24 0 SER 26 2 SER 27 -1
PBO 1 3 PBO 4 -1 PBO 7 2 PBO 9 3
PBO 11 -2 PBO 13 1 PBO 15 0 PBO 18 -1
PBO 19 -3 PBO 23 -2 PBO 25 1 PBO 28 0
;

proc nparlway wilcoxon data = rnksm;
    class trt; var score;
    title1 'The Wilcoxon Rank-Sum Test';
    title2 'Example 13.1: Seroxatene in Back Pain';
run;
```

OUTPUT 13.1 SAS Output for Example 13.1

The Wilcoxon Rank-Sum Test Example 13.1: Seroxatene in Back Pain								
The NPAR1WAY Procedure								
Wilcoxon Scores (Rank Sums) for Variable score Classified by Variable trt								
trt	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score			
SER	16	261.0	232.0	21.175008	16.312500			
PBO	12	145.0	174.0	21.175008	12.083333			
Average scores were used for ties.								
Wilcoxon Two-Sample Test								
Statistic		145.0000	①					
Normal Approximation								
Z		-1.3459	②					
One-Sided Pr < Z		0.0892	③					
Two-Sided Pr > Z		0.1783						
t Approximation								
One-Sided Pr < Z		0.0948						
Two-Sided Pr > Z		0.1895						
Z includes a continuity correction of 0.5.								
Kruskal-Wallis Test								
Chi-Square		1.8756	④					
DF		1						
Pr > Chi-Square		0.1708						

13.4 Details & Notes

- **13.4.1** The term ‘mean’ is used loosely in this chapter to refer to the appropriate location parameter. Because the mean is not always the best measure of a distribution’s center, the location parameter can refer to some other measure, such as one of the measures of central tendency described in Chapter 1 (Table 1.3).
- **13.4.2** For symmetric distributions, the population mean and median are the same. For skewed distributions with long tails to the right, the median is usually smaller than the mean and considered a better measure of the distributional ‘center’ or location. The geometric mean is also smaller than the arithmetic mean and is often used as a location parameter for exponentially distributed data. The *Wilcoxon rank-sum test* tests for a location or positional shift in distributions without the need to identify the best measure of central tendency. Thus, the parameter θ used in Section 13.2 is simply a symbol generically denoting a distribution’s location.
- **13.4.3** Although you need not make assumptions regarding the actual distributions of the data, the *Wilcoxon rank-sum test* does assume that the two population distributions have the same shape and differ only by a possible shift in location. Thus, you assume the same dispersion, which is analogous to the assumption of variance homogeneity required of the *two-sample t-test*. Unlike the *Wilcoxon signed-rank test* (Chapter 12) you need not assume the data come from a symmetric distribution.

The assumption of normality can be tested by using PROC UNIVARIATE in SAS. When normality cannot be assumed, the *Wilcoxon rank-sum test* is preferable to the *two-sample t-test*. Tests such as the *Kolmogorov-Smirnov test* and the *Cramer-Von Mises test*, available in SAS by specifying the EDF option in PROC NPAR1WAY, can be used to compare the equality of two distributions. Significance indicates a difference in location or scale between the two populations. NPAR1WAY also provides a number of other tests for location shifts (refer to the SAS Documentation for details).

- **13.4.4** The test statistic, R_1 , has a symmetric distribution about its mean, $n_1(N+1)/2$. Because of this symmetry, the one-tailed p-value is easily obtained by halving the two-tailed p-value.
- **13.4.5** The *Mann-Whitney U-test* is another non-parametric test for comparing location parameters based on two independent samples. Mathematically, it can be shown that the *Mann-Whitney test* is equivalent to the *Wilcoxon rank-sum test*.
- **13.4.6** Notice that the approximate test statistic, Z , can be computed from R_2 instead of R_1 . When using R_2 , the mean and standard deviation (μ_R , σ_R) are computed by reversing n_1 and n_2 in the formulas given.

- **13.4.7** Another approximation to the *Wilcoxon rank-sum test* is the use of the *two-sample t-test* on the ranked data. In SAS, use PROC RANK to rank the pooled data from the two samples, and then use PROC TTEST. A comparison of this procedure vs. the standard *Wilcoxon rank-sum test* is discussed by Conover and Iman (1981). Parametric methods, such as the *t-test* or *ANOVA*, used on the ranked data may lead to invalid conclusions in the presence of many ties or distributional anomalies. An aid in visualizing the distribution of the ranks using a box plot can easily be obtained with the ODS graphics feature of SAS. This is demonstrated in Chapter 14 (Example 14.1).
- **13.4.8** While the normal approximation for the *Wilcoxon rank-sum test* is usually quite good, even for samples as small as 8-10 per group, improvements in computational efficiencies make it easy to obtain exact tests even if the sample sizes are moderately larger. In SAS, an exact test for the *Wilcoxon rank-sum* is conducted by using the WILCOXON option with the EXACT STATEMENT following PROC NPAR1WAY, as shown below for Example 13.1:

```
proc npar1way data = rnksm;
  exact wilcoxon;
  class trt; var score;
run;
```

The resulting output gives an exact two-tailed p-value of 0.1804. For larger data sets, the NPAR1WAY procedure can use Monte Carlo methods to obtain exact p-values with fairly good efficiency.

- **13.4.9** In addition to testing for a significant shift in the distribution, you might want to report the mean or median response for each treatment group. In Example 13.1, the average pain ratings on the -3 to +3 scale can be calculated as 1.125 (interpreted as “a little better than slight improvement”) for the Seroxatene group, and 0.083 (interpreted as “hardly any better than no change”) for the placebo group. You can also compute a 95% confidence interval for the location shift between the groups using an estimator developed by Hodges and Lehmann (see, e.g., Hollander & Wolfe, 1999). This can be done in SAS (Version 9.2 and above) by including the HL option in the PROC NPAR1WAY statement, or in the EXACT statement if you want to use the exact tests. Adding the HL option to the PROC NPAR1WAY code of Example 13.1 provides a point estimate in the shift in average pain scores between Seroxatene and Placebo of -1.5 with a 95% confidence interval of 0 to -3, as seen in the resulting output:

Hodges-Lehmann Estimation			
Location Shift -1.0000			
95% Confidence Limits	Interval	Midpoint	Asymptotic Standard Error
-3.0000 0.0000	-1.5000	0.7653	

CHAPTER 14

The Kruskal-Wallis Test

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14.1 Introduction

The *Kruskal-Wallis test* is a non-parametric analogue of the *one-way ANOVA* (Chapter 6). It is used to compare population location parameters (mean, median, etc.) among two or more groups based on independent samples. Unlike an *ANOVA*, the assumption of normally distributed responses is not necessary. The *Kruskal-Wallis test* is an extension of the *Wilcoxon rank-sum test* (Chapter 13) for more than two groups, just as a *one-way ANOVA* is an extension of the *two-sample t-test*.

The *Kruskal-Wallis test* is based on the ranks of the data. This test is often useful in clinical trials for comparing responses among three or more dose groups or treatment groups using samples of non-normally distributed response data.

14.2 Synopsis

The data are collected as k (≥ 2) independent samples of size n_1, n_2, \dots, n_k , as shown in Table 14.1.

TABLE 14.1 Layout for the Kruskal-Wallis Test

Group 1	Group 2	...	Group k
y_{11}	y_{21}		y_{k1}
y_{12}	y_{22}		y_{k2}
...
y_{1n_1}	y_{2n_2}		y_{kn_k}

You want to test the hypothesis of equal mean responses among groups (H_0). The data are ranked, from lowest to highest, over the combined samples, and the test statistic is a function of the ranks and sample sizes.

For $i = 1, 2, \dots, k$ and $j = 1, 2, \dots, n_i$, let r_{ij} = rank of y_{ij} over the k combined samples. For each group ($i = 1, 2, \dots, k$), compute

$$R_i = \sum_{j=1}^{n_i} r_{ij}$$

The average rank of all $N = n_1 + n_2 + \dots + n_k$ observations is $\bar{R} = (N+1)/2$. Therefore, when the null hypothesis is true, the average rank for each group, namely $\bar{R}_i = (R_i / n_i)$, should be close to this value and the sum-of-squared deviations

$$\sum_{i=1}^k n_i (\bar{R}_i - \bar{R})^2$$

should be small.

This form is recognizable as the familiar ‘between-group’ sum of squares used in the *analysis of variance* (Chapters 6 and 7), after replacing the observed values with their ranks. The *Kruskal-Wallis test* statistic is a function of this sum of squares, which simplifies algebraically to the quantity

$$h^* = \frac{12}{N(N+1)} \cdot \left(\sum_{i=1}^k \frac{R_i^2}{n_i} \right) - 3(N+1)$$

When H_0 is true, h^* has an approximate *chi-square* distribution with $k-1$ degrees of freedom.

As with other ranking procedures, when you have tied data values, the average rank of the tied values is assigned to the corresponding r_{ij} values. Suppose there are g categories of tied values. For the L^{th} such category, compute $c_L = m(m^2-1)$, where m is the number of tied values for that category. A small adjustment can be made to the test statistic by using the correction factor $C = c_1 + c_2 + \dots + c_g$. With θ representing the population location parameter, the test is summarized as shown next.

null hypothesis:	$H_0: \theta_1 = \theta_2 = \dots = \theta_k$
alt. hypothesis:	$H_A: \theta_i \neq \theta_j$ for at least one pair (i,j)
test statistic:	$h = \frac{h^*}{\left(1 - \frac{C}{N(N^2 - 1)}\right)}$
decision rule:	reject H_0 if $h > \chi_{k-1}^2(\alpha)$

$\chi_{k-1}^2(\alpha)$ represents the critical chi-square value based on $k-1$ degrees of freedom and a significance level of α . Critical chi-square values can be found in tables of the chi-square distribution or by using the PROBCHI function in SAS.

14.3 Examples

Example 14.1—Psoriasis Evaluation in Three Groups

A study comparing a low dose (0.1%) and a high dose (0.2%) of a new, non-steroidal, anti-psoriasis medication was conducted using a parallel design, including a placebo group as control. Thirty-two patients were studied for 4 weeks of daily treatment. The primary efficacy response measure was the degree of psoriatic lesion reduction at study termination, rated on an ordinal scale, as follows:

Coded Response Category	Reduction in Lesion Size	Coded Response Category	Reduction in Lesion Size
1	< 0%	5	26-50%
2	0%	6	51-75%
3	1-10%	7	76-99%
4	11-25%	8	100%

Based on the data shown in Table 14.2, is there any difference in response among the three groups?

TABLE 14.2 Raw Data for Example 14.1

0.1% Solution		0.2% Solution		Placebo	
Patient Number	Category Code	Patient Number	Category Code	Patient Number	Category Code
1	5	3	5	2	5
6	4	5	8	4	3
9	1	7	2	8	7
12	7	10	8	11	1
15	4	14	7	13	2
19	3	18	4	16	4
20	6	22	5	17	2
23	7	26	4	21	1
27	8	28	6	24	4
32	7	31	4	25	5

Solution

This data set has an ordinal scale response with unequally spaced intervals. If you use a *one-way ANOVA*, the results might depend on the coding scheme used. Because the codes of 1 to 8 are arbitrary, the *Kruskal-Wallis test* is appropriate; the results do not depend on the magnitude of the coded values, only their ranks.

The data can be summarized in a frequency table format that includes the ranks, as shown in Table 14.3.

TABLE 14.3 Calculation of Ranks and Correction for Ties in Example 14.1

Response Category	Code	-- Frequencies --			Ranks	Average Rank	Total Frequency (m)	c = m(m ² -1)
		0.1%	0.2%	Placebo				
<0%	1	1	0	2	1-3	2	3	24
0%	2	0	1	2	4-6	5	3	24
1-10%	3	1	0	1	7-8	7.5	2	6
11-25%	4	2	3	3	9-16	12.5	8	504
26-50%	5	1	2	3	17-22	19.5	6	210
51-75%	6	1	1	0	23-24	23.5	2	6
76-99%	7	3	1	1	25-29	27	5	120
100%	8	1	2	0	30-32	31	3	24
		10	10	12			32	C = 918

The table of ranks and the rank sums for each group are shown in Table 14.4.

TABLE 14.4 Ranks of the Data in Example 14.1

0.1% Solution		0.2% Solution		Placebo	
Patient Number	Category Rank	Patient Number	Category Rank	Patient Number	Category Rank
1	19.5	3	19.5	2	19.5
6	12.5	5	31	4	7.5
9	2	7	5	8	27
12	27	10	31	11	2
15	12.5	14	27	13	5
19	7.5	18	12.5	16	12.5
20	23.5	22	19.5	17	5
23	27	26	12.5	21	2
27	31	28	23.5	24	12.5
32	27	31	12.5	25	19.5
$R_1 = 189.5$ ($n_1 = 10$)		$R_2 = 194.0$ ($n_2 = 10$)		$R_3 = 144.5$ ($n_3 = 12$)	

Compute the unadjusted test statistic as

$$h^* = \frac{12}{32(33)} \cdot \left(\frac{189.5^2}{10} + \frac{194.0^2}{10} + \frac{144.5^2}{12} \right) - 3(33) = 4.348$$

and the test is summarized as follows:

null hypothesis:	$H_0: \theta_1 = \theta_2 = \theta_3$
alt. hypothesis:	$H_A: \text{not } H_0$
test statistic:	$h = \frac{4.348}{\left(1 - \frac{918}{32 \cdot (32^2 - 1)}\right)} = \frac{4.348}{0.972} = 4.473$
decision rule:	reject H_0 if $h > \chi^2_2(0.05) = 5.991$
conclusion:	Because 4.473 is not > 5.991 , there is insufficient evidence to reject H_0 . You conclude that the data fail to reveal a statistical difference in psoriatic lesion reduction among the groups.

SAS Analysis of Example 14.1

In the preceding test summary, the PROBCHI function in SAS can be used to obtain the p-value associated with the test statistic 4.473 based on 2 degrees of freedom namely, $p = 0.1068 (=1 - \text{PROBCHI}(4.473,2))$.

The *Kruskal-Wallis test* can be performed in SAS by using PROC NPAR1WAY with the WILCOXON option, as shown in the SAS code for Example 14.1. SAS will automatically perform the *Wilcoxon rank-sum test* (Chapter 13) if there are only two groups and the *Kruskal-Wallis test* if there are more than two groups, as determined by the number of levels in the class variable. In this example, there are three treatment levels in the DOSE variable, which is used in the CLASS statement ❶.

The output shows the rank sums for each group ❷ and the chi-square statistic ❸, which corroborate the manual calculations demonstrated. The p-value ❹ is also printed out by SAS. The ODS graphics plot showing the box plots of the ranked data is also shown ❺ (see Section 14.4.6).

SAS Code for Example 14.1

```
data psor;
    input dose $ pat score @@;
    datalines;
    0.1 1 5 0.1 6 4 0.1 9 1 0.1 12 7
    0.1 15 4 0.1 19 3 0.1 20 6 0.1 23 7
    0.1 27 8 0.1 32 7 0.2 3 5 0.2 5 8
    0.2 7 2 0.2 10 8 0.2 14 7 0.2 18 4
    0.2 22 5 0.2 26 4 0.2 28 6 0.2 31 4
    PBO 2 5 PBO 4 3 PBO 8 7 PBO 11 1
    PBO 13 2 PBO 16 4 PBO 17 2 PBO 21 1
    PBO 24 4 PBO 25 5 PBO 29 4 PBO 30 5
;

ods graphics on;
proc npar1way wilcoxon data=psor plots=wilcoxon;
    class dose; ❶
    var score;
    title1 'The Kruskal-Wallis Test';
    title2 'Example 14.1: Psoriasis Evaluation in Three
        Groups';
run;
ods graphics off;
```

OUTPUT 14.1 SAS Output for Example 14.1

```

The Kruskal-Wallis Test
Example 14.1: Psoriasis Evaluation in Three Groups

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable score
Classified by Variable dose

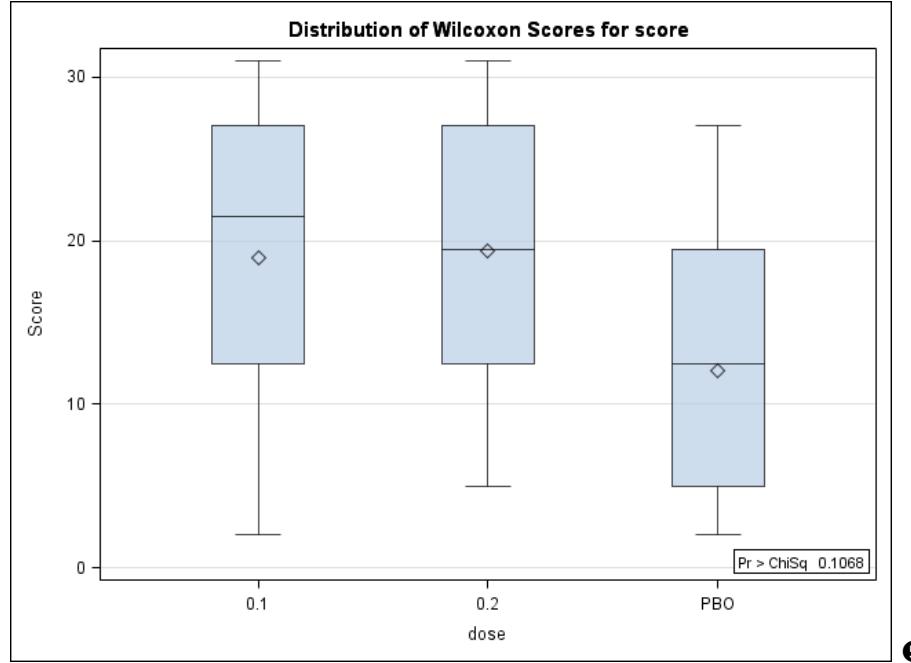
      Sum of      Expected      Std Dev      Mean
dose     N   Scores ❷ Under H0    Under H0   Score
-----
0.1      10    189.50    165.0    24.249418  18.950000
0.2      10    194.00    165.0    24.249418  19.400000
PBO      12    144.50    198.0    25.327691  12.041667

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square      4.4737 ❸
DF                  2
Pr > Chi-Square  0.1068 ❹

```



14.4 Details & Notes

- **14.4.1** When $k=2$, the *Kruskal-Wallis* chi-square value has 1 degree of freedom. This test is identical to the normal approximation used for the *Wilcoxon rank-sum test* (Chapter 13). As noted in previous sections, a chi-square with 1 degree of freedom can be represented by the square of a

standardized normal random variable (see Appendix B). For $k=2$, the h -statistic is the square of the *Wilcoxon rank-sum Z-test* (without the continuity correction).

- **14.4.2** The effect of adjusting for tied ranks is to slightly increase the value of the test statistic, h . Therefore, omission of this adjustment results in a more conservative test.
- **14.4.3** For small n_i 's (< 5), the chi-square approximation might not be appropriate. Special tables for the exact distribution of h are available in many non-parametric statistics books. These tables can be used to obtain the appropriate rejection region for small samples. (For example, *Nonparametric Statistical Methods* by Hollander and Wolfe (1999) provides tables of the critical values for the *Kruskal-Wallis test*, the *Wilcoxon rank-sum test*, and the *Wilcoxon signed-rank test*.)

Exact tests are also available in SAS by using the EXACT statement in PROC NPAR1WAY (see SAS/STAT documentation). Exact tests should be used in special cases when the distribution of the test statistic (h) is not well approximated with the chi-square, such as tests involving very small sample sizes or many ties.

- **14.4.4** By including the ANOVA option in PROC NPAR1WAY, *One-Way ANOVA* results can be obtained along with the *Kruskal-Wallis Test*. If the results are highly disparate, it probably means the parametric assumptions of the ANOVA are not met. The normality condition can be tested using the NORMAL option in PROC UNIVARIATE.
- **14.4.5** When the distribution of the response data is unknown and cannot be assumed to be normal, the median (rather than the mean) is frequently used as a measure of location or central tendency. The median is computed as the middle value (for an odd number of observations) or the average of the two middle values (for an even number of observations). Approximate 95% confidence intervals for the median can be constructed using non-parametric methods developed by Hodges and Lehmann (see Hollander and Wolfe, 1999).
- **14.4.6** Another approximate test based on the ranks uses a *one-way ANOVA* on the ranked data. This method is often used instead of the *Kruskal-Wallis test* because of its ease of use and the expectation of similar results, assuming sufficient sample size. However, you may want to take a look at the distributions of the ranked data before using this method. To do so, you can use the ODS graphics feature of SAS, as shown in Example 14.1 in which the `plots=wilcoxon` option is specified in the PROC NPAR1WAY statement ❶. The output ❷ shows similar dispersion in the ranks among groups and no distributional quirks that would prevent use of the ANOVA method. You may use the following SAS statements to rank the data for Example 14.1, and then perform the ANOVA:

```

proc rank data = psor out = rnk;
  var score;
  ranks rnkscore;
run;

proc glm data = rnk;
  class dose;
  model rnkscore = dose / ss3;
run;
quit;

```

The output (Output 14.2) shows the ANOVA results using the ranked data, which gives the p-value 0.1044 ❶ (compared with 0.1068 for the chi-square method).

OUTPUT 14.2 Analysis of Example 14.1 Using a One-Way ANOVA on the Ranks

The Kruskal-Wallis Test																														
Example 14.1: Psoriasis Evaluation in Three Groups																														
The GLM Procedure																														
Class Level Information																														
<table> <thead> <tr> <th>Class</th><th>Levels</th><th>Values</th></tr> </thead> <tbody> <tr> <td>dose</td><td>3</td><td>0.1 0.2 PBO</td></tr> </tbody> </table>							Class	Levels	Values	dose	3	0.1 0.2 PBO																		
Class	Levels	Values																												
dose	3	0.1 0.2 PBO																												
<table> <thead> <tr> <th>Number of observations Read</th><th>32</th></tr> </thead> <tbody> <tr> <td>Number of observations Used</td><td>32</td></tr> </tbody> </table>							Number of observations Read	32	Number of observations Used	32																				
Number of observations Read	32																													
Number of observations Used	32																													
Dependent Variable: rnkscore Rank for Variable score																														
<table> <thead> <tr> <th>Source</th><th>DF</th><th>Sum of Squares</th><th>Mean Square</th><th>F Value</th><th>Pr > F</th></tr> </thead> <tbody> <tr> <td>Model</td><td>2</td><td>382.645833</td><td>191.322917</td><td>2.45</td><td>0.1044</td></tr> <tr> <td>Error</td><td>29</td><td>2268.854167</td><td>78.236351</td><td></td><td></td></tr> <tr> <td>Corrected Total</td><td>31</td><td>2651.500000</td><td></td><td></td><td></td></tr> </tbody> </table>							Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	Model	2	382.645833	191.322917	2.45	0.1044	Error	29	2268.854167	78.236351			Corrected Total	31	2651.500000			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F																									
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Error	29	2268.854167	78.236351																											
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<table> <thead> <tr> <th>R-Square</th><th>Coeff Var</th><th>Root MSE</th><th>rnkscore Mean</th></tr> </thead> <tbody> <tr> <td>0.144313</td><td>53.60686</td><td>8.845131</td><td>16.50000</td></tr> </tbody> </table>							R-Square	Coeff Var	Root MSE	rnkscore Mean	0.144313	53.60686	8.845131	16.50000																
R-Square	Coeff Var	Root MSE	rnkscore Mean																											
0.144313	53.60686	8.845131	16.50000																											
<table> <thead> <tr> <th>Source</th><th>DF</th><th>Type III SS</th><th>Mean Square</th><th>F Value</th><th>Pr > F</th></tr> </thead> <tbody> <tr> <td>dose</td><td>2</td><td>382.6458333</td><td>191.3229167</td><td>2.45</td><td>0.1044❶</td></tr> </tbody> </table>							Source	DF	Type III SS	Mean Square	F Value	Pr > F	dose	2	382.6458333	191.3229167	2.45	0.1044❶												
Source	DF	Type III SS	Mean Square	F Value	Pr > F																									
dose	2	382.6458333	191.3229167	2.45	0.1044❶																									

- **14.4.7** When the *Kruskal-Wallis test* is significant, pairwise comparisons can be carried out with the *Wilcoxon rank-sum test* for each pair of groups. However, the multiplicity problem affecting the overall error rate must be considered for larger values of k. For example, the adjusted p-value method, such as a *Bonferroni* or *Holm's* method using PROC MULTTEST in SAS, can be performed as described in Appendix E. Other techniques for handling multiple comparisons when using non-parametric tests are discussed in Hollander and Wolfe (1999).

- **14.4.8** When the k levels of the Group factor can be ordered, you might want to test for association between response and increasing levels of Group. The dose-response study is an example. If the primary question is whether increasing response is related to larger doses, the *Jonckheere-Terpstra test* for ordered alternatives can be used. This is a non-parametric test that shares the same null hypothesis with the *Kruskal-Wallis test*, but the alternative is more specific:

$$H_A: \theta_1 \leq \theta_2 \leq \dots \leq \theta_k$$

with at least one strict inequality ($<$).

The *Jonckheere-Terpstra test* can be performed by specifying the JT option in the TABLES statement when using PROC FREQ in SAS. The SAS statements for this test using Example 14.1 are shown in the program that follows. (Notice that you first create a new variable, dos, so that the dose levels are in ascending order.)

```
data jttest; set psor;
  if dose = 'PBO' then dos = 0;
  else dos = dose;
run;

proc freq data = jttest;
  tables dos*score / jt;
run;
```

This test results in a p-value of 0.056 (compared with 0.107 for the *Kruskal-Wallis test*), which illustrates the increased power when using this test for the more targeted alternative. The *Jonckheere-Terpstra test* uses a chi-square approximation for large samples. Exact tests are available (see Hollander and Wolfe, 1999), and these tests can be performed by using the EXACT statement in PROC FREQ in SAS.

- **14.4.9** As illustrated in 14.4.5, using *ANOVA* methods based on the rank-transformed data is a common way to analyze data when the parametric assumptions might not be fulfilled. This approach can also be used for a two-way layout by first ranking the observations from lowest to highest across all treatment groups within each block, then using the usual *two-way ANOVA* methods (Chapter 7) on these ranks to test for significant treatment effects. Such a technique is often substituted for *Friedman's test*, which is the non-parametric analog of the *two-way ANOVA*. This approach assumes multiple observations per cell ($n_{ij} > 1$). Other types of layouts can be analyzed using similar ranking methods (see Conover and Iman, 1981).

CHAPTER 15

The *Binomial Test*

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15.1 Introduction

The *binomial test* is used to make inferences about a proportion or response rate based on a series of independent observations, each resulting in one of two possible mutually exclusive outcomes. The outcomes can be: response to treatment or no response, cure or no cure, survival or death, or in general, *event* or *non-event*. These observations are ‘binomial’ outcomes if the chance of observing the *event* of interest is the same for each observation.

In clinical trials, a common use of the *binomial test* is for estimating a response rate, p , using the number of patients (X) who respond to an investigative treatment out of a total of n studied. Special cases of the *binomial test* include two commonly used tests in clinical data analysis, *McNemar's test* (Chapter 18) and the *sign test* (discussed later in this chapter).

15.2 Synopsis

The experiment consists of observing n independent observations, each with one of two possible outcomes: *event* or *non-event*. For each observation, the probability of the *event* is denoted by p ($0 < p < 1$).

The total number of *events* in n observations, X , follows the binomial probability distribution (Chapter 1). Intuitively, the sample proportion, X/n , would be a good estimate of the unknown population proportion, p . Statistically, it is the best estimate.

The general formula for a binomial probability is:

$$\Pr(X=x) = \frac{n!}{x!(n-x)!} \cdot p^x(1-p)^{n-x}$$

where x can take integer values 0, 1, 2, ..., n .

The symbol ‘!’ is read ‘factorial’ and indicates multiplication by successively smaller integer values down to 1, i.e., $a! = (a) \cdot (a-1) \cdot (a-2) \dots (3) \cdot (2) \cdot (1)$.

(For example, $5! = 5 \times 4 \times 3 \times 2 \times 1 = 120$).

You want to determine whether the population proportion, p , differs from a hypothesized value, p_0 . If the unknown proportion, p , equals p_0 , then the estimated proportion, X/n , should be close to p_0 , i.e., X should be close to $n \cdot p_0$. When p differs from p_0 , X might be much larger or smaller than $n \cdot p_0$. Therefore, you reject the null hypothesis if X is ‘too large’ or ‘too small’.

The test summary is:

null hypothesis:	$H_0: p = p_0$
alt. hypothesis:	$H_A: p \neq p_0$
test statistic:	$X = \text{the number of events in } n \text{ observations}$
decision rule:	reject H_0 if $X \leq X_L$ or $X \geq X_U$, where X_L and X_U are chosen to satisfy $\Pr(X_L < X < X_U) \geq 1-\alpha$

Tables of binomial probabilities found in most introductory statistical texts or the SAS function PROBBNML can be used to determine X_L and X_U . This method is demonstrated in Example 15.1. Another option, using a normal approximation, is also discussed followed by how you can perform this analysis in SAS.

15.3 Examples

Example 15.1—Genital Wart Cure Rate

A company markets a therapeutic product for genital warts with a known ‘cure’ rate of 40% in the general population. In a study of 25 patients with genital warts treated with this product, patients were also given high doses of vitamin C. As shown in Table 15.1, 14 patients were ‘cured’. Is this consistent with the ‘cure’ rate in the general population?

TABLE 15.1. Raw Data for Example 15.1

Patient Number	‘Cured’ ?						
1	YES	8	YES	15	YES	22	YES
2	NO	9	NO	16	NO	23	NO
3	YES	10	NO	17	NO	24	YES
4	NO	11	YES	18	YES	25	YES
5	YES	12	NO	19	YES		
6	YES	13	YES	20	NO		
7	NO	14	NO	21	YES		

Solution

Let p represent the probability of a ‘cure’ in any randomly selected patient with genital warts and treated with Vitamin C concomitantly with the company’s product. You want to know if this unknown rate differs from the established rate of $p_0 = 0.4$. Thus,

|| **null hypothesis:** $H_0: p = 0.4$
alt. hypothesis: $H_A: p \neq 0.4$

test statistic: $X = 14$ (the number ‘cured’ out of the $n = 25$ patients treated)

decision rule: reject H_0 if $X \leq 4$ or $X \geq 15$

conclusion: Do not reject the null hypothesis. Because 14 does not lie in the rejection region, you conclude that there is insufficient evidence from this study to indicate that concomitant Vitamin C treatment has an effect on the product’s ‘cure’ rate.

The decision rule is established by finding a range of X values such that the probability of X being outside that range when the null hypothesis is true is approximately equal to the significance level. With a nominal significance level of $\alpha = 0.05$, the limits of the rejection region, 4 and 15, can be found from a table of binomial probabilities, satisfying $\Pr(X \leq 4) + \Pr(X \geq 15) \leq 0.05$. The PROBBNML function in SAS can also be used (see Section 15.4.5). Because of the discrete nature of the binomial distribution, the true significance level will usually be less than α (0.05). In this case, it is 0.044. (See Sections 15.4.1 and 15.4.2 for additional discussion on exact p-values.)

Normal Approximation

For large values of n and non-extreme values of p , a binomial response, X , can be approximated by a normal distribution with mean $n \cdot p$ and variance $n \cdot p \cdot (1-p)$. This approximation improves as n gets larger or as p gets closer to 0.5. Based on this statistical principle, another way to establish the rejection region for $\alpha = 0.05$ and large n is by computing

$$X_L = (n \cdot p_0) - 1.96(n \cdot p_0 \cdot (1 - p_0))^{1/2}$$

and

$$X_U = (n \cdot p_0) + 1.96(n \cdot p_0 \cdot (1 - p_0))^{1/2}$$

Applying these formulas to Example 15.1, you obtain

$$X_L = (25 \cdot 0.4) - 1.96(25(0.4)(0.6))^{1/2} = 5.2$$

and

$$X_U = (25 \cdot 0.4) + 1.96(25(0.4)(0.6))^{1/2} = 14.8$$

Because X can only take integer values, the rejection region becomes $X \leq 5$ and $X \geq 15$. Because of the small sample size, this approximation produces a slightly larger lower rejection region and yields an actual significance level of 0.064 (based on exact binomial probabilities). Since $X = 14$, the conclusion for Example 15.1 is to not reject H_0 at a nominal significance level of 0.05 (actual significance level of 0.064).

A more common form of the normal approximation to the *binomial test* uses as the test statistic the actual estimate of p , namely $\hat{p} = X/n$. Under the assumption of approximate normality, the test summary becomes:

null hypothesis: $H_0: p = p_0$
alt. hypothesis: $H_A: p \neq p_0$

test statistic: $Z = \frac{|\hat{p} - p_0| - \frac{1}{2n}}{\sqrt{\frac{p_0 \cdot (1-p_0)}{n}}}$

decision rule: reject H_0 if $|Z| > Z_{\alpha/2}$

Because the binomial distribution is a discrete distribution (i.e., takes integer values), while the normal distribution is continuous, the $1/(2n)$ in the numerator of the test statistic is used as a ‘continuity correction’ to improve the approximation. When H_0 is true, Z has an approximate standard normal distribution. For the commonly used value of $\alpha = 0.05$, $Z_{0.025} = 1.96$.

In Example 15.1, the test statistic based on the normal approximation is

$$Z = \frac{\left| \frac{14}{25} - 0.4 \right| - \frac{1}{50}}{\sqrt{\frac{0.4 \cdot 0.6}{25}}} = \frac{0.140}{0.098} = 1.429$$

Because 1.429 is less than 1.96, the null hypothesis is not rejected. The actual 2-sided p-value is 0.153 (Appendix A.1 or use the PROBNORM function in SAS as shown in Section 15.4.5).

SAS Analysis of Example 15.1

You can use PROC FREQ in SAS to perform the normal approximation to the *binomial test* as shown below. The variable of interest is ‘cured’ which takes the value ‘YES’ or ‘NO’. You create a 1-way table in PROC FREQ using the TABLES statement with the variable cured. The BINOMIALC, (p=) and ALPHA= options are used to perform the ‘corrected’ *binomial test* using the normal approximation with a hypothesized response rate of 0.4 and a significance level of $\alpha = 0.05$ ❶. The SAS output confirms the Z-test of 1.429 ❷ with a 2-sided p-value of 0.153 ❸.

SAS Code for Example 15.1

```
data gwart;
    input patient $ cured $ @@;
    datalines;
1 YES    2 _NO   3 YES    4 _NO   5 YES    6 YES
7 _NO   8 YES   9 _NO   10 _NO  11 YES   12 _NO
13 YES  14 _NO  15 YES  16 _NO  17 _NO  18 YES
19 YES  20 _NO  21 YES  22 YES  23 _NO  24 YES
25 YES;
run;

proc freq data=gwart;
    tables cured / binomialc (p = 0.4) alpha=0.05; ①
    exact binomial; ④
    title1 "Binomial Test";
    title2 "Example 15.1: Genital Warts Cure Rate";
run;
```

OUTPUT 15.1 SAS Output for Example 15.1

Example 15.1: Genital Warts Cure Rate				
The FREQ Procedure				
cured	Frequency	Percent	Cumulative Frequency	Cumulative Percent
YES	14	56.00	14	56.00
_NO	11	44.00	25	100.00
Binomial Proportion for cured = YES				
Proportion			0.5600	
ASE			0.0993	
95% Lower Conf Limit			0.3454	⑤
95% Upper Conf Limit			0.7746	
Exact Conf Limits				
95% Lower Conf Limit			0.3493	⑥
95% Upper Conf Limit			0.7560	
Test of H0: Proportion = 0.4				
ASE under H0			0.0980	
Z			1.4289	②
One-sided Pr > Z			0.0765	
Two-sided Pr > Z			0.1530	③
Exact Test				
One-sided Pr >= P			0.0778	
Two-sided = 2 * One-sided			0.1556	④
The asymptotic confidence limits and test include a continuity correction.				
Sample Size = 25				

15.4 Details & Notes

- **15.4.1** In Example 15.1, instead of establishing a rejection region based on the X values, an alternative approach is to compute the actual p-value, and reject H_0 if it is less than $\alpha = 0.05$. An exact one-tailed p-value can be found by computing the probability that $X \geq 14$ when $p_0 = 0.4$, which is 0.078. You double this to get an approximate 2-tailed p-value, i.e., $p = 0.156$. Since this is greater than $\alpha = 0.05$, you would not reject H_0 .

This is the method SAS uses to compute exact p-values for the *binomial test*. You may obtain these results by including the EXACT statement with the BINOMIAL option as shown in the SAS code for Example 15.1 ④.

- **15.4.2** The binomial probability distribution is the most commonly encountered discrete distribution in statistics. You've seen how exact p-values for hypothesis testing can be obtained using your knowledge of binomial probabilities. This chapter also shows how to obtain the p-values based on an asymptotic distribution. A binomial response, X , has an asymptotic normal distribution, i.e., the distribution of X becomes closer to a normal distribution as n gets large. In this case, 'large' depends on the binomial probability, p . For values of p close to 0 or 1, n needs to be larger to approach normality than when p is closer 0.5 (see Section 15.4.3).

The normal approximation to the binomial distribution is a result of the *Central Limit Theorem* (Chapter 1). Let y_i represent a binomial response for Patient i ($i = 1, 2, \dots, n$) with numeric values of 0 ('non-event') and 1 ('event'). Note that X , the number of 'events' in n trials, is the sum of the y_i 's. The probability of 'event', p , is simply the population mean of the distribution from which the y_i 's have been selected. That distribution is the binomial distribution with mean, p , and variance, $p(1-p)$. Therefore, $\hat{p} = X/n = \bar{y}$ has an approximate normal distribution with mean p and variance $p(1-p)/n$ for large n , according to the *Central Limit Theorem* (see Section 1.2). The Z-test statistic for the normal approximation to the *binomial test* uses p_0 , the hypothesized value of the unknown parameter p because the test is conducted assuming H_0 is true.

Many statistical methods, including those discussed in Chapters 13-19, are based on a binomial or other discrete distribution which also has the feature of asymptotic normality. As such, you have the option of analyzing the data using an exact test or an asymptotic test. Most of the methods in this book focus on the asymptotic tests since clinical trials, especially Phase II and III trials, usually have large enough sample sizes, and the asymptotic distributions are easier to work with. In cases where exact tests are more appropriate, you can use the EXACT statement in SAS under PROC FREQ for all of the methods discussed in Chapters 13-19.

- **15.4.3** The normal approximation to the binomial is generally a good approximation if

$$\text{and } \begin{aligned} (n \cdot p) - 2\sqrt{n \cdot p \cdot (1-p)} &\geq 0 \\ (n \cdot p) + 2\sqrt{n \cdot p \cdot (1-p)} &\leq n \end{aligned}$$

or equivalently, if

$$n \geq 4 \times \max \{(p/(1-p)), ((1-p)/p)\}$$

The minimum sample size, n , satisfying this inequality is shown in Table 15.2 for various values of p . You can see that larger n 's are required for response probabilities (p) closer to 0 or 1.

TABLE 15.2 Minimum n Required by Normal Approximation as a Function of p

P	n
0.5	4
0.4 or 0.6	6
0.3 or 0.7	10
0.2 or 0.8	16
0.1 or 0.9	36

- **15.4.4** An approximate 95% confidence interval for estimating the proportion, p , is given by

$$\hat{p} \pm 1.96 \cdot \sqrt{\frac{\hat{p} \cdot (1-\hat{p})}{n}}$$

where $\hat{p} = X/n$. If you use the continuity correction, this becomes

$$\hat{p} \pm 1.96 \cdot \sqrt{\frac{\hat{p} \cdot (1-\hat{p})}{n}} + \frac{1}{2 \cdot n}$$

With $X/n = 14/25 = 0.56$ in Example 15.1, a 95% confidence interval for p is

$$0.56 \pm (1.96 ((0.56 \times 0.44) / 25)^{1/2} + 1/50) =$$

$$0.56 \pm 0.21 = (0.35 - 0.77)$$

This is the continuity corrected asymptotic 95% confidence interval for p , sometimes referred to as the Wald confidence limits, and is shown in the SAS output ⑤. Exact confidence intervals, sometimes known as the Clopper-Pearson limits, are also provided by SAS ⑥. With SAS 9.2 and higher, you

may also obtain confidence limits using other asymptotic methods, including the Wilson, Agresti-Coull and Jeffreys methods (see SAS Documentation for details).

- **15.4.5** The SAS function PROBBNML can be used to obtain binomial probabilities for specified p and n . The PROBBNML function, PROBBNML(p,n,x), returns the probability that a binomial random variable with probability p and sample size n is less than or equal to x . The actual significance level 0.044 in Example 15.1 is found by using the SAS statement $\text{PROBBNML}(0.4,25,4) + (1 - \text{PROBBNML}(0.4,25,14))$.

The PROBNORM function in SAS can be used with the normal approximation to obtain p-values. The approximate p-value for Example 15.1 with a *Z-test* statistic of 1.429 is found in SAS as $p=2*(1 - \text{PROBNORM}(1.429)) = 0.1530$.

- **15.4.6** When $p_0 = 0.5$, the *binomial test* is sometimes called the *sign test*. A common application of the *sign test* is in testing for pre- to post-treatment changes, given information only about whether a measurement increases or decreases following treatment. The number of increases is a binomial random variable with $p = 0.5$ when the null hypothesis of no pre- to post-treatment changes is true. In SAS, you can conduct the sign test by using ($p = 0.5$) in the BINOMIAL option of the TABLES statement of PROC FREQ (or simply omit it since 0.5 is the default).
- **15.4.7** Because Example 15.1 tests for a difference from the hypothesized value in either direction, a *two-tailed test* is used. A *one-tailed test* would be used when you want to test whether the population proportion, p , is strictly *greater than* or strictly *less than* the threshold level, p_0 . Use the rejection region according to the alternative hypothesis as follows:

Type of Test	Alternative Hypothesis	Corresponding Rejection Region
two-tailed	$H_A: p \neq p_0$	reject H_0 if $Z > Z_{\alpha/2}$ or $Z < -Z_{\alpha/2}$
one-tailed (right)	$H_A: p > p_0$	reject H_0 if $Z > Z_\alpha$
one-tailed (left)	$H_A: p < p_0$	reject H_0 if $Z < -Z_\alpha$

CHAPTER 16

The Chi-Square Test

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16.1 Introduction

The *chi-square test* is used to compare two independent binomial proportions, p_1 and p_2 . In the analysis of clinical data, the binomial proportion typically represents a response rate, cure rate, survival rate, abnormality rate, or some other ‘*event*’ rate as introduced in the previous chapter. Often, you want to compare such ‘response’ rates between a treated group and a parallel control group.

The *chi-square test* is an approximate test, which may be used when the normal approximation to the binomial distribution is valid (see Chapter 15). A popular alternative, *Fisher's exact test* (Chapter 17), is based on exact probabilities and it is often applied when conditions for using the *chi-square test* are not met (see Section 16.4.3 for details).

16.2 Synopsis

Observation is made of X_1 responders out of n_1 patients who are studied in one group, and X_2 responders out of n_2 patients in a second, independent group, as shown in Table 16.1.

TABLE 16.1 Layout for the Chi-Square Test

	Number of Responders	Number of Non-Responders	Total
Group 1	X_1	$n_1 - X_1$	n_1
Group 2	X_2	$n_2 - X_2$	n_2
Combined	$X_1 + X_2$	$N - (X_1 + X_2)$	$N = n_1 + n_2$

Assume that each of the n_i patients in Group i ($i = 1, 2$) have the same chance, p_i , of responding, so that X_1 and X_2 are independent binomial random variables (see Chapter 15). The goal is to compare population ‘response’ rates (p_1 vs. p_2) based on these sample data. Compute

$$\text{NUM} = \frac{(X_1 \cdot n_2 - X_2 \cdot n_1)}{N}$$

and

$$\text{DEN} = \frac{n_1 \cdot n_2 \cdot (X_1 + X_2) \cdot (N - X_1 - X_2)}{N^3}$$

Assuming that the normal approximation to the binomial distribution is applicable (see Section 16.4.3), the *chi-square test* summary is

null hypothesis:	$H_0: p_1 = p_2$
alt. hypothesis:	$H_A: p_1 \neq p_2$
test statistic:	$\chi^2 = \frac{\text{NUM}^2}{\text{DEN}}$
decision rule:	reject H_0 if $\chi^2 > \chi^2_1(\alpha)$

The rejection region is found by obtaining the critical chi-square value based on 1 degree of freedom, denoted as $\chi^2_1(\alpha)$, from chi-square tables (Appendix A.3) or by using the SAS function CINV($1-\alpha, df$). For $\alpha = 0.05$, the critical chi-square value is 3.841 (=CINV(0.95,1) from SAS).

This computing formula for the chi-square statistic can be shown (Section 16.4.2) to be equivalent to the more popular form

$$\chi^2 = \sum_{i=1}^4 \frac{(O_i - E_i)^2}{E_i}$$

where the O_i 's and E_i 's are the observed and expected cell frequencies, respectively, as shown in Table 16.2.

TABLE 16.2 Observed (O) and Expected (E) Cell Frequencies

<i>i</i>	O_i	E_i
1	X_1	$n_1(X_1+X_2)/N$
2	X_2	$n_2(X_1+X_2)/N$
3	n_1-X_1	$n_1(N-X_1-X_2)/N$
4	n_2-X_2	$n_2(N-X_1-X_2)/N$

16.3 Examples

❖ Example 16.1—ADR Frequency with Antibiotic Treatment

A study was conducted to monitor the incidence of gastro-intestinal (GI) adverse drug reactions of a new antibiotic used in lower respiratory tract infections (LRTI). Two parallel groups were included in the study. One group consisted of 66 LRTI patients randomized to receive the new treatment and a reference group of 52 LRTI patients randomized to receive erythromycin. There were 22 patients in the test group and 28 patients in the control (erythromycin) group who reported one or more GI complaints during 7 days of treatment. Is there evidence of a difference in GI side effect rates between the two groups?

Solution

Define ‘response’ as the event that a patient develops one or more GI reactions during the study, and let p_1 and p_2 represent the probabilities that a randomly selected LRTI patient has such a ‘response’ when treated with the test drug and the control drug, respectively. The data are often summarized in a 2×2 contingency table as shown in Table 16.3.

TABLE 16.3 ‘Response’ Frequencies for Example 16.1

	Number of ‘Responders’	Number of Non-‘Responders’	Total
Test Drug	22 (33.3%)	44	66
Control	28 (53.8%)	24	52
Combined	50 (42.4%)	68	118

Compute

$$\text{NUM} = \frac{(22 \cdot 52) - (28 \cdot 66)}{118} = -5.966$$

and

$$\text{DEN} = \frac{66 \cdot 52 \cdot 50 \cdot 68}{118^3} = 7.102$$

At a significance level of 0.05, the test summary is

null hypothesis:	$H_0: p_1 = p_2$
alt. hypothesis:	$H_A: p_1 \neq p_2$
test statistic:	$\chi^2 = \frac{(-5.966)^2}{7.102} = 5.012$
decision rule:	reject H_0 if $\chi^2 > \chi^2_1(0.05) = 3.841$
conclusion:	Because $5.012 > 3.841$, you reject H_0 and conclude there is a significant difference in the incidence of GI adverse effects between treatment groups at a 0.05 level of significance.

The p-value can be obtained from SAS by using the PROBCHI function as, $p = 1 - \text{PROBCHI}(5.012, 1)$, which returns the value of 0.0252.

SAS Analysis of Example 16.1

The SAS code for conducting the *chi-square test* using PROC FREQ is shown next. The TABLES statement ❶ identifies the row and column variables that are used to form the 2×2 table. The row variable (specified first in the TABLES statement) is the treatment group (grp), and the column variable is the response (resp). The CHISQ option in the TABLES statement specifies that you want the *chi-square test*. Although not shown here, you may also have SAS print the expected cell frequencies by including the EXPECTED option in the TABLES statement.

The WEIGHT statement ❷ specifies that the response variable, CNT, is read into the data set by SAS as cell frequencies. If the response data (i.e., YES or NO) are input individually for each patient, the CNT variable is not used and the WEIGHT statement is omitted from PROC FREQ.

Output 16.1 confirms the chi-square statistic of 5.012, with a p-value of 0.0252 ❸.

SAS Code for Example 16.1

```
data adr;
    input grp resp $ cnt @@;
    datalines;
1 YES 22    1 _NO 44
2 YES 28    2 _NO 24
;

/* grp 1 = Test Drug, grp 2 = Control */
proc freq data = adr;
    tables grp*resp / chisq nopercent nocol;      ❶
    weight cnt;                                     ❷
    title1 'The Chi-Square Test';
    title2 'Example 16.1: ADR Frequency with Antibiotic
Treatment';
run;
```

OUTPUT 16.1 SAS Output for Example 16.1

```
The Chi-Square Test
Example 16.1: ADR Frequency with Antibiotic Treatment

The FREQ Procedure
Table of grp by resp

      grp          resp

Frequency,
Row Pct |     YES |     _NO |   Total
-----+-----+-----+
      1 |     22 |     44 |     66
      | 33.33 | 66.67 |
-----+-----+
      2 |     28 |     24 |     52
      | 53.85 | 46.15 |
-----+-----+
Total      50      68      118

Statistics for Table of GRP by RESP
Statistic           DF      Value      Prob
-----
Chi-Square           1      5.0119 ❸ 0.0252
Likelihood Ratio Chi-Square   1      5.0270 0.0250
Continuity Adj. Chi-Square   1      4.2070 ❹ 0.0403
Mantel-Haenszel Chi-Square   1      4.9694 0.0258
Phi Coefficient        -0.2061
Contingency Coefficient     0.2018
Cramer's V            -0.2061

Fisher's Exact Test
-----
Cell (1,1) Frequency (F)      22
Left-sided Pr <= F          0.0201
Right-sided Pr >= F         0.9925

Table Probability (P)        0.0125
Two-sided Pr <= P          0.0385
Sample Size = 118
```

Chi-Square Tests for the $g \times r$ Contingency Table

This discussion about the *chi-square test*, so far, has focused on its simplest form, namely that of a 2×2 table. In **statistics**, *chi-square test* is a generic reference for any inferential statistic that has a chi-square or approximate chi-square probability distribution. There are many situations that involve a hypothesis that can be tested with a *chi-square test*, and several different forms can be used with frequency tables.

A frequency table with g rows and r columns is called a ‘ $g \times r$ contingency table’. The row and column variables can be nominal or ordinal. Nominal levels are descriptive; ordinal levels are quantitative and have a hierarchical ordering. Examples of nominal and ordinal variables are shown in Table 16.4.

TABLE 16.4 Examples of Nominal and Ordinal Variables

Type	Variable	Levels (Categories)
Nominal	RACE	Caucasian, Black, Hispanic, Oriental
	LESION SITE	Leg, Back, Arm, Face, Chest
	TREATMENT	Active, Placebo, Reference
Ordinal	IMPROVEMENT	Worse, None, Some, Marked, Cured
	SEVERITY	None, Mild, Moderate, Severe
	DOSAGE	10 mg, 20 mg, 40 mg, 80 mg

In comparative clinical trials, it is often the case that the row variable refers to a group, such as treatment group or dose group, and the column variable refers to a response category. A typical $g \times r$ contingency table has the following layout, with X_{ij} representing the frequency of events in Group i and Response Category j .

TABLE 16.5 Layout for a $g \times r$ Contingency Table

Group	----- Response Category -----			
	1	2	...	r
1	X_{11}	X_{12}	...	X_{1r}
2	X_{21}	X_{22}	...	X_{2r}
...	...			
g	X_{g1}	X_{g2}	...	X_{gr}

The most common form of the *chi-square test* for contingency table analysis is for testing ‘general association’ between the Group and Response variables. The null hypothesis is that there is no association. The alternative hypothesis is that the distribution over response categories differs among Group levels, or more generally, that Response is ‘associated’ with Group. This is tested by using a version of the *chi-square test* in the form already introduced.

If the Response variable is ordinal, you can compare mean responses among the Group levels by assigning numerically coded values called ‘scores’ to the response categories. Here, ‘table’ or ‘integer’ scores are used, which are simply the ranks of the categories when ordered from smallest to largest. In this case, the alternative hypothesis is that mean scores differ among groups. A different form of the *chi-square test* can be used to test this hypothesis. A mean score can be computed as the sum of all scores for a specific row (i.e., score \times frequency summed over all scores) divided by the row total.

If the Group and Response variables are both ordinal, you can use another type of *chi-square* statistic to test whether there is a trend or correlation between increasing response scores and the Group scores.

The following three cases are tested using the *chi-square test* for ‘general association’ (χ^2_{GA}) based on $(g-1)(r-1)$ degrees of freedom, the *chi-square test* for ‘row mean scores differ’ (χ^2_{MSD}) based on $g-1$ degrees of freedom, and the *chi-square test* for ‘non-zero correlation’ (χ^2_{COR}) based on 1 degree of freedom, respectively, as shown in Table 16.6. Notice that the ‘correlation’ test is only meaningful when both the Response and Group categories are ordinal, and ‘mean scores’ within a row make sense only when the Response category is ordinal.

TABLE 16.6 Types of Chi-Square Tests for Contingency Tables

Response (columns)	Group (rows)	Alternative Hypothesis		
		General Association	Row Mean Scores Differ	Non-Zero Correlation
Nominal	Nominal	χ^2_{GA}	--	--
Nominal	Ordinal	χ^2_{GA}	--	--
Ordinal	Nominal	χ^2_{GA}	χ^2_{MSD}	--
Ordinal	Ordinal	χ^2_{GA}	χ^2_{MSD}	χ^2_{COR}

As an example, the 3×3 tables which follow illustrate patterns of cell frequencies that can be associated with the three alternative hypotheses above. Table (i) shows a significant general association ($p = 0.0423$), which indicates a difference among groups in the distribution of responses. Table (ii) indicates a significant difference in mean scores among groups ($p = 0.0071$). The mean scores are 1.67, 2.33, and 2.00 for Groups A, B, and C, respectively (based on ‘table’ scores). A pattern resulting in significant correlation is shown in Table (iii) ($p = 0.0017$), under the assumption that the Group factor is ordinal with A, B, and C representing increasing values (e.g., increasing dosage). Notice that the only difference between Tables (ii) and (iii) is that the row frequencies for the B and C groups have been exchanged, so that the row mean scores increase with increasing Group levels. Response frequencies for these tables are shown in Figure 16.1 by Group.

Table (i): General Association

Group	Response			Test	p-Value
	1	2	3		
A	10	15	5	χ^2_{GA}	0.0423
B	10	10	10	χ^2_{MSD}	0.2905
C	10	5	15	χ^2_{COR}	0.1159

Table (ii): Row Mean Scores Differ

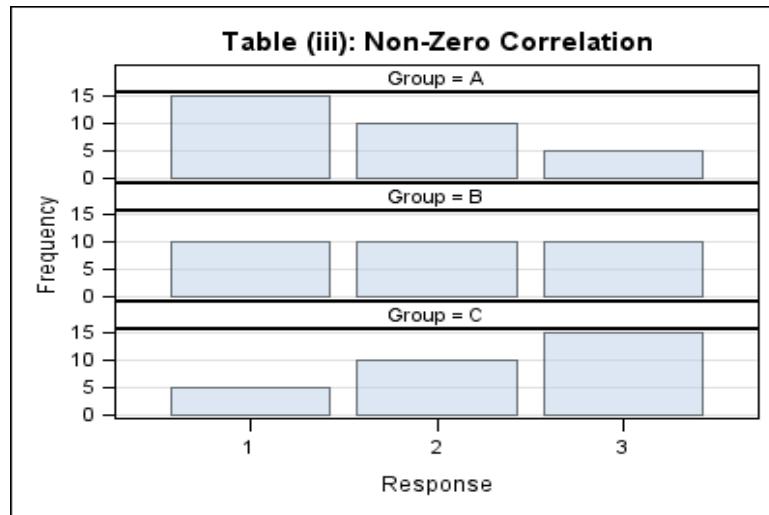
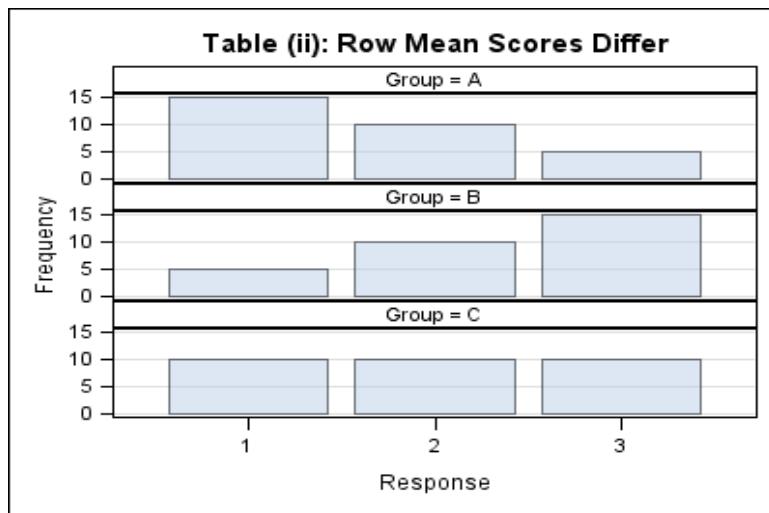
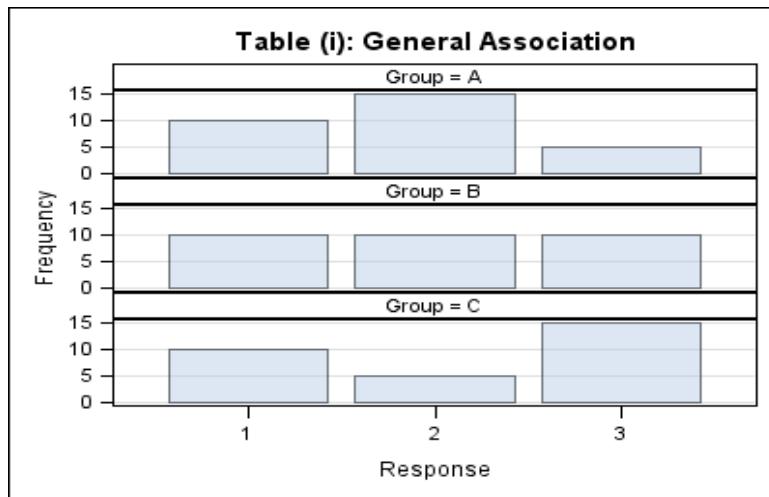
Group	Response			Test	p-Value
	1	2	3		
A	15	10	5	χ^2_{GA}	0.0423
B	5	10	15	χ^2_{MSD}	0.0071
C	10	10	10	χ^2_{COR}	0.1159

Table (iii): Non-Zero Correlation

Group	Response			Test	p-Value
	1	2	3		
A	15	10	5	χ^2_{GA}	0.0423
B	10	10	10	χ^2_{MSD}	0.0071
C	5	10	15	χ^2_{COR}	0.0017

The *chi-square statistic* for general association can be computed in a way similar to that demonstrated for the 2×2 table. The mean scores chi-square value is a function of the sample group means and variances, and the correlation statistic is, computationally, a multiple of the correlation coefficient. These can easily be obtained in SAS using the CMH option in the TABLES statement in PROC FREQ, as demonstrated in the code for the sample data in Tables (i), (ii), and (iii) that follows.

Figure 16.1 Distribution of Response by Group



**Example SAS Code for Chi-Square Tests for Tables (i), (ii), and (iii)
Using SAS**

```

data g_by_r;
    input tbl group $ resp $ cnt @@;
    datalines;
1 A 1 10    1 A 2 15    1 A 3 5
1 B 1 10    1 B 2 10    1 B 3 10
1 C 1 10    1 C 2 5     1 C 3 15
2 A 1 15    2 A 2 10    2 A 3 5
2 B 1 5     2 B 2 10    2 B 3 15
2 C 1 10    2 C 2 10    2 C 3 10
3 A 1 15    3 A 2 10    3 A 3 5
3 B 1 10    3 B 2 10    3 B 3 10
3 C 1 5     3 C 2 10    3 C 3 15
;

ods select cmh;
proc freq order = data data = g_by_r;
    by tbl;
    tables group*resp / cmh nopercent nocol;
    weight cnt;
    title1 'Chi-Square Test for 3-by-3 Table';
run;

```

OUTPUT 16.2 CMH Tests for 3-by-3 Tables

Chi-Square Test for 3-by-3 Table				
The FREQ Procedure				
Summary Statistics for group by resp Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
<hr/> -----tbl=1-----				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	2.4722	0.1159
2	Row Mean Scores Differ	2	2.4722	0.2905
3	General Association	4	9.8889	0.0423
<hr/> -----tbl=2-----				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	2.4722	0.1159
2	Row Mean Scores Differ	2	9.8889	0.0071
3	General Association	4	9.8889	0.0423
<hr/> -----tbl=3-----				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	9.8889	0.0017
2	Row Mean Scores Differ	2	9.8889	0.0071
3	General Association	4	9.8889	0.0423

Binomial Response with More Than Two Groups

The binomial response ($r=2$) for more than two groups ($g>2$) is a special, but frequently encountered, case of the $g \times r$ table just discussed. In this case, the null hypothesis is that response rates are the same among all groups ($p_1 = p_2 = \dots = p_g$).

One alternative hypothesis is that response rates differ among groups. With binomial responses, the hypothesis of ‘general association’ is equivalent to the hypothesis of ‘row mean scores differ’ because the row mean is a linear function of the probability of response, as binomial response categories can always be coded 0 (‘no response’) and 1 (‘response’). When the Group factor is ordinal, the test for ‘non-zero correlation’ is actually a linear ‘trend’ test. This can be used to test for dose response, for example, when the Group factor represents dose level.

Example 16.2—Comparison of Dropout Rates for 4 Dose Groups

The following table summarizes dropout rates for patients randomized to 4 dose groups in a clinical study. (i) Is there a difference in dropout rates among groups, and (ii) do dropout rates have a linear correlation with dosage?

TABLE 16.7 Frequency Table for Example 16.2

Group	----- Dropout? -----	
	YES	NO
10 mg	5	35
20 mg	6	29
40 mg	10	28
80 mg	12	27

SAS Analysis of Example 16.2

The CMH option in the TABLES statement of PROC FREQ, shown in the SAS code which follows, produces the output entitled ‘Cochran-Mantel-Haenszel Statistics’, which shows the tests for ‘general association’, ‘row mean scores differ’ and ‘non-zero correlation’ (Output 16.3). You see $\chi^2_{GA} = \chi^2_{MSD} = 4.7516$ based on 3 degrees of freedom with a p-value of 0.1909 ❸. From this, you can conclude that response rates are not significantly different among the dose groups.

However, the *chi-square test* for non-zero correlation, χ^2_{COR} is 4.217 based on 1 degree of freedom with a p-value of 0.0400 ❹, which indicates a significant trend in response rates with increasing dosage. It’s not unusual to be unable to detect a difference in response rates among groups, yet find that a dose-response trend exists, as this example illustrates.

The *chi-square test* produced by the CHISQ option in the TABLES statement is also a test of ‘general association’ ⑦. This is simply a multiple ($N/(N-1)$) of χ^2_{GA} , and the two forms are essentially equivalent for large N. The Mantel-Haenszel *chi-square test* ⑧ is equivalent to χ^2_{COR} ⑥ in a non-stratified layout as presented here.

SAS Code for Example 16.2

```
data dorate;
    input dose_mg resp $ cnt @@;
    datalines;
10 YES 5    10 _NO 35
20 YES 6    20 _NO 29
40 YES 10   40 _NO 28
80 YES 12   80 _NO 27
;

proc freq data = dorate;
    tables dose_mg*resp / chisq cmh trend nopercent nocol;
    weight cnt;
    title1 'The Chi-Square Test';
    title2 'Example 16.2: Comparison of Dropout Rates for 4
Dose Groups';
run;
```

OUTPUT 16.3 SAS Output for Example 16.2

The Chi-Square Test				
Example 16.2: Comparison of Dropout Rates for 4 Dose Groups				
The FREQ Procedure				
Table of dose_mg by resp				
dose_mg resp				
Frequency,				
Row	Pct	YES	_NO	Total
-----+-----+-----+-----+				
10	5	35	40	
12.50	87.50			
-----+-----+-----+-----+				
20	6	29	35	
17.14	82.86			
-----+-----+-----+-----+				
40	10	28	38	
26.32	73.68			
-----+-----+-----+-----+				
80	12	27	39	
30.77	69.23			
-----+-----+-----+-----+				
Total		33	119	152

OUTPUT 16.3 SAS Output for Example 16.2 (continued)

Statistics for Table of dose_mg by resp				
Statistic	DF	Value	Prob	
Chi-Square	3	4.7831	0.1884	7
Likelihood Ratio Chi-Square	3	4.9008	0.1792	
Mantel-Haenszel Chi-Square	1	4.2171	0.0400	8
Phi Coefficient		0.1774		
Contingency Coefficient		0.1747		
Cramer's V		0.1774		
 Cochran-Armitage Trend Test				
Statistic (Z)		2.0603	0.9	
One-sided Pr > Z		0.0197		
Two-sided Pr > Z		0.0394		
 Sample Size = 152				
 Summary Statistics for dose_mg by resp				
Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	4.2171	0.0400 6
2	Row Mean Scores Differ	3	4.7516	0.1909
3	General Association	3	4.7516	0.1909 5
 Total Sample Size = 152				

Multinomial Responses

Comparison of two groups ($g=2$) when there are more than two response categories ($r > 2$) is also a special case of the $g \times r$ table that was discussed previously. In this case, you use a $2 \times r$ table, where $r (>2)$ is the number of response levels. For example, if patients randomized to active and placebo groups had responses categorized as ‘none’, ‘partial’ or ‘complete’, the 2×3 table would be

TABLE 16.8 Layout for a $2 \times r$ Contingency Table

Group	Response		
	None	Partial	Complete
Active	X_{11}	X_{12}	X_{13}
Placebo	X_{21}	X_{22}	X_{23}

The hypothesis of ‘general association’ is equivalent to the hypothesis that the distribution of responses differs between groups. This test can be used regardless of whether the response levels are ordinal or nominal. When the response is ordinal, the ‘row mean scores differ’ hypothesis is equivalent to the ‘non-zero correlation’ hypothesis in the $2 \times r$ table.

❖ Example 16.3—Active vs. Placebo Comparisons of Degree of Response

The following table contains the number of patients who show complete, partial, or no response after treatment with either active medication or a placebo. Is there a difference between treatment groups in response distributions?

TABLE 16.9 Frequency Table for Example 16.3

Group	Response		
	None	Partial	Complete
Active	16	26	29
Placebo	24	26	18

SAS Analysis of Example 16.3

The CMH option in the TABLES statement in PROC FREQ requests the *chi-square tests* for general association and linear correlation, as shown in the SAS code that follows. In Output 16.4, you see a chi-square value of 4.0727 based on 1 degree of freedom, and a p-value of 0.0436 ⑩, which indicates a significant correlation or ‘trend’ between treatment group and response. The test for general association ⑪ is non-significant ($p=0.1299$), which indicates a lack of evidence of differing response distributions between the active and the placebo groups.

SAS Code for Example 16.3

```

data multi;
    input trt $ resp $ cnt @@;
    datalines;
ACT NONE 16 ACT PART 26 ACT FULL 29
PBO NONE 24 PBO PART 26 PBO FULL 18
;

proc freq order = data data = multi;
    tables trt*resp / cmh chisq trend nopercent nocol;
    weight cnt;
    title1 'The Chi-Square Test';
    title2 'Example 16.3: Active vs. Placebo Comparison of
Degree of Response';
run;

```

OUTPUT 16.4 SAS Output for Example 16.3

The Chi-Square Test
Example 16.3: Active vs. Placebo Comparison of Degree of Response

The FREQ Procedure

Table of trt by resp

trt	resp			
Frequency				
Row Pct	NONE	PART	FULL	Total
ACT	16	26	29	71
	22.54	36.62	40.85	
PBO	24	26	18	68
	35.29	38.24	26.47	
Total	40	52	47	139

Statistics for Table of trt by resp

Statistic	DF	Value	Prob

Chi-Square	2	4.1116	0.1280
Likelihood Ratio Chi-Square	2	4.1446	0.1259
Mantel-Haenszel Chi-Square	1	4.0727	0.0436
Phi Coefficient		0.1720	
Contingency Coefficient		0.1695	
Cramer's V		0.1720	

Cochran-Armitage Trend Test

Statistic (Z) 2.0254
One-sided Pr > Z 0.0214
Two-sided Pr > |Z| 0.0428

⑫

Sample Size = 139

Summary Statistics for trt by resp

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob

1	Nonzero Correlation	1	4.0727	0.0436 ⑩
2	Row Mean Scores Differ	1	4.0727	0.0436
3	General Association	2	4.0821	0.1299 ⑪

Total Sample Size = 139

16.4 Details & Notes

- **16.4.1** An equivalent test to the *chi-square test* for comparing two binomial proportions is based on the Z-test statistic as follows:

$$Z = \frac{\hat{p}_1 - \hat{p}_2}{\sqrt{\bar{p}(1-\bar{p}) \cdot \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

where $\hat{p}_i = \frac{X_i}{n_i}$ and $\bar{p} = \frac{(X_1 + X_2)}{(n_1 + n_2)}$

When H_0 is true and values for n_1 and n_2 are large, Z has an approximate standard normal distribution, and you reject H_0 if $|Z| > Z_{\alpha/2}$.

Because the square of a standard normal variable is a chi-square with 1 degree of freedom, Z^2 has a chi-square distribution (see Appendix B) and can be shown, algebraically, to be equivalent to the chi-square test statistic introduced in Section 16.2. In Example 16.1, the response rate estimates are found as follows:

TABLE 16.10 Estimating Response Rates for Example 16.1

	Test Group (i=1)	Control Group (i=2)	Combined
X_i	22	28	50
n_i	66	52	118
Est. of p	0.3333	0.5385	0.4237

and the Z-test statistic is

$$\begin{aligned} Z &= \frac{0.3333 - 0.5385}{\sqrt{(0.4237) \cdot (0.5763) \cdot \left(\frac{1}{66} + \frac{1}{52}\right)}} \\ &= -2.2387 \end{aligned}$$

Because $2.2387 > 1.96$, you reject H_0 based on the Z-statistic at a 0.05 level of significance.

Notice that $Z^2 = (-2.2387)^2 = 5.012$ is the chi-square value previously obtained. The critical chi-square value with 1 degree of freedom (from the chi-square tables) corresponds to the square of the critical Z value from the normal tables. For $\alpha = 0.05$, the critical value is $\chi^2(0.05) = 3.841 = 1.96^2 = (Z_{0.025})^2$.

- **16.4.2** Section 16.2 notes the equivalence of the two forms of the chi-square statistic:

$$\chi^2 = \sum_{i=1}^4 \frac{(O_i - E_i)^2}{E_i} = \frac{\text{NUM}^2}{\text{DEN}}$$

You can show this algebraically by first observing that $\text{NUM}^2 = (O_i - E_i)^2$, and that $(O_i - E_i)^2$ is the same for all i ($= 1, 2, 3, 4$ in Table 16.2). Furthermore,

$$\sum_{i=1}^4 \frac{1}{E_i} = \frac{1}{\text{DEN}}$$

Note also that the formulas for the expected cell frequencies in Table 16.2,

$$E_i = n_i \cdot \bar{p}$$

are based on the assumption that H_0 is true. The variance of X_i is given by

$$\text{var}(X_i) = n_i \cdot \bar{p} \cdot (1 - \bar{p}) = \frac{n_i}{N^2} \cdot (X_1 + X_2)(N - (X_1 + X_2))$$

so that

$$\begin{aligned} \text{var}(\text{NUM}) &= \text{var}\left(\frac{X_1 \cdot n_2 - X_2 \cdot n_1}{N}\right) \\ &= \frac{1}{N^2} \cdot (n_2^2 \cdot \text{var}(X_1) + n_1^2 \cdot \text{var}(X_2)) \\ &= \text{DEN} \end{aligned}$$

Because O_i has an asymptotic normal distribution (see Section 15.4.2), you can see that, for sufficiently large sample size, the hypothesis test statistic is simply the square of a standard normal random variable under H_0 , which is a chi-square statistic with 1 degree of freedom (Appendix B).

- **16.4.3** In conducting a two-sample binomial comparison using either the *chi-square test* or the Z-statistic, the normal approximation to the binomial distribution must be valid. The tests described here might not be valid if X_i or $n_i - X_i$ is small for $i = 1$ or 2. Generally, the analyst should be wary of results that use this approximate test if any cell frequency is less than 5. *Fisher's exact test* (Chapter 17) might be applicable for cases that involve small cell frequencies.

The normal approximation to the binomial is usually a good approximation if

$$n_i \cdot p_i - 2\sqrt{n_i \cdot p_i \cdot (1 - p_i)} \geq 0$$

and

$$n_i \cdot p_i + 2\sqrt{n_i \cdot p_i \cdot (1 - p_i)} \leq n_i$$

for $i = 1$ and $i = 2$ (see Section 15.4.2).

For small values of n_1 and n_2 , the normal approximation can be improved by adjusting the test statistic with a ‘continuity correction’, $C = 0.5(1/n_1 + 1/n_2)$. The adjustment is made by subtracting C from the numerator of the Z-test statistic if the numerator is greater than 0, or by adding C to the numerator of Z if it is less than 0.

In Example 16.1, the Z-statistic using the continuity correction is

$$Z = \frac{(0.3333 - 0.5385) + 0.5\left(\frac{1}{66} + \frac{1}{52}\right)}{\sqrt{0.4237 \cdot (0.5763) \cdot \left(\frac{1}{66} + \frac{1}{52}\right)}} = -2.051$$

The continuity-corrected chi-square value is the square of this Z-value, $(-2.051)^2 = 4.207$. This adjusted value is shown in Output 16.1, with a p-value of 0.0403 ❷.

- **16.4.4** An approximate 95% confidence interval for the proportion, p_i , is given by

$$\hat{p}_i \pm 1.96 \cdot \sqrt{\frac{\hat{p}_i \cdot (1-\hat{p}_i)}{n_i}} \quad \text{where} \quad \hat{p}_i = \frac{X_i}{n}$$

An approximate 95% confidence interval for the difference in proportions, $p_1 - p_2$, is given by

$$(\hat{p}_1 - \hat{p}_2) \pm 1.96 \cdot \sqrt{\frac{\hat{p}_1 \cdot (1-\hat{p}_1)}{n_1} + \frac{\hat{p}_2 \cdot (1-\hat{p}_2)}{n_2}}$$

With $X_1/n_1 = 22/66 = 0.333$ and $X_2/n_2 = 28/52 = 0.538$ in Example 16.1, a 95% confidence interval for $p_1 - p_2$ is

$$0.333 - 0.538 \pm 1.96 \cdot \left(\frac{(0.333 \cdot 0.667)}{66} + \frac{(0.538 \cdot 0.462)}{52} \right)^{\frac{1}{2}} =$$

$$-0.205 \pm 0.177 = (-0.382 \text{ to } -0.028)$$

You can use the RISKDIFF option in the TABLES statement of PROC FREQ to obtain these confidence limits using SAS, or the RISKDIFFC option if you wish to include a continuity correction (recommended for smaller values of n_1 and n_2).

- **16.4.5** Because Example 16.1 tests for a difference from the hypothesized value in either direction, a two-tailed test is used. A one-tailed test would be used if you want to test whether one population proportion is strictly *greater than* or *strictly less* than the other. Use the rejection region according to the alternative hypothesis as follows:

Type of Test	Alternative Hypothesis	Corresponding Rejection Region
two-tailed	$H_A: p \neq p_2$	reject H_0 if $Z > Z_{\alpha/2}$ or $Z < -Z_{\alpha/2}$
one-tailed (right)	$H_A: p > p_2$	reject H_0 if $Z > Z_\alpha$
one-tailed (left)	$H_A: p < p_2$	reject H_0 if $Z < -Z_\alpha$

Notice that the *chi-square test* is a two-tailed test because large chi-square values within the rejection region occur when Z is a very large positive *or* large negative value. To conduct a one-tailed *chi-square test*, the nominal significance level, α , should be doubled when looking up the critical chi-square values. The critical values for a rejection region that correspond to a significance level of $\alpha = 0.05$ are

Test	One-Tailed	Two-Tailed
Z-Test	1.645	1.960
Chi-Square	2.706	3.841

The p-value for a *chi-square test* in the SAS output corresponds to a two-tailed test. The one-tailed p-value is found by halving this value.

- **16.4.6** For the case of $n_1 = n_2$, an approximate method for estimating sample sizes to detect a difference in response rates, $\Delta = p_1 - p_2$, is shown in Chapter 2.
- **16.4.7** In addition to the *chi-square test* for ‘non-zero correlation’, the *Cochran-Armitage test* for trend can also be used to test for linear correlation between a binomial response and an ordinal Group variable. This test is based on a *Z-test* statistic that has the standard normal distribution for large N. The *Mantel-Haenszel* chi-square and the *Cochran-Armitage test* for trend can be used interchangeably when Response and Group are ordinal factors and one of these factors has only two levels. When the total sample size, N, is large, they are equivalent. The *Mantel-Haenszel* chi-square is simply Z^2 adjusted by a factor of $(N-1)/N$.

For Example 16.2, the *Cochran-Armitage test* results are printed by using the TREND option in the TABLES statement in PROC FREQ. Output 16.3 shows a Z statistic of 2.0603 ⑨. With N=152, the *Mantel-Haenszel* chi-square can be confirmed as $(151/152) \cdot 2.0603^2 = 4.217$.

Example 16.3 also includes the TREND option in the TABLES statement to show the results of the *Cochran-Armitage test*. As shown in Output 16.4 ⑫, the trend test is significant with a two-tailed p-value of 0.0428.

- **16.4.8** When comparing binomial proportions among more than two groups, a significant test for general association would imply that response rates differ for at least one pair of group levels. Multiple comparisons using a series of 2×2 tables can be used to determine which pairs actually differ. This might require multiplicity adjustments for multiple comparisons, which are discussed in Appendix E.
- **16.4.9** When there are only two categories (i.e., $g=2$ or $r=2$), the scores of 0 and 1 can be assigned to the levels regardless of whether the factor is nominal or ordinal. Therefore, when there are only two groups ($g=2$), the alternative hypothesis of ‘row mean scores differ’ is equivalent to the ‘non-zero correlation’ hypothesis. When there are only two response categories ($r=2$), the hypothesis of ‘general association’ is equivalent to the ‘row mean scores differ’ hypothesis.
- **16.4.10** ‘Table’ scores are the simplest and most commonly used scores for ordinal response categories when conducting *chi-square tests*. SAS uses table scores by default in PROC FREQ, as noted in Example 16.3. One criticism in using table scores is that, because they are equally spaced, they might not give an accurate representation of the ordinal nature of categories, which are thought not to be equally spaced. For example, if response is ‘degree of improvement’ with categories ‘none’, ‘some’, ‘marked’, and ‘cured’, the relative difference between ‘marked’ and ‘cured’ might be clinically more important than the difference between ‘none’ and ‘some’.

Rank, log-rank, ridit, and modified ridit scores are alternatives to the table scores available with PROC FREQ in SAS (specified by including the “SCORES = ” option). Of these, modified ridit scores are probably the most frequently encountered in clinical trials. These scores are based on the mid-ranks of the column totals, standardized by dividing by $(N+1)$.

For example, suppose 50 patients were classified in four response categories as ‘none’ ($n=20$), ‘some’ ($n=13$), ‘marked’ ($n=10$), and ‘cured’ ($n=7$). Computation of the modified ridit scores is shown in Table 16.11.

TABLE 16.11 Sample Calculation of Modified Ridit Scores

	None	Some	Marked	Cured
Column Total	20	13	10	7
Midrank	10.5	27	38.5	47
Mod. Ridit Score	0.206	0.529	0.755	0.922

The SCORES=MODRIDIT option in the TABLES statement in PROC FREQ is used to perform the *chi-square test* based on modified ridit scores in SAS.

Note: Mid-ranks are computed as

None:	$(20 + 1) / 2 = 10.5$
Some:	$(20 + (13 + 1) / 2) = 27$
Marked	$(20 + 13 + (10 + 1)/2) = 38.5$
Cured:	$(20 + 13 + 10 + (7 + 1) / 2) = 47$

Custom scoring systems can also be used when the relative importance of the response categories is well defined. In the preceding example, you can assign scores of 0, 2, 5, and 10, for example, to reflect the clinical importance of the response relative to other responses. However, in the rare cases when such relative importance can be meaningfully assigned, it is usually easier to treat the response as a numeric rather than categorical variable when determining the best analysis to use. In such cases, many analysts prefer to simply reassign numeric values to each response category in the input data set. If the relative clinical importance of the response categories is not clear, you should examine the robustness of the results under various scoring assignments before making a conclusion.

Caution! When using SAS with a character-valued ordinal response variable, you must check that SAS picks up the correct ordering before PROC FREQ assigns the scores. There are a number of ways to do this, such as using the ORDER option in PROC FREQ, or simply reassigning values in the data step as noted above. The same caveat applies to the Group factor when there are more than 2 groups of an ordinal nature.

- **16.4.11** The *chi-square tests* that were discussed for ordinal responses are invariant over linear transformations of the scores. For example, if there are four ordered-response categories as shown in Table 16.11, you can code the categories by using table scores of 1-2-3-4, or scores based on any linear transformation of these scores, such as 0-1-2-3 or 5-10-15-20, and obtain the same results. The same invariance principle holds when using modified ridit scores. The modified ridit scores found in Table 16.11 can be compared with the table scores using the linear transformation shown in Table 16.12.

TABLE 16.12 Comparison of Ridit Scores with Table Scores

	None	Some	Marked	Cured
Mod. Ridit Score	0.206	0.529	0.755	0.922
Subtract 0.206	0	0.323	0.549	0.716
Multiply by 4.19	0	1.35	2.30	3.00
Table Scores	0	1	2	3

This representation more easily depicts the spacing of the modified ridit scores relative to the table scores. Using a similar approach, examples of modified ridit scores transformed to a 0-3 interval for comparison with the table scores are shown in Table 16.13 for various overall distributions of the response category (i.e., column totals). Notice that the modified ridit scores are equivalent to table scores when the distribution is uniform.

TABLE 16.13 Comparison of Ridit Scores for Various Marginal Distributions

Distribution		None	Some	Marked	Cured
Uniform	Column Total	25	25	25	25
	Scaled Mod. Ridit	0	1	2	3
Normal	Column Total	10	40	40	10
	Scaled Mod. Ridit	0	0.83	2.17	3
Bimodal	Column Total	40	10	10	40
	Scaled Mod. Ridit	0	1.25	1.75	3
Exponential	Column Total	60	20	14	6
	Scaled Mod. Ridit	0	1.80	2.55	3
Skewed	Column Total	5	15	55	25
	Scaled Mod. Ridit	0	0.35	1.59	3

- **16.4.12** In a $g \times r$ contingency table with ordinal responses, the *chi-square test* for the ‘row mean scores differ’ hypothesis is identical to the *Kruskal-Wallis test* (Chapter 14) when rank or modified ridit scores are used. This is clear when you recognize that a contingency table is simply a summary of responses in which frequencies represent the numbers of tied values. The *Kruskal-Wallis test* is based on ranks of the data with average ranks assigned to tied values. These are the same as the mid-ranks used in the modified ridit scores.
- **16.4.13** The *chi-square test* is generally a good approximation in a $g \times r$ table when none of the cell frequencies are zero and fewer than 20% of the cells have frequency less than 5, as a rule of thumb. Tables with many smaller frequencies can be analyzed using exact methods, which are based on complex enumeration algorithms.

A *generalized Fisher's exact test*, as discussed in Chapter 17 (see Section 17.4.4), exact Pearson chi-square and a likelihood ratio exact test, each used to test general association, and a Mantel-Haenszel exact test for 'linear association', are all available in SAS. These tests can be conducted by including the FISHER, PCHI, LRCHI, or MHCHI option, respectively, in the EXACT statement in PROC FREQ. (For more information, see the *SAS/STAT User's Guide* for PROC FREQ.)

- **16.4.14** ODS Graphics can be used to quickly obtain histograms of the response frequencies using PROC FREQ. Figure 16.1 was created by simply turning on ODS GRAPHICS in PROC FREQ, and then using the ODS Graphics Editor, available in SAS 9.2 and later, to make subtle cosmetic adjustments.

CHAPTER 17

Fisher's Exact Test

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17.1 Introduction

Fisher's exact test is an alternative to the *chi-square test* (Chapter 16) for comparing two independent binomial proportions, p_1 and p_2 . This method is based on computing exact probabilities of observing a given result or a more extreme result, when the hypothesis of equal proportions is true. *Fisher's exact test* is useful when the normal approximation to the binomial might not be applicable, such as in the case of small cell sizes or extreme proportions.

17.2 Synopsis

Using the same notation as in Chapter 16, you observe X_1 'responders' out of n_1 patients studied in one group and X_2 'responders' of n_2 patients in a second independent group, as shown in Table 17.1.

TABLE 17.1 Layout for Fisher's Exact Test

	Number of Responders	Number of Non-Responders	Total
Group 1	X_1	n_1-X_1	n_1
Group 2	X_2	n_2-X_2	n_2
Combined	X_1+X_2	$N-(X_1+X_2)$	$N = n_1 + n_2$

Given equal proportions, $p_1 = p_2$, the probability of observing the configuration shown in Table 17.1, when the marginal totals are fixed, is found by the ‘hypergeometric probability distribution’ as

$$\text{prob} = \frac{\binom{n_1}{X_1} \cdot \binom{n_2}{X_2}}{\binom{N}{X_1+X_2}} \quad \text{where } \binom{a}{b} = \frac{a!}{b! \cdot (a-b)!}$$

is the combinatorial symbol that represents “the number of ways ‘b’ items can be selected from a set of ‘a’ items”. (Note: The symbol ‘!’ is read ‘factorial’ with $a! = a(a-1)(a-2)\dots(3)(2)(1)$. For example, $5! = (5)(4)(3)(2)(1) = 120$.) The probability of the table configuration simplifies to

$$\text{prob} = \frac{(X_1+X_2)! \cdot (N - X_1 - X_2)! \cdot (n_1)! \cdot (n_2)!}{N! \cdot X_1! \cdot X_2! \cdot (n_1 - X_1)! \cdot (n_2 - X_2)!}$$

The p-value for the test, Fisher's exact probability, is the probability of the observed configuration plus the sum of the probabilities of all other configurations with a more extreme result for fixed row and column totals.

17.3 Examples

Example 17.1—CHF Incidence in CABG after ARA

A new adenosine-releasing agent (ARA), thought to reduce side effects in patients undergoing coronary artery bypass surgery (CABG), was studied in a pilot trial that enrolled 35 patients who received active medication and 20 patients who received a placebo. Follow-up observation revealed that 2 patients who received active medication and 5 patients who received the placebo had shown symptoms of congestive heart failure (CHF) within 90 days post surgery. Is this evidence of a reduced rate of CHF for patients treated with the ARA compound?

Solution

Let p_1 and p_2 represent the CHF rates for the active-medication and placebo groups, respectively. You want to test for equal proportions vs. the one-tailed alternative because you are looking for improvement.

$$H_0: p_1 = p_2$$

$$H_A: p_1 < p_2$$

The summary results are shown in Table 17.2.

TABLE 17.2 CHF Frequency Summary for Example 17.1

	Active Group (i=1)	Placebo Group (i=2)	Combined
X _i	2	5	7
n _i	35	20	55
Estimate of p	0.057	0.250	0.127

The conditions for using the *chi-square test* (Chapter 16) are not met in this case because of the small cell sizes. However, *Fisher's exact test* can be used as demonstrated next. The observed table and tables with a more extreme result that have the same row and column totals are shown in Table 17.3.

TABLE 17.3 Configuration of 'Extreme' Tables for Calculation of Fisher's Exact Probability

Table (i)		Table (ii)		Table (iii)		Row Total
		X	n-X	X	n-X	n
ACT	2	33		1	34	
	5	15		6	14	
PBO			7	48		
					7	48
Column Total		7	48	7	48	55

Under the null hypothesis, H₀, the probability for Table (i) is found by

$$\text{prob}_1 = \frac{(7)! \cdot (48)! \cdot (35)! \cdot (20)!}{(55)! \cdot (2)! \cdot (5)! \cdot (33)! \cdot (15)!} = 0.04546$$

Similarly, the probabilities for Tables (ii) and (iii) can be computed as prob₂ = 0.00669 and prob₃ = 0.00038, respectively. The exact one-tailed p-value is p = 0.04546 + 0.00669 + 0.00038 = 0.05253. Because this value is greater than 0.05, you would not reject the hypothesis of equal proportions at a significance level of 0.05. (This is close to 0.05, however, and it might encourage a researcher to conduct a larger study).

SAS Analysis of Example 17.1

Fisher's exact test can be performed by using the FISHER option in the TABLES statement in PROC FREQ, as shown in the SAS code for Example 17.1 ❶. This example uses the WEIGHT statement ❷ because the summary results are input. If you use the data set that contains the observations for individual patients, the WEIGHT statement would be omitted. Notice that, with a 2×2 table, either the FISHER or the CHISQ option in the TABLES statement will print both the *chi-square test* and the *Fisher's exact test* results.

Fisher's exact probability is printed in Output 17.1, with a one-tailed value of 0.0525 ❸. Notice that SAS prints a warning about using the *chi-square test* when cell sizes are too small.

SAS Code for Example 17.1

```
data cabg;
    input grp resp $ cnt  @@;
    datalines;
1 YES   2     1 _NO 33
2 YES   5     2 _NO 15
;

/* grp 1 = Active Group,  grp 2 = Placebo */
proc freq data = cabg;
    tables grp*resp / fisher nopercent nocol;      ❶
    weight cnt;                                     ❷
    title1 "Fisher's Exact Test";
    title2 'Example 17.1:  CHF Incidence in CABG after ARA';
run;
```

OUTPUT 17.1 SAS Output for Example 17.1

Fisher's Exact Test
 Example 17.1: CHF Incidence in CABG after ARA

The FREQ Procedure

Table of grp by resp

grp	resp			
Row	Pct	YES	_NO	Total
1		2	33	35
		5.71	94.29	
2		5	15	20
		25.00	75.00	
Total		7	48	55

Statistics for Table of grp by resp

Statistic	DF	Value	Prob
Chi-Square	1	4.2618	0.0390
Likelihood Ratio Chi-Square	1	4.1029	0.0428
Continuity Adj. Chi-Square	1	2.7024	0.1002
Mantel-Haenszel Chi-Square	1	4.1843	0.0408
Phi Coefficient		-0.2784	
Contingency Coefficient		0.2682	
Cramer's V		-0.2784	

WARNING: 50% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Fisher's Exact Test

Cell (1,1) Frequency (F)	2	
Left-sided Pr <= F	0.0525	③
Right-sided Pr >= F	0.9929	
Table Probability (P)	0.0455	⑤
Two-sided Pr <= P	0.0857	④

Sample Size = 55

17.4 Details & Notes

- **17.4.1** *Fisher's exact test* is considered a non-parametric test because it does not rely on any distributional assumptions.
- **17.4.2** Manual computations for Fisher's exact probabilities can be very tedious if you use a calculator, even with relatively small cell sizes. A statistical program, such as SAS, is recommended to facilitate the computations. *Fisher's exact test* is valid no matter how large the sample sizes

are, but it is most useful for small cell frequencies due to limitations on computational resources. As cell sizes get larger, SAS applies computational algorithms to make the calculations more efficient. You may also use Monte Carlo techniques with PROC FREQ (see SAS documentation) to gain efficiency in the computations of Fisher's p-values. As noted in Chapter 16, the *chi-square test* produces very accurate results for comparing binomial proportions even when some cell frequencies are 5 or less (see Section 16.4.3).

- **17.4.3** Example 17.1 is carried out as a 1-tailed or 1-sided test because the interest is in whether the side effect rate for the active treatment is less than that of placebo, i.e., 'improvement'. If you want to test for an overall difference in side effect proportions without specifying which treatment is better, you can use *Fisher's exact test* with a two-sided alternative. Some analysts will simply double the one-sided p-value to obtain the two-sided result. However Fisher's probabilities are not necessarily symmetric, and this method is usually overly conservative.

To obtain the two-sided p-value, first compute the probabilities associated with all possible tables that have the same row and column totals. The 2-sided p-value is then found by adding the probability of the observed table with the sum of the probabilities of each table whose probability is less than that of the observed table. To obtain the *two-sided test* for Example 17.1 in this way, first compute the probabilities for each table as follows:

TABLE 17.4 Individual Table Probabilities for Example 17.1

Table	Group	Responders	Non- Responders	Probabilities	
1	Active	0	35	0.0004	+
	Placebo	7	13		
2	Active	1	34	0.0067	+
	Placebo	6	14		
3	Active	2	33	0.0455	+ (Observed)
	Placebo	5	15		
4	Active	3	32	0.1563	
	Placebo	4	16		
5	Active	4	31	0.2941	
	Placebo	3	17		
6	Active	5	30	0.3040	
	Placebo	2	18		
7	Active	6	29	0.1600	
	Placebo	1	19		
8	Active	7	28	0.0331	+
	Placebo	0	20		
				TOTAL:	1.0000

+ = Probability included in two-tailed p-value

The observed Table 3 has probability 0.0455 of occurring when H_0 is true. Tables 1, 2, and 8 each have probabilities less than 0.0455 and are included in the p-value for the two-sided alternative. Thus, the two-sided p-value is

$$P = 0.0004 + 0.0067 + 0.0455 + 0.0331 = 0.0857$$

As noted in SAS Output 17.1, both the one-sided ❸ and two-sided results ❹ for *Fisher's exact test* are provided. The observed table probability, 0.0455, is also printed ❺.

- **17.4.4** *Fisher's exact test* can be extended to situations that involve more than two treatment groups or more than two response levels. This is sometimes referred to as the *generalized Fisher's exact test* or the *Freeman-Halton test*. With g treatment groups, you would establish a $g \times 2$ table of responses by extending the 2×2 table. You may also extend this method to multinomial responses, i.e., responses that can result in one of r possible outcomes ($r > 2$). With two treatment groups, the 2×2 table would be extended to a $2 \times r$ table, or more generally, to a $g \times r$ table. The alternative hypothesis that is tested by the generalized *Fisher's exact test* is the hypothesis that corresponds to 'general association', as discussed in Chapter 16. If you want to conduct tests on 'linear correlation' or 'row mean scores', you can use other exact tests as mentioned in Section 16.4.13.

In SAS, you can include the FISHER option in the EXACT statement in PROC FREQ to perform the generalized *Fisher's exact test* when the table has more than two rows or two columns.

CHAPTER 18

McNemar's Test

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18.1 Introduction

McNemar's test is a special case of comparing two binomial proportions when using paired samples. *McNemar's test* is often used in a clinical trials application when dichotomous outcomes are recorded for each patient under two different conditions. The conditions might represent different treatments, different measurement times, different body locations, etc. The pair of measurements might also come from two different individuals who are matched based on some characteristic in common. Such matched pairs are usually established using a random pairing of patients who have a common characteristic such as age, or as a natural pairing such as two identical twins. The goal is to compare response rates under the two sets of conditions or matched observations. In these cases, the assumption of independent groups is not met, so you cannot use the *chi-square test* (Chapter 16) or *Fisher's exact test* (Chapter 17).

Typical examples where *McNemar's test* might be applicable include testing for a shift in the proportion of abnormal responses from before treatment to after treatment in the same group of patients, or comparing response rates of two ophthalmic treatments when both are given to each patient, one treatment in each eye.

18.2 Synopsis

In general, there are n pairs of observations, each observation in the pair resulting in a dichotomous outcome, say 'response' or 'non-response'. The results can be partitioned into 4 subgroups: both observations of the pair are 'response' (A), the first observation of the pair is 'response' and the second is 'non-response' (B), the first observation of the pair is 'non-response' and the second is 'response' (C), and both observations are 'non-response' (D), as shown in the 2×2 table that follows.

TABLE 18.1 Layout for McNemar's Test

		2 nd Observation		Total
		Response	Non-Response	
1 st Observation	Response	A	B	A+B
	Non-Response	C	D	C+D
	Total	A+C	B+D	n = A+B+C+D

Let p_1 represent the probability that the first observation of the pair is a ‘response’, and p_2 the probability that the second observation is a ‘response’. The hypothesis of interest is the equality of the response proportions, p_1 and p_2 . The test statistic is based on the difference in the discordant cell frequencies (B and C) as shown in the test summary below. This statistic has an approximate chi-square distribution when H_0 is true.

null hypothesis:	$H_0: p_1 = p_2$
alt. hypothesis:	$H_A: p_1 \neq p_2$
test statistic:	$\chi^2 = \frac{(B - C)^2}{B + C}$
decision rule:	reject H_0 if $\chi^2 > \chi^2_1(\alpha)$

The rejection region is found by obtaining the critical chi-square value based on 1 degree of freedom, $\chi^2_1(\alpha)$, from chi-square tables or by using the SAS function CINV(1- α , df). For $\alpha = 0.05$, the critical chi-square value is 3.841 (see Table A.3 in Appendix A.3, or the SAS function CINV(0.95,1)).

18.3 Examples

Example 18.1—Bilirubin Abnormalities Following Drug Treatment

Eighty-six patients were treated with an experimental drug for 3 months. Pre- and post-study clinical laboratory results showed abnormally high total bilirubin values (above the upper limit of the normal range) as indicated in Table 18.2. Is there evidence of a change in the pre- to post-treatment rates of abnormalities?

TABLE 18.2 Raw Data for Example 18.1

Patient Number	Pre-	Post-	Patient Number	Pre-	Post-	Patient Number	Pre-	Post-
1	N	N	31	N	N	61	N	N
2	N	N	32	N	N	62	N	N
3	N	N	33	Y	N	63	N	N
4	N	N	34	N	N	64	N	N
5	N	N	35	N	N	65	N	N
6	N	Y	36	N	N	66	N	N
7	Y	Y	37	N	N	67	N	N
8	N	N	38	N	Y	68	N	N
9	N	N	39	N	Y	69	N	Y
10	N	N	40	N	N	70	N	Y
11	N	N	41	N	N	71	Y	Y
12	Y	N	42	N	Y	72	N	N
13	N	N	43	N	N	73	N	N
14	N	Y	44	Y	N	74	N	Y
15	N	N	45	N	N	75	N	N
16	N	N	46	N	N	76	N	Y
17	N	N	47	Y	Y	77	N	N
18	N	N	48	N	N	78	N	N
19	N	N	49	N	N	79	N	N
20	N	Y	50	N	Y	80	N	N
21	N	N	51	Y	N	81	Y	Y
22	Y	N	52	N	N	82	N	N
23	N	N	53	N	Y	83	N	N
24	N	N	54	N	N	84	N	N
25	Y	N	55	Y	Y	85	N	N
26	N	N	56	N	N	86	N	N
27	N	N	57	N	N			
28	Y	Y	58	N	N			
29	N	Y	59	N	N			
30	N	Y	60	N	N			

N = normal, Y = abnormally high

Solution

Let p_1 and p_2 represent the proportions of patients who have abnormally high bilirubin values (Y) before and after treatment, respectively. The data are summarized in Table 18.3.

TABLE 18.3 Abnormality Frequency Summary for Example 18.1

		Post-Treatment		
		N	Y	Total
Pre-Treatment	N	60	14	74
	Y	6	6	12
	Total	66	20	86

Y = Total bilirubin above upper limit of normal range

The test summary is

null hypothesis:	$H_0: p_1 = p_2$
alt. hypothesis:	$H_A: p_1 \neq p_2$
test statistic:	$\chi^2 = (14 - 6)^2 / (14 + 6)$
	$= 64 / 20 = 3.20$
decision rule:	reject H_0 if $\chi^2 > 3.841$
conclusion:	Because 3.20 is not > 3.841 , you do not reject H_0 , concluding that, at a significance level of 0.05, there is insufficient evidence that a shift in abnormality rates occurs with treatment.

The actual p-value of 0.074 for the chi-square value of 3.20 can be found by using the SAS expression `1-PROBCHI(3.20, 1)`, which returns a value of 0.074.

SAS Analysis of Example 18.1

To conduct *McNemar's test* in SAS, use the AGREE option in the TABLES statement in PROC FREQ ❶. The SAS code and output for Example 18.1 are shown below. Note that the individual response data are input for each patient. As demonstrated in previous chapters (Chapters 16, 17), if the counts were input instead, you would include a WEIGHT statement with PROC FREQ (this is also shown in Example 18.2). The chi-square value of 3.20 ❷ with a p-value of 0.074 ❸ are shown in the output.

SAS Code for Example 18.1

```
proc format;
  value rsltfmt 0 = 'N' 1 = 'Y';
run;

data bili;
  input pat pre pst @@;
  datalines;
  1 0 0 2 0 0 3 0 0 4 0 0 5 0 0 6 0 1
  7 1 1 8 0 0 9 0 0 10 0 0 11 0 0 12 1 0
  13 0 0 14 0 1 15 0 0 16 0 0 17 0 0 18 0 0
  19 0 0 20 0 1 21 0 0 22 1 0 23 0 0 24 0 0
  25 1 0 26 0 0 27 0 0 28 1 1 29 0 1 30 0 1
  31 0 0 32 0 0 33 1 0 34 0 0 35 0 0 36 0 0
  37 0 0 38 0 1 39 0 1 40 0 0 41 0 0 42 0 1
  43 0 0 44 1 0 45 0 0 46 0 0 47 1 1 48 0 0
  49 0 0 50 0 1 51 1 0 52 0 0 53 0 1 54 0 0
  55 1 1 56 0 0 57 0 0 58 0 0 59 0 0 60 0 0
  61 0 0 62 0 0 63 0 0 64 0 0 65 0 0 66 0 0
  67 0 0 68 0 0 69 0 1 70 0 1 71 1 1 72 0 0
  73 0 0 74 0 1 75 0 0 76 0 1 77 0 0 78 0 0
  79 0 0 80 0 0 81 1 1 82 0 0 83 0 0 84 0 0
  85 0 0 86 0 0
;

ods exclude
  simplekappa;
proc freq data = bili;
  tables pre*pst / agree norow nocol;           ❶
  exact mcnem;                                     ❷
  format pre pst rsltfmt.;                         ❸
  title1 "McNemar's Test";
  title2 'Example 18.1: Bilirubin Abnormalities Following
    Drug Treatment';
run;
```

OUTPUT 18.1 SAS Output for Example 18.1

McNemar's Test				
Example 18.1: Bilirubin Abnormalities Following Drug Treatment				
The FREQ Procedure				
Table of pre by pst				
pre	pst			
Frequency				
Percent	N	Y	Total	
-----+-----+-----+-----+				
N	60	14	74	74
69.77	16.28	86.05		86.05
-----+-----+-----+-----+				
Y	6	6	12	12
6.98	6.98	13.95		13.95
-----+-----+-----+-----+				
Total	66	20	86	86
76.74	23.26	100.00		
Statistics for Table of pre by pst				
McNemar's Test				

Statistic (S)	3.2000			②
DF	1			
Asymptotic Pr > S	0.0736			③
Exact Pr >= S	0.1153			④
Sample Size = 86				

Three Response Categories

Many situations arise in clinical data analysis in which the data are paired and the response has three categorical levels. The bilirubin response in Example 18.1, for example, can be classified as ‘normal’, ‘above normal’, or ‘below normal’. Clinical laboratory data are often analyzed using pre- to post-study ‘shift’ tables, which show the frequencies of patients shifting among these three categories from baseline to some point in the study following treatment. Such data can be analyzed using the *Stuart-Maxwell test*, which is an extension of *McNemar’s test*.

The *Stuart-Maxwell test* is also a *chi-square test* based on the cell frequencies from a layout similar to that shown in Table 18.4. The null hypothesis is that the distribution of responses among the three categories is the same under both conditions, in this case, pre- and post-study.

TABLE 18.4 Layout for a Paired Experiment with a Trinomial Response

		Post-Study		
		Low	Normal	High
Pre-Study	Low	n_{11}	n_{12}	n_{13}
	Normal	n_{21}	n_{22}	n_{23}
	High	n_{31}	n_{32}	n_{33}

Compute

$$d_1 = (n_{12} + n_{13}) - (n_{21} + n_{31})$$

$$d_2 = (n_{21} + n_{23}) - (n_{12} + n_{32})$$

$$d_3 = (n_{31} + n_{32}) - (n_{13} + n_{23})$$

and, for $i \neq j$,

$$\bar{n}_{ij} = (n_{ij} + n_{ji}) / 2$$

For $i = 1, 2, 3$, let $p_i(1)$ represent the probability of being classified in Category i under Condition 1 and $p_i(2)$ represent the probability of being classified in Category i under Condition 2, the test summary is

null hypothesis: $H_0: p_i(1) = p_i(2)$ for $i = 1, 2$, and 3

alt. hypothesis: $H_A: p_i(1) \neq p_i(2)$ for $i = 1, 2$, or 3

test statistic: $S = \frac{(\bar{n}_{23} \cdot d_1^2) + (\bar{n}_{13} \cdot d_2^2) + (\bar{n}_{12} \cdot d_3^2)}{2 \cdot ((\bar{n}_{12} \cdot \bar{n}_{23}) + (\bar{n}_{12} \cdot \bar{n}_{13}) + (\bar{n}_{13} \cdot \bar{n}_{23}))}$

decision rule: reject H_0 if $S > \chi^2_2(\alpha)$

When H_0 is true, S has an approximate chi-square distribution with 2 degrees of freedom. For $\alpha = 0.05$, the critical chi-square value, $\chi^2_2(\alpha)$, is 5.991 from Table A.3 (Appendix A) or by using the SAS function =CINV(0.95,2).

Example 18.2—Symptom Frequency Before and After Treatment

Patients characterized their craving of certain high-fat food products before and two weeks after an experimental diet therapy as ‘never’, ‘occasional’ or ‘frequent’, as summarized in Table 18.5. Does the diet appear to have an effect on the frequency of these cravings?

TABLE 18.5 Cell Frequencies for Example 18.2

		Two Weeks		
		Never	Occasional	Frequent
Pre-Study	Never	14	6	4
	Occasional	9	17	2
	Frequent	6	12	8

Solution

Compute

$$d_1 = (6 + 4) - (9 + 6) = -5$$

$$d_2 = (9 + 2) - (6 + 12) = -7$$

$$d_3 = (6 + 12) - (4 + 2) = 12$$

and

$$\bar{n}_{12} = (n_{12} + n_{21}) / 2 = (6 + 9) / 2 = 7.5$$

$$\bar{n}_{13} = (n_{13} + n_{31}) / 2 = (4 + 6) / 2 = 5$$

$$\bar{n}_{23} = (n_{23} + n_{32}) / 2 = (2 + 12) / 2 = 7$$

The test statistic is

$$S = \frac{(7 \cdot (-5)^2) + (5 \cdot (-7)^2) + (7.5 \cdot (12)^2)}{2 \cdot ((7.5 \cdot 7) + (7.5 \cdot 5) + (5 \cdot 7))} = 6.00$$

which is larger than the critical chi-square value of 5.991. Therefore, there has been a significant shift in the distribution of responses at the $\alpha = 0.05$ level. The marginal response probabilities are estimated as shown in Table 18.6.

TABLE 18.6 Marginal Response Probabilities for Example 18.2

	Pre-Study	2 Weeks
Never	$24 / 78 = 30.8\%$	$29 / 78 = 37.2\%$
Occasional	$28 / 78 = 35.9\%$	$35 / 78 = 44.9\%$
Frequent	$26 / 78 = 33.3\%$	$14 / 78 = 17.9\%$

SAS Analysis of Example 18.2

The *Stuart-Maxwell test* can be approximated by *Bhapkar's test* in SAS, which is carried out using PROC CATMOD to perform a repeated measures test for marginal homogeneity. To do this, you use 'MARGINALS' in the RESPONSE statement in PROC CATMOD. A REPEATED statement is used to let SAS know the responses are repeated measures, to assign a factor name ('TIME'), and to indicate the number of levels of the factor (2), as shown below for Example 18.2. The results show a chi-square value of 6.5 with a p-value of 0.0388 \textcircled{S} , indicating a significant difference in the distribution of responses over time. Since *Bhapkar's test* is asymptotically equivalent to the *Stuart-Maxwell test*, the results of these two tests will be closer for larger sample sizes.

SAS Code for Example 18.2

```
data diet;
    input pre wk2 cnt @@;
    datalines;
    0 0 14    0 1 6    0 2 4
    1 0 9     1 1 17   1 2 2
    2 0 6     2 1 12   2 2 8
    ;
    run;

proc catmod data=diet;
    weight cnt;
    response marginals;
    model pre*wk2=_response_ / freq;
    repeated TIME 2;
    title1 "Bhapkar's Test";
    title2 "Example 18.2: Craving Level Following Diet";
    run;
    quit;
```

OUTPUT 18.2 SAS Output for Example 18.2

Bhapkar's Test
Example 18.2: Craving Level Following Diet

The CATMOD Procedure

Data Summary

Response	pre*wk2	Response Levels	9
Weight Variable	cnt	Populations	1
Data Set	DIET	Total Frequency	78
Frequency Missing	0	Observations	9

Population Profiles

Sample	Sample Size
1	78

Response Profiles

Response	pre	wk2
1	0	0
2	0	1
3	0	2
4	1	0
5	1	1
6	1	2
7	2	0
8	2	1
9	2	2

Response Frequencies

Sample	Response Number								
	1	2	3	4	5	6	7	8	9
1	14	6	4	9	17	2	6	12	8

Analysis of Variance

Source	DF	Chi-Square	Pr > ChiSq
Intercept	2	379.45	<.0001
TIME	2	6.50	0.0388
Residual	0	.	.

Analysis of Weighted Least Squares Estimates

Effect	Parameter	Estimate	Standard Error	Chi-Square	Pr > ChiS
Intercept	1	0.3397	0.0430	62.44	<.0001
	2	0.4038	0.0435	86.07	<.0001
TIME	3	-0.0321	0.0318	1.01	0.314
	4	-0.0449	0.0341	1.73	0.188

18.4 Details & Notes

- **18.4.1** For *McNemar's test*, the estimate of p_1 is $(A+B)/n$, and the estimate of p_2 is $(A+C)/n$. The difference in proportions, $p_1 - p_2$, is estimated by $((A+B)/n) - ((A+C)/n) = (B-C)/n$. An approximate 95% confidence interval for $p_1 - p_2$ is given by

$$\frac{(B-C)}{n} \pm 1.96 \cdot \left(\frac{1}{n}\right) \cdot \sqrt{B+C - \frac{(B-C)^2}{n}}$$

For Example 18.1, the approximate 95% confidence interval for $p_1 - p_2$ is

$$\frac{(14 - 6)}{86} \pm 1.96 \cdot \left(\frac{1}{86}\right) \cdot \sqrt{14+6 - \frac{(14 - 6)^2}{86}}$$

$$= 0.093 \pm 0.100 \text{ or } (-0.007 \text{ to } 0.193)$$

- **18.4.2** Note that the chi-square test statistic is based only on the discordant cell sizes (B, C) and ignores the concordant cells (A, D). However, the estimates of p_1 , p_2 and the size of the confidence interval are based on all cells because they are inversely proportional to the total sample size, n.
- **18.4.3** A well-known relationship in probability theory is that if a random variable, Z, has a standard normal distribution (i.e., mean 0 and variance 1), then Z^2 has a chi-square distribution with 1 degree of freedom (see Appendix B). This principle can be used to show the relationship of the *McNemar chi-square* to the *binomial test* using the normal approximation as follows.

When the null hypothesis is true, the discordant values, B and C, should be about the same, that is $B/(B+C)$ should be about 1/2. *McNemar's test* is equivalent to using the *binomial test* (Chapter 15) to test $H_0: p = 0.5$, where p equals the fraction of the events that fall in 1 of the 2 discordant cells. Using the setup of Chapter 15 with $n = B+C$ and $X = B$, the normal approximation to the *binomial test* (expressed as a standard normal Z when H_0 is true), is

$$Z = \frac{(|\hat{p} - 0.5| - \frac{1}{2(B+C)})}{\sqrt{\frac{0.5 \cdot 0.5}{(B+C)}}} = \frac{|B - C| - 1}{\sqrt{B+C}}$$

because $\hat{p} = \frac{B}{(B+C)}$

Therefore,

$$Z^2 = \frac{(|B - C| - 1)^2}{(B + C)} = \chi^2$$

This test includes the continuity correction discussed in Chapter 15. For Example 18.1, you can compute

$$Z^2 = \frac{(|14 - 6| - 1)^2}{(14 + 6)} = 2.45 = \chi^2$$

which has a p-value of 0.1175 (computed in SAS as `p=1-probchi(2.45, 1)`).

For small values of B and C, you may also compute the exact binomial probabilities as the probability of observing the discordant pairs, B and C, in Table 18.3 or those more extreme. This is $\Pr(B \leq 6) + \Pr(C \geq 14)$, where B and C are binomial random variables with $n = 20$ and $p = 0.5$. Computed in SAS, this becomes:

```
probbnml(0.5, 20, 6) + (1 - probbnml(0.5, 20, 13))
```

which returns a value of 0.1153. An exact procedure for the *McNemar test* can be performed in SAS by using the EXACT statement with the MCNEM parameter in PROC FREQ. The SAS code is shown in Example 18.1 ④, resulting in the p-value of 0.1153.

- **18.4.4** Because the chi-square approximation for *McNemar's test* is identical to the *binomial test* using a normal approximation, it should be used only when the conditions for the normal approximation apply (see Chapter 15). Using the notation here, these conditions can be simplified as $B^2 \geq C(4-B)$ and $C^2 \geq B(4-C)$. Notice that these conditions need to be checked only if either B or C is less than 4. If the conditions are not satisfied, the exact binomial probabilities should be used rather than the normal approximation or the chi-square statistic.
- **18.4.5** The above application of the *binomial test* is an example of the *sign test* mentioned in Chapter 15. In applying the *sign test*, the number of increases (+) are compared with the number of decreases (-), ignoring tied values. In Example 18.1, B represents the number of increases, C represents the number of decreases, and the concordant values (A and D) represent the tied values, which are ignored.
- **18.4.6** The p-value in the SAS output corresponds to a *two-sided hypothesis test*. The *one-sided* p-value can be found by halving this value.

- **18.4.7** *McNemar's test* is often used to detect shifts in response rates between pre- and post-treatment measurement times within a single treatment group. A comparison in shifts can be made between two treatment or dose groups (e.g., Group 1 and Group 2) using a normal approximation as follows. As in Table 18.1, let A_i , B_i , C_i , D_i , and n_i represent the cell and overall frequencies for Group i ($i = 1, 2$). The proportional shift for Group i is

$$D_i = \frac{A_i + C_i}{n_i} - \frac{A_i + B_i}{n_i} = \frac{C_i - B_i}{n_i}$$

To test for a difference between groups in these proportional shifts, you can use

$$Z = \frac{|D_1 - D_2|}{\sqrt{\sigma_{D_1}^2 + \sigma_{D_2}^2}}$$

as a test statistic that has an approximate standard normal distribution under the hypothesis of no difference in shifts between groups. Above, the variance of D_i is computed as

$$\sigma_{D_i}^2 = \frac{n_i(B_i + C_i) - (B_i - C_i)^2}{n_i^3}$$

- **18.4.8** The *Stuart-Maxwell test* is sometimes referred to as a test for ‘marginal homogeneity’ because it tests for equality between the two conditions in the marginal response probabilities. Another alternative hypothesis that might be of interest is that of symmetry. Symmetry occurs when the off-diagonal cell probabilities are the same for each 2×2 sub-table. This implies that changes in response levels between the conditions (e.g., pre- and post-) occur in both directions with the same probability.

With dichotomous outcomes, the conditions of marginal homogeneity and symmetry are equivalent. With more than two response categories, symmetry is a stronger condition and implies marginal homogeneity. The 3×3 tables in Table 18.6 show a pattern of marginal homogeneity with symmetry (Table i) and without symmetry (Table ii).

TABLE 18.6 Examples of Symmetry (i) and non-Symmetry (ii) in a 3 x 3 Table

		2 nd Observation			
		1	2	3	
1 st Observation	1	30	15	5	50
	2	15	5	10	30
	3	5	10	5	20
		50	30	20	100

		2 nd Observation			
		1	2	3	
1 st Observation	1	30	0	20	50
	2	20	10	0	30
	3	0	20	0	20
		50	30	20	100

Bowker's test can be used to test for symmetry in 3×3 or larger tables. The test statistic is found by adding the *McNemar* chi-square statistics for each 2×2 sub-table. For a table with k categories, there are $k \cdot (k-1)/2$ 2×2 sub-tables. For example, the 3×3 table contains three 2×2 sub-tables with categories 1 and 2, 1 and 3, and 2 and 3. *Bowker's test* for k categories is a *chi-square test* with $k \cdot (k-1)/2$ degrees of freedom.

Bowker's test for symmetry can be performed in SAS in the same way that *McNemar's test* is run (by using the AGREE option in the TABLES statement in PROC FREQ). If SAS detects $k > 2$, the test for symmetry is automatically performed. *Bowker's test* results for Example 18.2 are found by using the SAS statements that follow. The output (Output 18.3) shows a test statistic of 8.1429 **6**, which indicates a significant departure from the hypothesis of symmetry ($p=0.0431$).

```

ods select
    symmetrytest;
proc freq data = diet;
    tables pre*wk2 / agree nocol norow;
    weight cnt;
run;

```

OUTPUT 18.3 Output for Symmetry Test in Example 18.2

Statistics for Table of pre by wk2		
Test of Symmetry		
Statistic (S)	8.1429	❶
DF	3	
Pr > S	0.0431	

- **18.4.9** Notice that Section 18.4.7 shows one method for comparing the magnitude of shifts in conditions based on correlated responses among independent groups. If there are more than two groups or more than two response levels, or the design involves other stratification factors, PROC CATMOD in SAS may be used to handle these more complex designs. Stokes, Davis, and Koch (2000) illustrate the analysis of examples that involve *repeated measures* using PROC CATMOD.

CHAPTER 19

The Cochran-Mantel-Haenszel Test

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19.1 Introduction

The *Cochran-Mantel-Haenszel test* is used in clinical trials to compare two binomial proportions from independent populations based on stratified samples. This test provides a means of combining a number of 2×2 tables of the type discussed in Chapters 16 and 17 when each is from a separate, independent stratum.

The stratification factor can represent patient subgroups, such as study centers, gender, age group, or disease severity, and acts similar to the blocking factor in a *two-way ANOVA* (Chapter 7). The *Cochran-Mantel-Haenszel test* obtains an overall comparison of response rates adjusted for the stratification variable. The adjustment is simply a weighting of the 2×2 tables in proportion to the within-strata sample sizes.

The *Cochran-Mantel-Haenszel test* is often used in the comparison of response rates between two treatment groups in a multi-center study using the study centers as strata.

19.2 Synopsis

Assume there are k strata ($k \geq 2$). Within Stratum j , there are N_j patients ($j = 1, 2, \dots, k$), randomly allocated to one of two groups. In Group 1, there are n_{j1} patients, X_{j1} of whom are considered ‘responders’. Similarly, Group 2 has n_{j2} patients with X_{j2} ‘responders’, as shown in Table 19.1.

TABLE 19.1 Layout for the Cochran-Mantel-Haenszel Test

Stratum	Group	Responders	Non-Responders	Total
1	1	X_{11}	$n_{11} - X_{11}$	n_{11}
	2	X_{12}	$n_{12} - X_{12}$	n_{12}
	Total	$X_{11} + X_{12}$	$N_1 - (X_{11} + X_{12})$	N_1
2	1	X_{21}	$n_{21} - X_{21}$	n_{21}
	2	X_{22}	$n_{22} - X_{22}$	n_{22}
	Total	$X_{21} + X_{22}$	$N_2 - (X_{21} + X_{22})$	N_2
.				
k	1	X_{k1}	$n_{k1} - X_{k1}$	n_{k1}
	2	X_{k2}	$n_{k2} - X_{k2}$	n_{k2}
	Total	$X_{k1} + X_{k2}$	$N_k - (X_{k1} + X_{k2})$	N_k

Let p_1 and p_2 denote the overall response rates for Group 1 and Group 2, respectively. For Stratum j , compute the quantities

$$\text{NUM}_j = \frac{X_{j1} \cdot n_{j2} - X_{j2} \cdot n_{j1}}{N_j}$$

and

$$\text{DEN}_j = \frac{n_{j1} \cdot n_{j2} \cdot (X_{j1} + X_{j2}) \cdot (N_j - X_{j1} - X_{j2})}{N_j \cdot (N_j - 1)}$$

The *Cochran-Mantel-Haenszel test* summary is

null hypothesis:	$H_0: p_1 = p_2$
alt. hypothesis:	$H_A: p_1 \neq p_2$
test statistic:	$\chi^2_{\text{CMH}} = \frac{\left(\sum_{j=1}^k \text{NUM}_j \right)^2}{\sum_{j=1}^k \text{DEN}_j}$
decision rule:	reject H_0 if $\chi^2_{\text{CMH}} > \chi^2_1(\alpha)$

Sections 19.4.3 and 19.4.4 provide further details and variations of these formulas. As in previous chapters, $\chi^2_1(\alpha)$ represents the critical chi-square value with significance level α and 1 degree of freedom.

19.3 Examples

Example 19.1—Dermotel Response in Diabetic Ulcers

A multi-center study with 4 centers is testing an experimental treatment, Dermotel, used to accelerate the healing of dermal foot ulcers in diabetic patients. Sodium hyaluronate was used in a control group. Patients who showed a decrease in ulcer size after 20 weeks treatment of at least 90% by surface area measurements were considered ‘responders’. The numbers of responders in each group are shown in Table 19.2 for each study center. Is there an overall difference in response rates between the Dermotel and control groups?

TABLE 19.2 Response Frequencies by Study Center for Example 19.1

Study Center	Treatment Group	Response	Non-Response	TOTAL
1	Dermotel	26	4	30
	Control	18	11	29
	Total	44	15	59

2	Dermotel	8	3	11
	Control	7	5	12
	Total	15	8	23

3	Dermotel	7	5	12
	Control	4	6	10
	Total	11	11	22

4	Dermotel	11	6	17
	Control	9	5	14
	Total	20	11	31

Solution

Consider the study centers as separate strata. For Study Center 1, compute

$$\begin{aligned} \text{NUM}_1 &= \frac{X_{11} \cdot n_{12} - X_{12} \cdot n_{11}}{N_1} \\ &= \frac{26 \cdot 29 - 18 \cdot 30}{59} = 3.6271 \end{aligned}$$

and

$$\begin{aligned} \text{DEN}_1 &= \frac{n_{11} \cdot n_{12} \cdot (X_{11} + X_{12}) \cdot (N_1 - X_{11} - X_{12})}{N_1^2 \cdot (N_1 - 1)} \\ &= \frac{30 \cdot 29 \cdot 44 \cdot 15}{59^2 \cdot 58} = 2.8440 \end{aligned}$$

These quantities can be computed in a similar way for the other centers, and the results are shown in Table 19.3.

TABLE 19.3 Computational Summary for CMH Test Statistic of Example 19.1

STUDY CENTER (j)	NUM _j	DEN _j
1	3.6271	2.8440
2	0.8261	1.3611
3	1.000	1.4286
4	0.0322	1.8162
TOTAL	5.4855	7.4500

The test summary using the *Cochran-Mantel-Haenszel test* at a significance level of 0.05 is

null hypothesis: $H_0: p_1 = p_2$
alt. hypothesis: $H_A: p_1 \neq p_2$

test statistic:
$$\chi^2_{CMH} = \frac{\left(\sum_{j=1}^4 NUM_j \right)^2}{\sum_{j=1}^4 DEN_j}$$
$$= 5.4855^2 / 7.4500 = 4.039$$

decision rule: reject H_0 if $\chi^2_{CMH} > 3.841$

conclusion: Because $4.039 > 3.841$, you reject H_0 at a significance level of $\alpha=0.05$ and conclude that there is a significant difference in response rates between the *Dermotel* treatment and the control.

SAS Analysis of Example 19.1

The CMH option in the FREQ procedure in SAS can be used to conduct the *Cochran-Mantel-Haenszel test*, as shown on the following pages. The stratification factor (CNTR) must be specified first in the TABLES statement as shown in the SAS code ❶. Use the NOPERCENT and NOCOL options to suppress printing of unneeded results (the row percentages will be printed because the NOROW option is not specified). This example uses the WEIGHT statement to indicate that frq represents the cell counts that are input in the data set. If the data have not already been aggregated, you would input responses for each patient as a separate observation in the data set, in which case the WEIGHT statement would be omitted.

The output shows the 2×2 tables for each study center ❷. These can be omitted using the NOFREQ option in the TABLES statement. The row percentages are printed under the cell frequencies, and the percent for RESP = YES represents the estimated response rate. The *Cochran-Mantel-Haenszel test* results are shown on the subsequent output page ❸, confirming the chi-square statistic of 4.039. The critical chi-square value with 1 degree of freedom for rejection of H_0 , 3.841, can be obtained using the CINV function in SAS as follows:

```

data _null_;
  chi=cinv(0.95,1);
  put 'chi=' chi;
run;

```

However, the p-value of 0.0445❸ printed on the SAS output is less than 0.05, indicating significance at the $\alpha = 0.05$ level. Note that the tests for ‘general association’, ‘row mean scores differ’ and ‘non-zero correlation’ are all equivalent for 2x2 tables.

The SAS code includes an overall *chi-square test* (Chapter 16), ignoring the stratification factor (Study Center), which is not significant ($p=0.0513$) ❹. This is discussed more in Section 19.4.2.

SAS Code for Example 19.1

```

data ulcr;
  input cntr $ trt $ resp $ freq @@;
  datalines;
1 ACT YES 26 1 CTL YES 18
1 ACT _NO 4 1 CTL _NO 11
2 ACT YES 8 2 CTL YES 7
2 ACT _NO 3 2 CTL _NO 5
3 ACT YES 7 3 CTL YES 4
3 ACT _NO 5 3 CTL _NO 6
4 ACT YES 11 4 CTL YES 9
4 ACT _NO 6 4 CTL _NO 5
;

/* Analysis using cntr as stratification factor */
ods exclude
  CommonRelRisks;
proc freq data = ulcr;
  tables cntr*trt*resp / cmh nopercent nocol;      ❶
  weight freq;
  title1 'The Cochran-Mantel-Haenszel Test';
  title2 'Example 19.1: Response to Dermotel in Diabetic
          Ulcers';
run;

/* Analysis without stratification (ignoring CNTR) */
proc freq data = ulcr;                                ❷
  tables trt*resp / chisq nopercent nocol;
  weight freq;
run;

```

OUTPUT 19.1 SAS Output for Example 19.1

The Cochran-Mantel-Haenszel Test
Example 19.1: Response to Dermotel in Diabetic Ulcers

The FREQ Procedure

Table 1 of trt by resp
Controlling for cntr=1

②

trt	resp		
Frequency	YES	_NO	Total
Row Pct			
ACT	26	4	30
	86.67	13.33	
CTL	18	11	29
	62.07	37.93	
Total	44	15	59

Table 2 of trt by resp
Controlling for cntr=2

②

trt	resp		
Frequency	YES	_NO	Total
Row Pct			
ACT	8	3	11
	72.73	27.27	
CTL	7	5	12
	58.33	41.67	
Total	15	8	23

Table 3 of trt by resp
Controlling for cntr=3

②

trt	resp		
Frequency	YES	_NO	Total
Row Pct			
ACT	7	5	12
	58.33	41.67	
CTL	4	6	10
	40.00	60.00	
Total	11	11	22

OUTPUT 19.1 SAS Output for Example 19.1 (continued)

The Cochran-Mantel-Haenszel Test
Example 19.1: Response to Dermotol in Diabetic Ulcers

Table 4 of trt by resp
Controlling for cntr=4

②

trt	resp			
Frequency	Row Pct	YES	_NO	Total
ACT	11 6 17			
	64.71 35.29			
CTL	9 5 14			
	64.29 35.71			
Total	20	11		31

Summary Statistics for trt by resp
Controlling for cntr

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

③

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	4.0391	0.0445
2	Row Mean Scores Differ	1	4.0391	0.0445
3	General Association	1	4.0391	0.0445

Breslow-Day Test for
Homogeneity of the Odds Ratios

Chi-Square 1.8947
DF 3
Pr > ChiSq 0.5946

⑤

Total Sample Size = 135

Table of trt by resp

trt	resp			
Frequency	Row Pct	YES	_NO	Total
ACT	52 18 70			
	74.29 25.71			
CTL	38 27 65			
	58.46 41.54			
Total	90	45		135

OUTPUT 19.1 SAS Output for Example 19.1 (continued)

The Cochran-Mantel-Haenszel Test
Example 19.1: Response to Dermotel in Diabetic Ulcers

Statistic	DF	Value	Prob
Chi-Square	1	3.7978	0.0513
Likelihood Ratio Chi-Square	1	3.8136	0.0508
Continuity Adj. Chi-Square	1	3.1191	0.0774
Mantel-Haenszel Chi-Square	1	3.7697	0.0522
Phi Coefficient		0.1677	
Contingency Coefficient		0.1654	
Cramer's V		0.1677	

Fisher's Exact Test

Cell (1,1) Frequency (F)	52
Left-sided Pr <= F	0.9837
Right-sided Pr >= F	0.0385

Table Probability (P)	0.0222
Two-sided Pr <= P	0.0676

Sample Size = 135

Example 19.2—Flexisyl in Fibromyalgia

A study was conducted to investigate the effects of the muscle relaxant, Flexisyl, in patients with fibromyalgia. Patients with an established diagnosis of fibromyalgia were randomized to receive Flexisyl or a competing agent, Norbend, or placebo for 8 weeks. Since Flexisyl is known to have an antidepressant effect, patients were stratified by history of depression. Global pain scores were rated as 'much improved', 'somewhat improved', 'no change', or 'worse'. The results for the study's 189 patients are shown in Table 19.4. Is there enough evidence to indicate a difference among the treatment groups in pain improvement?

TABLE 19.4 Response Frequencies by History of Depression for Example 19.2

History of Depression?	Treatment Group	'Worse'	'No Change'	'Somewhat Improved'	'Much Improved'
NO	Flexisyl	4	11	14	9
	Norbend	5	13	15	8
	Placebo	7	14	14	4
YES	Flexisyl	4	7	9	5
	Norbend	3	6	11	3
	Placebo	5	9	6	3

Solution

You can use an extended version of the *Cochran-Mantel-Haenszel test* with history of depression as a stratification factor. The treatment group is a nominal factor with three levels (*Flexisyl*, *Norbend*, and *Placebo*), and the pain improvement is an ordinal factor with four response levels (Worse, No Change, Somewhat Improved, and Much Improved).

SAS Analysis of Example 19.2

In SAS, use PROC FREQ with the CMH option in the TABLES statement. The stratification factor (*hxdep*) must be specified first in the TABLES statement as shown in the SAS code ❶. Since the response (improvement in fibromyalgia pain) is ordinal, you would use the ‘row mean scores differ’ hypothesis, as discussed in Chapter 16. Here, the modified ridit scores are used.

The output shows the 3×4 tables for each stratum ❷, including the response percentages for each treatment group. The *Cochran-Mantel-Haenszel test* results are shown in the output ❸. The p-value of 0.112 indicates no significant differences in mean responses among the three treatment groups. A weighted least squares (WLS) approach to solve this problem is also shown in the output and is discussed further in Section 19.4.8. Finally, the bar chart equivalent of Table 19.4 is shown at the end of the output. This is generated by the addition of the ODS GRAPHICS statements in the SAS code.

SAS Code for Example 19.2

```
data fmpain;
    input hxdep $ trt $ imprv cnt @@;
    datalines;
    _NO FLEX -1 4 _NO FLEX 0 11 _NO FLEX 1 14 _NO FLEX 2 9
    _NO NORB -1 5 _NO NORB 0 13 _NO NORB 1 15 _NO NORB 2 8
    _NO PLAC -1 7 _NO PLAC 0 14 _NO PLAC 1 14 _NO PLAC 2 4
    YES FLEX -1 4 YES FLEX 0 7 YES FLEX 1 9 YES FLEX 2 5
    YES NORB -1 3 YES NORB 0 6 YES NORB 1 11 YES NORB 2 3
    YES PLAC -1 5 YES PLAC 0 9 YES PLAC 1 6 YES PLAC 2 3
;

proc format;
    value imprv
        2 = "much improved"
        1 = "somewhat improved"
        0 = "no change"
        -1 = "worse";
run;

ods graphics on;
proc freq data = fmpain;
    weight cnt;
    tables hxdep*trt*imprv /
        cmh nopercent nocol scores = modridit;      ❶
    format imprv imprv. ;
    title1 "Extended Cochran-Mantel-Haenszel Test";
    title2 "Example 19.2: Flexisyl in Fibromyalgia Pain";
run;
ods graphics off;

/* Analysis using PROC CATMOD */
proc catmod data = fmpain;
    weight cnt;
    response means;                                ❹
    model imprv = hxdep trt;
    title3 " ";
    title4 "WLS Using CATMOD: comparison of means";
run;
```

OUTPUT 19.2 SAS Output for Example 19.2

Extended Cochran-Mantel-Haenszel Test
 Example 19.2: Flexisyl in Fibromyalgia Pain

The FREQ Procedure

Table 1 of trt by imprv
 Controlling for hxdep=YES

trt	imprv					
Frequency	Row Pct	-1	0	1	2	Total
FLEX		4	7	9	5	25
		16.00	28.00	36.00	20.00	
NORB		3	6	11	3	23
		13.04	26.09	47.83	13.04	
PLAC		5	9	6	3	23
		21.74	39.13	26.09	13.04	
Total		12	22	26	11	71

Table 2 of trt by imprv
 Controlling for hxdep=NO

7

trt	imprv					
Frequency	Row Pct	-1	0	1	2	Total
FLEX		4	11	14	9	38
		10.53	28.95	36.84	23.68	
NORB		5	13	15	8	41
		12.20	31.71	36.59	19.51	
PLAC		7	14	14	4	39
		17.95	35.90	35.90	10.26	
Total		16	38	43	21	118

The FREQ Procedure

Summary Statistics for trt by imprv
 Controlling for hxdep

Cochran-Mantel-Haenszel Statistics (Modified Ridit Scores) 8

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	3.8227	0.0506
2	Row Mean Scores Differ	2	4.3860	0.1116
3	General Association	6	4.9284	0.5530

Total Sample Size = 189

OUTPUT 19.2 SAS Output for Example 19.2 (continued)

Extended Cochran-Mantel-Haenszel Test
Example 19.2: Flexisyl in Fibromyalgia Pain

WLS Using CATMOD: comparison of means

The CATMOD Procedure

Data Summary

Response	imprv	Response Levels	4
Weight Variable	cnt	Populations	6
Data Set	FMPAIN	Total Frequency	189
Frequency Missing	0	Observations	24

Population Profiles

Sample	hxdep	trt	Sample Size
1	YES	FLEX	25
2	YES	NORB	23
3	YES	PLAC	23
4	NO	FLEX	38
5	NO	NORB	41
6	NO	PLAC	39

Response Profiles

Response	imprv
1	-1
2	0
3	1
4	2

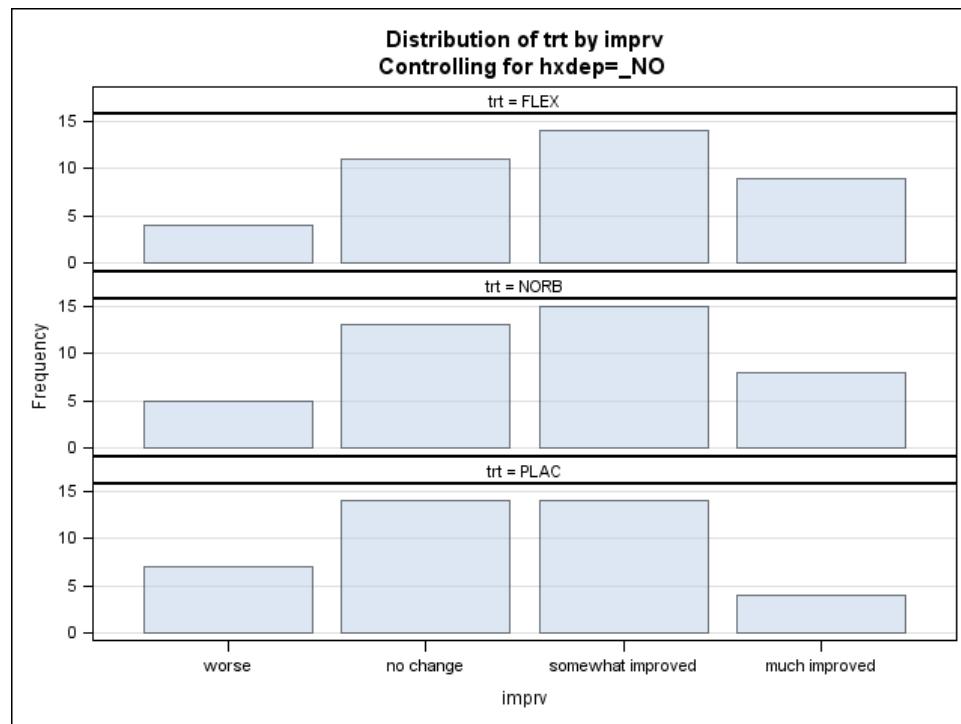
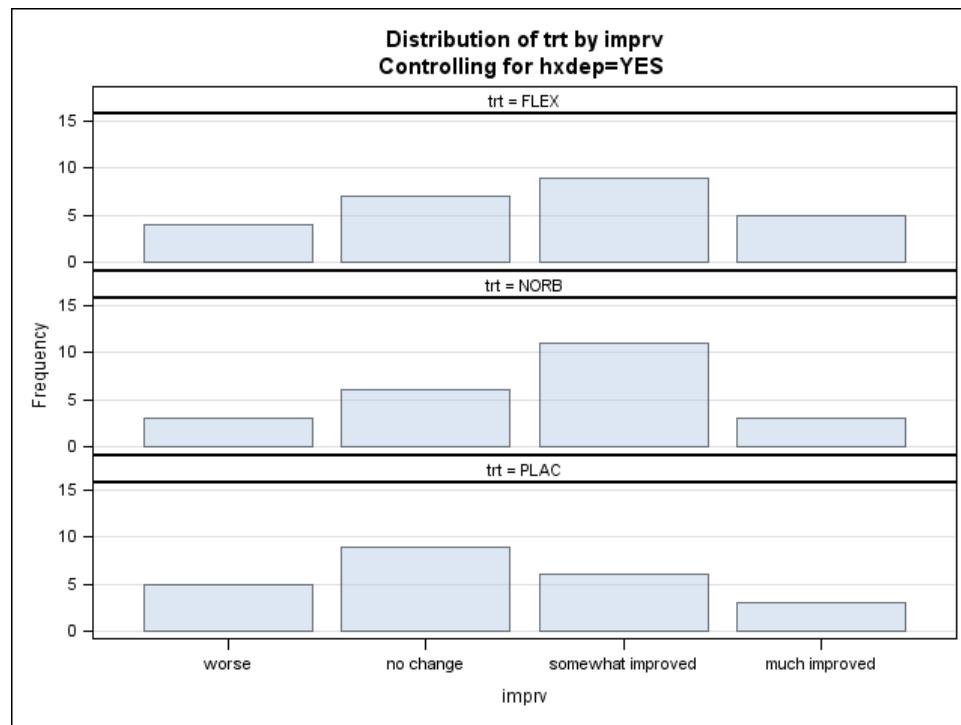
Analysis of Variance

Source	DF	Chi-Square	Pr > ChiSq
Intercept	1	61.10	<.0001
hxdep	1	0.32	0.5737
trt	2	4.53	0.1037 10
Residual	2	0.11	0.9477

Analysis of Weighted Least Squares Estimates

Parameter	Estimate	Standard Error	Chi-Square	Pr > ChiSq
Intercept	0.5453	0.0698	61.10	<.0001
hxdep	YES	-0.0392	0.0697	0.32
trt	FLEX	0.1303	0.0967	1.82
	NORB	0.0702	0.0940	0.56

OUTPUT 19.2 SAS Output for Example 19.2 (continued)



19.4 Details & Notes

- **19.4.1** A convenient way to summarize the test results by stratum is shown for Example 19.1 in Table 19.5. The response rate for Group i, Stratum j is estimated by $100(X_{ji}/n_{ji})\%$. The overall response rate for Group i is estimated by $100((X_{1i}+X_{2i}+X_{3i}+X_{4i})/(n_{1i}+n_{2i}+n_{3i}+n_{4i}))\%$.

TABLE 19.5 Summary of Results for Example 19.1

Study Center	Response Rates					
	Active	(n)	Control	(n)	Chi-Square	p-Value
1	86.7%	(30)	62.1%	(29)	4.706	0.030*
2	72.7%	(11)	58.3%	(12)	0.524	0.469
3	58.3%	(12)	40.0%	(10)	0.733	0.392
4	64.7%	(17)	64.3%	(14)	0.001	0.981
Overall	74.3%	(70)	58.5%	(65)	4.039	0.044*

Although not included in the SAS output for this example, the chi-square values and p-values can be output for each stratum using the CHISQ option in the TABLES statement of PROC FREQ.

- **19.4.2** By ignoring the strata and combining all the data of Example 19.1 into one simple *chi-square test* (Chapter 16), you obtain the following results, as shown in Output 19.1④:

TABLE 19.6 Chi-Square Test for Example 19.1, Ignoring Strata

Group	Response	Non-Response	Total
Active	52 (74.3%)	18	70
Control	38 (58.5%)	27	65
Total	90 (100.0%)	45	135

chi-square value = 3.798, p = 0.051

The test statistic does not quite attain significance at the 0.05 level as with the *Cochran-Mantel-Haenszel test*. In this example, the within-strata information used by the *Cochran-Mantel-Haenszel test* is advantageous in revealing greater statistical significance. This will often be the case when there is a big difference in sample sizes among strata and the largest strata show the biggest response rate differences.

- **19.4.3** Under the null hypothesis, the response rate for Stratum j is estimated as

$$\hat{p}_j = \frac{x_{j1} + x_{j2}}{N_j}$$

and the expected value of X_{ji} is

$$n_{ji} \cdot \hat{p}_j = \frac{n_{ji}}{N_j} \cdot (x_{j1} + x_{j2})$$

In Section 19.2, NUM_j represents the difference between the observed and expected cell frequencies in the j th stratum (see Section 16.4.2). With some algebraic manipulation,

$$NUM_j = \frac{X_{j1} \cdot n_{j2} - X_{j2} \cdot n_{j1}}{N_j}$$

can be expressed as

$$w_j \cdot (\hat{p}_{j1} - \hat{p}_{j2})$$

where $\hat{p}_{ji} = \frac{X_{ji}}{n_{ji}}$ is the estimate of p_{ji} , and w_j is a function of the group sample sizes

$$w_j = \left(\frac{1}{n_{j1}} + \frac{1}{n_{j2}} \right)^{-1}$$

Written in this way, the *Cochran-Mantel-Haenszel* statistic is seen to be based on the within-strata response rate differences combined over all strata, weighted by w_j . Because w_j increases with the n_{ij} 's, it is seen that greater weights are assigned to those strata that have larger sample sizes.

- **19.4.4** The *Cochran-Mantel-Haenszel test* was originally developed for use with retrospective data in epidemiological applications. The same methodology has been widely applied to prospective clinical trials, such as those illustrated here. A number of alternative statistics, similar but with minor variations to the version presented, have also been used.

For example, *Cochran's (chi-square) test* is computed in the same way as the *Cochran-Mantel-Haenszel* statistic with the exception that the denominator of DEN_j is N_j^3 instead of $N_j^2(N_j-1)$. The *Cochran-Mantel-Haenszel test* is an attempt to improve on *Cochran's test* by giving the strata with fewer patients less weight in the overall analysis, while leaving strata with large N_j 's relatively unaltered. Notice that if *Cochran's test* is used with only one stratum ($k = 1$),

the chi-square value is $(\text{NUM}_1)^2 / \text{DEN}_1$, which is identical to the *chi-square test* discussed in Chapter 16.

Another variation of the *Cochran-Mantel-Haenszel* statistic is the continuity-corrected value as follows:

$$\chi^2_{\text{CMH}} = \frac{\left(\left| \sum_{j=1}^k \text{NUM}_j \right| - 0.5 \right)^2}{\sum_{j=1}^k \text{DEN}_j}$$

The continuity correction should be used if there are many small cell sizes. However, use of the continuity correction might produce overly conservative results, and it can generally be omitted for reasonable cell sizes.

- **19.4.5** An interaction exists if the differences in response rates between groups are not consistent among strata. An example of an interaction is an Active vs. Control response rate comparison of 60% vs. 30% in one stratum and 30% vs. 60% in another stratum. Combining strata might mask this interaction, which leads to offsetting responses and no overall differences.

In the presence of an interaction or lack of ‘homogeneity’ among response differences, each stratum should be analyzed separately, and further analyses might be pursued in an attempt to explain the interaction.

The *Breslow-Day test* for homogeneity among strata is included in the SAS output when conducting the *Cochran-Mantel-Haenszel test*. This test provides an indication of the presence of an interaction even though it is based on the odds ratios (see Chapter 20) rather than the differences in response rates. A significant *Breslow-Day test* does not invalidate the results of the *CMH test*, but indicates caution should be used in its interpretation, and that perhaps individual strata should be further investigated. The SAS output for Example 19.1 shows a non-significant *Breslow-Day test* with a p-value of 0.5946 ⑤.

Note: *Zelen’s test* may be used to test for homogeneity of odds ratios when using exact tests for stratified 2×2 tables. This test is available in SAS 9.2 and later by specifying the EQOR option when using the EXACT statement.

- **19.4.6** Combining data across strata into a single 2×2 table should only be considered when the individual tables have like proportions. To show the problems that can arise by automatically combining tables without carefully examining each, consider the results for two strata as shown in Table 19.7.

The response rates for Group A are greater than those of Group B within each stratum. But the overall response rate for Group B is greater for the combined strata. This situation can occur if there is a big difference in response rates among strata, and sample sizes are imbalanced between groups in opposite ways among strata.

TABLE 19.7 Example of 2x2 Tables with Counter-Intuitive Combined Results

Stratum	Group	Responders	Non-Responders	Total	Response Rate
1	A	10	38	48	21%
	B	4	21	25	16%
2	A	20	10	30	67%
	B	27	17	44	61%
Combined	A	30	48	78	38%
	B	31	38	69	45%

- **19.4.7** In Chapter 16, you see that the *chi-square test* for 2×2 tables is equivalent to using a normal approximation to the binomial distribution, and for general use, the cell frequencies should be large enough to validate that approximation. A conservative “rule-of-thumb” is to require expected cell frequencies of at least 5. This rule can be loosened somewhat to ‘at least 5 in each of at least 80% of the cells’ for stratified data, as long as the combined cell frequencies over all strata are sufficiently large. The requirement for larger cell frequencies becomes even less important when the response variable is ordinal and has more than two categories.
- **19.4.8** The *Cochran-Mantel-Haenszel test* can be extended to contingency tables larger than 2×2 tables, as shown in Example 19.2. The general layout is based on k strata of $g \times r$ contingency tables, where g (number of rows) and r (columns), in a typical clinical study, represent the number of treatment groups and the number of response categories, respectively. The FREQ procedure in SAS can also be used to analyze results for this more general setup. The CMH option in the TABLES statement prints the results for the ‘general association’, ‘row mean scores differ’ and ‘non-zero correlation’ hypotheses when samples are stratified in the same way as the unstratified case discussed in Chapter 16. Caution must be used with larger values of g and r due to interpretation difficulties, smaller cell sizes, and the increased potential for interactions.

In Example 19.2, you have $g = 3$ (treatment groups), $r = 4$ (response levels), and $k = 2$ (strata). The modified ridit scores used result in a p-value of 0.112. The table scores, which are already coded as -1, 0, 1, 2, could also be used (this is default in SAS, so the “scores = table” option is not necessary). In this example, the table scores analysis would result in a p-value of 0.115, almost

identical to that of the modified ridit scores. If the response were nominal instead of ordinal, you would use the ‘general association’ hypothesis (see Chapter 16).

For larger samples, you can also use a weighted least squares (WLS) approach using PROC CATMOD in SAS. The SAS code is shown in Example 19.2 with the RESPONSE = MEANS statement ❸. Note that the model is very similar to that used in the GLM procedure. You can easily check for an interaction between treatment group (trt) and depression history (hxdep) by including the trt*hxdep term in the MODEL statement. The WLS results show a p-value for treatment effect of 0.104 ❾.

PROC GENMOD can also be used to analyze categorical data and is very useful for modeling more complex layouts of stratified contingency tables. The newer procedure PROC GLIMMIX can be used as a more general approach to fitting generalized linear models which include mixed effects and binary or categorical responses. In Example 19.1, you can include Study Center as a random effect by including Center in a RANDOM statement in PROC GLIMMIX. Be aware, however, that using these modeling techniques generally require larger sample sizes. For each effect in the model, you should have at least 50-100 patients, as a rule of thumb.

Details about sample size guidelines for applying the *CMH test* and performing modeling techniques for categorical data using PROC CATMOD, PROC GENMOD and PROC GLIMMIX are discussed in a number of books published under the SAS Press program.

- **19.4.9** The *Cochran-Mantel-Haenszel test* as presented here is a *two-sided test*. The p-value from the SAS output can be halved to obtain a *one-sided test*.

CHAPTER 20

Logistic Regression

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20.1 Introduction

Logistic regression analysis is a statistical modeling method for analyzing categorical response data while accommodating adjustments for one or more explanatory variables or ‘covariates’. This method is analogous to *linear regression analysis* for continuous normally distributed responses (Chapter 10), or to *ANCOVA* (Chapter 11), which is useful for comparing two or more groups while adjusting for various background factors (covariates). Although all of these methods include covariate adjustments, *linear regression* and *ANCOVA* analyze *means* of numeric response measures; *logistic regression* analyzes *proportions* based on categorical responses, most commonly binary responses (e.g., success rates, survival rates, or cure rates).

Historically, *logistic regression* techniques have been widely used for identifying risk factors associated with disease in epidemiological studies. *Logistic regression* is also popular for analyzing prospective clinical trials and in identifying potentially important covariates in exploratory analyses of clinical research data.

Examples from clinical research where *logistic regression* might be useful include:

- comparing survival rates in cancer patients among various treatment groups adjusted for age and duration of disease.
- comparing proportions of patients whose dermal ulcers show complete healing between an active and placebo group adjusted for baseline ulcer size.
- comparing the proportion of normalized hypertensive patients between two anti-hypertensive treatment groups adjusted for age, cholesterol level, tobacco use, and exercise habits.

20.2 Synopsis

The Logit Model: One Covariate

Consider an experiment whose outcome can take one of two possible values (yes-no, normal-abnormal, present-absent, cured-not cured, died-survived, etc.). Let Y represent a random variable with coded values 1 and 0 where $Y=1$ indicates that the *event* of interest occurs (event), and $Y=0$ indicates that the *event* does not occur (non-event). If you suspect that one or more background factors (X) will affect the response, you want the analysis to reflect this relationship, and you must incorporate the covariates (X) into the analysis. Initially, we consider only continuous numeric valued covariates or those that can be numerically coded, e.g., binary or ordinal.

The methods used to develop *ANCOVA* procedures (Chapter 11) are not applicable because the responses are not normally distributed. Instead, you will model a transformation of the data called the ‘logit function’ which takes the form:

$$Y^* = \ln\left(\frac{P}{1-P}\right), \quad \text{for } 0 < P < 1.$$

Here, ‘ \ln ’ represents the natural-logarithm function, P represents the probability of event occurrence, and $P/(1-P)$ represents the odds of occurrence. The terms ‘logit’ and ‘log odds’ are used interchangeably.

For now, assume there is just one covariate, X . Because Y only takes the values 0 or 1 for a given value of X , the expected value or mean of Y equals the probability that $Y=1$. You denote this probability by P_x , where $0 < P_x < 1$. *Logistic regression* assumes that the logit function is linearly related to X as:

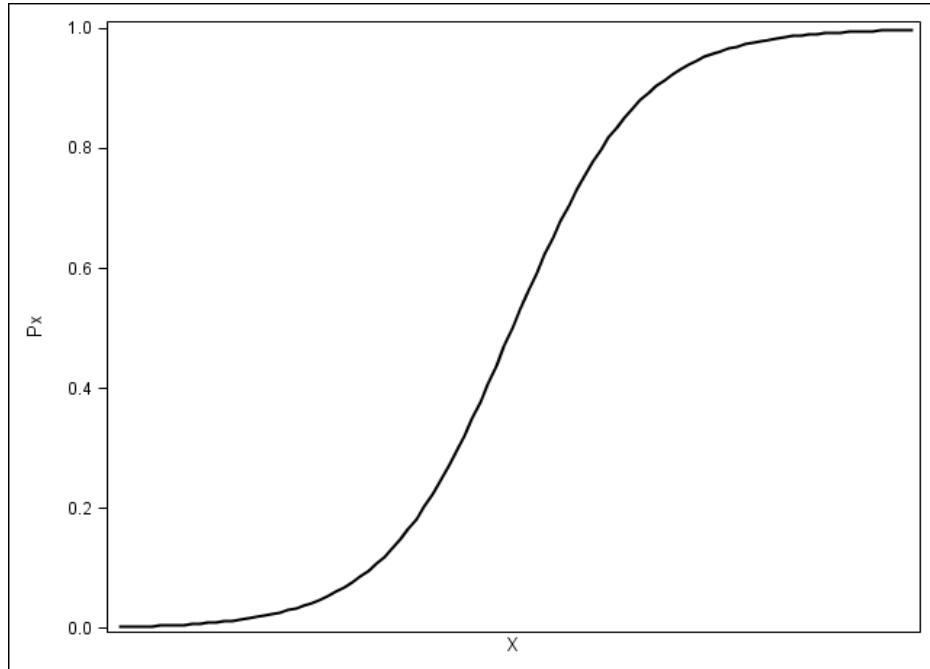
$$\ln\left(\frac{P_x}{1-P_x}\right) = \alpha + \beta X$$

With some algebraic manipulation, this can be re-expressed in terms of P_x as

$$P_x = \frac{1}{1 + e^{-(\alpha + \beta X)}}$$

If you plot this function, you see that P_x is related to X in a sigmoidal fashion as shown in Figure 20.1. $P_x/(1-P_x)$ is the ‘odds’ that $Y=1$ when $X=x$, i.e., the odds that the *event* of interest occurs for a given value of X .

FIGURE 20.1 Logistic Probability Function



The Odds Ratio

Note that the odds for a specific covariate value of $X=x$ is

$$\text{Odds}_x = e^{\alpha+\beta x}$$

and the odds for a covariate value of $X=x+1$ is

$$\text{Odds}_{x+1} = e^{\alpha+\beta(x+1)}$$

so that the ratio of the odds based on a 1-unit increment in X is simply

$$\frac{\text{Odds}_{x+1}}{\text{Odds}_x} = e^\beta$$

This is called the ‘odds ratio’ (OR) for the covariate X , and as seen, does not depend on x . By subtracting 1 from the OR and multiplying by 100, you obtain the percent change in the odds of *event* occurrence when the covariate (X) increases by 1 unit, i.e., $100(e^\beta - 1)$. If X is a dichotomous variable, such as Gender with values ‘Male’ and ‘Female’, you can assign numeric values to its levels, e.g., 0=Male and 1=Female, in which case the OR represents the factor by which the odds of *event* increases for females relative to males.

Model Estimation

For a fixed value of the covariate, such as $X = x$, one way to estimate P_x is by using $\hat{p}_x = y_x/n_x$, where n_x is the number of observations at $X = x$ and y_x is the number of events out of the n_x observations. Observe that y_x is a binomial random variable so that \hat{p}_x will be a better estimate when n_x is large.

Suppose 12 patients with ulcers secondary to *H. pylori* were cured out of 20 patients who were treated with an antibiotic. The study results were broken down by the ulcer history (X , in years) as shown in Table 20.1:

TABLE 20.1 Naïve Estimates of P_x

X	y_x	n_x	P_x (est)	Logit
1	4	6	0.667	0.693
2	6	10	0.600	0.405
3	2	4	0.500	0.000
TOTAL	12	20	0.600	

You might estimate the chance of cure with the antibiotic for patients with a 1-year history ($x = 1$) by $\hat{p}_1 = 4/6 = 0.667$. The logit of \hat{p}_1 is $\ln(0.667/0.333) = 0.693$.

Another way to estimate P_x is to obtain an estimate of the *logistic regression* model, then plug in the value of x and solve for P_x . To estimate the model, you can use an approach known as maximum likelihood, which uses all the data to determine the estimate of each model parameter. These estimates are determined in a way that maximizes the likelihood of observing the data collected, and they are called ‘maximum likelihood estimates’ (MLEs). In addition to having some powerful statistical properties, MLEs have the advantage that data need not be grouped, as in the above example, but are based on the individual observations for each subject with a 0 or 1 response. The resulting model can be used to estimate P_x even for unobserved values of X .

Although the mathematical derivations based on the maximum likelihood method are complex and beyond the scope of this book, this method yields a set of simultaneous equations that can be solved for a and b , the estimates of α and β . These equations, which do not have a closed solution, have the form

$$\sum_{i=1}^N Y_i = \sum_{i=1}^N \left(1 + e^{-(a+b_{xi})}\right)^{-1}$$

and

$$\sum_{i=1}^N X_i \cdot Y_i = \sum_{i=1}^N X_i \cdot \left(1 + e^{-(a+b_{xi})}\right)^{-1}$$

Numerical techniques such as Newton-Raphson algorithms and iteratively weighted least-squares methods are used by SAS and other computer programs to solve these equations.

The Logit Model: Multiple Covariates

In general, the *logistic regression* layout has N patients and k covariates, X_1, X_2, \dots, X_k , and a typical data set can have the following layout:

TABLE 20.2 Layout for Logistic Regression

Patient Number	Response	Covariates			
	Y	X_1	X_2	...	X_k
1	y_1	X_{11}	X_{21}	...	X_{k1}
2	y_2	X_{12}	X_{22}	...	X_{k2}
...
N	y_N	X_{1N}	X_{2N}	...	X_{kN}

Each y_i takes a value of 0 (non-event) or 1 (event). The X_i 's can be known, numeric-valued, concomitant factors, such as age, WBC, or fasting glucose level, or they can represent levels of ordinal categorical variables, such as small-medium-large or none-mild-moderate-severe. You can also use nominal level categorical explanatory variables, as discussed later (see Section 20.4.6). In controlled clinical studies, at least one of the X_i 's is included to represent the treatment or dose group.

The model for the probability of 'event', P, is

$$P = \frac{1}{1 + e^{-(\alpha + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_k x_k)}}$$

and the logit is the linear function

$$\ln \left(\frac{P}{1-P} \right) = \alpha + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_k x_k$$

If all the X's are continuous numeric covariates, the odds can be expressed as

$$\left(\frac{P}{1-P} \right) = e^{(\alpha + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_k x_k)}$$

and the odds ratio for X_i is

$$OR_{x_i} = e^{\beta_i}$$

with the interpretation that

$$100 \cdot (e^{\beta_i} - 1)$$

represents the percent increase in the odds of ‘event’ occurrence when X_i increases by 1 unit and all other X ’s are held constant.

The importance of any particular covariate (X_i) for predicting the *event* probability is measured by the magnitude of the parameter coefficient, β_i . Estimates of these parameters can be found by the method of maximum likelihood using PROC LOGISTIC in SAS. For large samples, these estimates (b_i) have an approximate normal distribution. If s_{β_i} represents the standard error of the estimate, b_i , then b_i / s_{β_i} has an approximate standard normal distribution under the null hypothesis that $\beta_i=0$, and its square has the chi-square distribution with 1 degree of freedom. The test summary for each model parameter, β_i , is based on this *Wald chi-square*, summarized as follows:

null hypothesis:	$H_0: \beta_i = 0$
alternative hypothesis:	$H_A: \beta_i \neq 0$
test statistic:	$\chi_w^2 = \left(\frac{b_i}{s_{b_i}} \right)^2$
decision rule:	reject H_0 if $\chi_w^2 > \chi_1^2(\alpha)$

Example 20.1 illustrates a *logistic regression* analysis for $k=2$ using PROC LOGISTIC in SAS.

20.3 Examples

¶ Example 20.1—Relapse Rate Adjusted for Remission Time in AML

One hundred and two patients with acute myelogenous leukemia (AML) in remission were enrolled in a study of a new antisense oligonucleotide (asODN). The patients were randomly assigned to receive a 10-day infusion of asODN or no treatment (Control), and the effects were followed for 90 days. The time of remission from diagnosis or prior relapse (X, in months) at study enrollment was considered an important covariate in predicting response. The response data are shown in Table 20.3 with Y=1 indicating relapse, death, or major intervention, such as bone marrow transplant before Day 90. Is there any evidence that administration of asODN is associated with a decreased relapse rate?

TABLE 20.3 Raw Data for Example 20.1

-----asODN Group-----

Patient Number	X	Y	Patient Number	X	Y	Patient Number	X	Y
1	3	0	32	9	0	67	12	0
2	3	1	33	6	1	69	12	0
4	3	1	36	6	0	71	12	0
6	6	1	39	6	0	73	9	1
7	15	0	42	6	0	74	6	1
10	6	1	44	3	1	77	12	0
11	6	1	46	18	0	79	6	0
14	6	1	49	9	0	81	15	1
15	15	0	50	12	1	83	9	0
17	15	0	52	6	0	85	3	1
20	12	0	54	9	1	88	9	0
21	18	0	56	9	1	90	9	0
22	6	1	58	3	0	92	9	0
25	15	0	60	9	1	94	9	0
26	6	1	62	12	0	95	9	1
28	15	0	63	12	0	98	12	1
29	12	1	66	3	0	99	3	1
						102	6	1

-----Control Group-----

Patient Number	X	Y	Patient Number	X	Y	Patient Number	X	Y
3	9	1	38	15	0	72	9	1
5	3	0	40	15	1	75	15	0
8	12	1	41	9	0	76	15	0
9	3	1	43	9	0	78	12	0
12	3	1	45	12	1	80	9	0
13	15	1	47	3	1	82	12	0
16	9	1	48	6	1	84	15	0
18	12	1	51	6	1	86	18	1
19	3	1	53	12	0	87	12	0
23	9	1	55	12	0	89	15	1
24	15	1	57	12	1	91	15	0
27	9	1	59	3	1	93	15	0
30	6	1	61	12	1	96	18	0
31	9	1	64	3	1	97	18	1
34	6	1	65	12	1	100	18	0
35	12	0	68	6	1	101	18	0
37	9	0	70	6	1			

X = time in remission (months)

Solution

You want to compare relapse rates between treatment groups adjusted for prior remission time. By constructing a series of 2×2 contingency tables from the data (one table for each X value), you obtain the following summary table:

TABLE 20.4 Naïve Estimates of Response Rates for Example 20.1

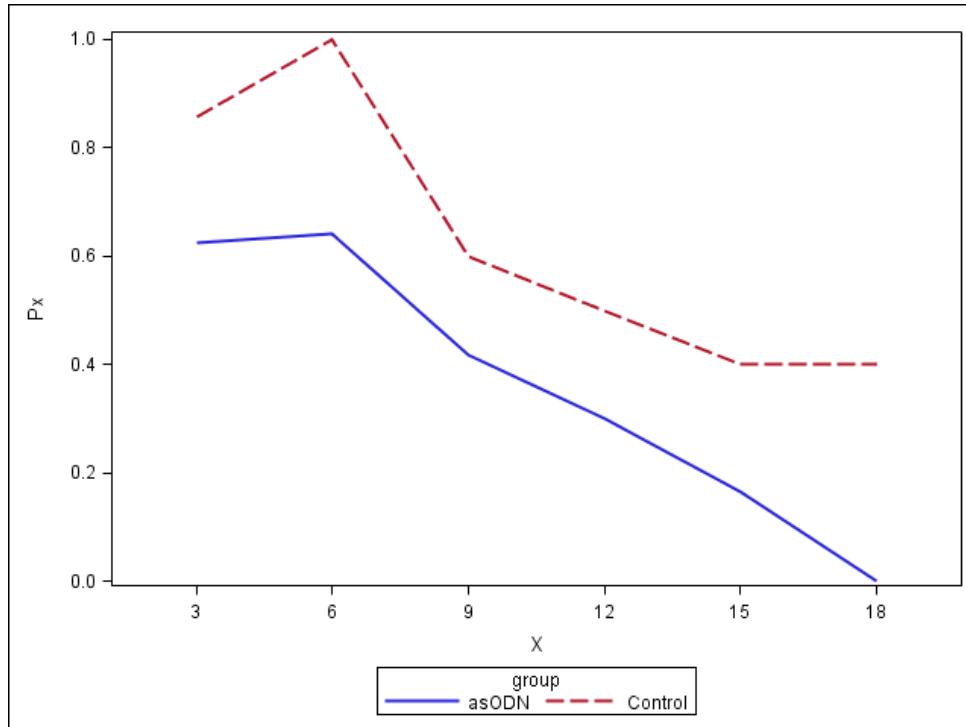
X (months)	asODN Group			Control Group		
	Number of Events	N	Estimated P_x	Number of Events	N	Estimated P_x
3	5	8	0.625	6	7	0.857
6	9	14	0.643	6	6	1.000
9	5	12	0.417	6	10	0.600
12	3	10	0.300	6	12	0.500
15	1	6	0.167	4	10	0.400
18	0	2	0.000	2	5	0.400
TOTAL	23	52	0.442	30	50	0.600

If prior remission time (X) is ignored, overall relapse rates are estimated as 0.442 for the asODN group and 0.600 for the Control group. This is not a significant difference if you use the *chi-square test* discussed in Chapter 16 ($\chi^2 = 2.54$, $p = 0.111$; see ⑥ in Output 20.1).

You can also proceed by treating prior remission time as a grouped stratification factor and applying the *Cochran-Mantel-Haenszel test* (Chapter 19). This approach would satisfy the goal of comparing relapse rates between groups by controlling for prior remission time. However, the ordinality of the stratification variable is ignored with the *CMH test*, and logistic modeling might be preferable in such situations. The modeling technique allows for interpolation based on unobserved values of the numeric covariate, provides estimates of the odds ratios, and permits the inclusion of interactions and additional covariates.

A plot of the summary data (Figure 20.2) shows that the probability of relapse (P_x) seems to depend on the length of prior remission, with higher probabilities of relapse associated with shorter remission times. Using this information in a *logistic regression* analysis provides an adjusted treatment group comparison that is found to be significant ($p=0.0165$), as shown in Output 20.1 ⑦.

FIGURE 20.2 Probability of Relapse (P_x) vs. Remission Time (X) for Example 20.1



To perform *logistic regression*, include a treatment factor, say ‘Group’, and a remission time covariate, X. Group is a nominal factor, and prior remission time (X) is considered a continuous numeric covariate. The *logistic regression* model can be written as

$$\text{logit} = (\alpha + \beta_1 \cdot \text{Group} + \beta_2 \cdot X)$$

Use PROC LOGISTIC in SAS to fit this model, as shown in the analysis that follows.

SAS Analysis of Example 20.1

The SAS code for performing the *logistic regression* analysis is shown under “SAS Code for Example 20.1”, and the results are given in Output 20.1. In the input data set, the treatment effect, group, takes values ‘ACT’ for the active group and ‘PBO’ for the placebo group. The response, relapse, takes values ‘YES’ or ‘NO’ ①.

The LOGISTIC procedure is used much like PROC GLM or MIXED discussed in earlier chapters. Include a CLASS statement for the nominal valued group factor as shown. By using the REF=‘PBO’ option, you’re telling SAS to treat the placebo

group as the reference group. The PARAM=REF option forces SAS to use the ‘reference’ parameterization, which is discussed in more detail in Appendix I. Briefly, the α in the model represents the effect of the reference group (PBO), and β_1 represents the incremental effect of ACT over PBO.

In the MODEL statement, you designate the response variable, relapse, on the left side of the equality sign and the effects, group, and x, on the right. Using the MODEL variable option EVENT='YES' ensures that SAS models values of 'YES' for relapse as the event of interest. The ODDSRATIO statements for group and x are used in conjunction with the ODDSRATIO plot-request keyword in the PLOTS option of PROC LOGISTIC ❸ to identify the effects whose odds ratios are to be plotted by ODS graphics (produces Figure 20.3). The EFFECT keyword in the PLOTS option is used to obtain an effects plot shown in Figure 20.5 (see Section 20.4.11) For comparison, the analysis is also conducted using PROC FREQ to perform a *chi-square test* ignoring the covariate, X, remission time ❹.

From Output 20.1, the MLEs of the model parameters, α , β_1 , and β_2 , are seen to be $a = 2.6135$, $b_1 = -1.1191$, and $b_2 = -0.1998$ ❺, which result in an estimated *logistic regression* equation of

$$\text{logit} = (2.6135 - 1.1191 \cdot \text{Group} - 0.1998 \cdot X)$$

so that the estimated probability of relapse is

$$\hat{P} = \frac{1}{1 + e^{-(2.6135 - 1.1191 \cdot \text{Group} - 0.1998 \cdot X)}}$$

One question of interest is whether X = remission time is an important covariate, tested as follows:

null hypothesis:	$H_0: \beta_2 = 0$
alt. hypothesis:	$H_A: \beta_2 \neq 0$
test statistic:	$\chi_w^2 = 12.72$
decision rule:	reject H_0 if $\chi_w^2 > \chi_1^2(0.05)$ ($= 3.841$)
conclusion:	Because $12.72 > 3.841$, you reject H_0 indicating a significant covariate, X.

From SAS, you can find the p-value associated with 12.72 as $1 - \text{PROBCHI}(12.72, 1) = 0.0004$.

As seen in the output, the time since prior remission (X) is a highly significant background factor for predicting the probability of a relapse within 90 days ($p=0.0004$) ⑤. This is not taken into account in the unadjusted *chi-square test*, which yields relapse rates of 44.2% and 60.0% for the Active and Control groups, respectively ($p=0.111$) ⑥.

Similarly, comparison of relapse rates between treatment groups (group) adjusted for the covariate is tested by the hypothesis

$$H_0: \beta_1 = 0$$

The SAS output indicates a significant Wald chi-square value of 5.7446 with a p-value of 0.0165 ⑦. The estimate of β_1 is -1.1191 , and the estimate of the treatment OR is $e^{-1.1191} = 0.327$ ⑧. This means that, adjusted for prior remission time, there is a $100 \cdot (0.327 - 1) = -67.3\%$ increase, or 67.3% reduction, in the odds of relapse associated with the active treatment group (ACT) compared with the reference placebo group (PBO). The odds ratios and 95% confidence intervals are printed in the SAS output [0.131 – 0.815] ⑨. Figure 20.3, generated with ODS Graphics and the associated ODDSRATIO statements, depicts the OR and 95% confidence intervals for the two model effects, Group and X.

SAS Code for Example 20.1

```
data aml;
  input pat group $ x  relapse $ @@;
  datalines;
  1 ACT 3 NO      2 ACT 3 YES     4 ACT 3 YES
  6 ACT 6 YES     7 ACT 15 NO    10 ACT 6 YES
  11 ACT 6 YES    14 ACT 6 YES   15 ACT 15 NO
  17 ACT 15 NO    20 ACT 12 NO  21 ACT 18 NO
  22 ACT 6 YES    25 ACT 15 NO  26 ACT 6 YES
  28 ACT 15 NO    29 ACT 12 YES 32 ACT 9 NO
  33 ACT 6 YES    36 ACT 6 NO   39 ACT 6 NO
  42 ACT 6 NO     44 ACT 3 YES  46 ACT 18 NO
  49 ACT 9 NO     50 ACT 12 YES 52 ACT 6 NO
  54 ACT 9 YES    56 ACT 9 YES  58 ACT 3 NO
  60 ACT 9 YES    62 ACT 12 NO  63 ACT 12 NO
  66 ACT 3 NO     67 ACT 12 NO  69 ACT 12 NO
  71 ACT 12 NO    73 ACT 9 YES  74 ACT 6 YES
  77 ACT 12 NO    79 ACT 6 NO   81 ACT 15 YES
  83 ACT 9 NO     85 ACT 3 YES  88 ACT 9 NO
  90 ACT 9 NO     92 ACT 9 NO   94 ACT 9 NO
  95 ACT 9 YES    98 ACT 12 YES 99 ACT 3 YES
  102 ACT 6 YES   3 PBO 9 YES   5 PBO 3 NO
  8 PBO 12 YES    9 PBO 3 YES   12 PBO 3 YES
  13 PBO 15 YES   16 PBO 9 YES  18 PBO 12 YES
  19 PBO 3 YES    23 PBO 9 YES  24 PBO 15 YES
  27 PBO 9 YES    30 PBO 6 YES  31 PBO 9 YES
  34 PBO 6 YES    35 PBO 12 NO  37 PBO 9 NO
```

```

38 PBO 15 NO      40 PBO 15 YES     41 PBO  9 NO
43 PBO  9 NO      45 PBO 12 YES     47 PBO  3 YES
48 PBO  6 YES      51 PBO  6 YES     53 PBO 12 NO
55 PBO 12 NO      57 PBO 12 YES     59 PBO  3 YES
61 PBO 12 YES      64 PBO  3 YES     65 PBO 12 YES
68 PBO  6 YES      70 PBO  6 YES     72 PBO  9 YES
75 PBO 15 NO      76 PBO 15 NO     78 PBO 12 NO
80 PBO  9 NO      82 PBO 12 NO     84 PBO 15 NO
86 PBO 18 YES      87 PBO 12 NO     89 PBO 15 YES
91 PBO 15 NO      93 PBO 15 NO     96 PBO 18 NO
97 PBO 18 YES     100 PBO 18 NO    101 PBO 18 NO
;

ods graphics on;
proc logistic data=aml plots(only)=(effect oddsratio); ❸
  class group(ref='PBO') / param=ref;
  model relapse(event='YES')= group x;
  oddsratio group;
  oddsratio x;
  title1 'Logistic Regression';
  title2 'Example 20.1: Relapse Rate Adjusted for
          Remission Time in AML';
run;
ods graphics off;

proc freq data = aml; ❹
  tables group*relapse / chisq nocol nopercent nocum;
  title3 'Chi-Square Test Ignoring the Covariate';
run;

```

OUTPUT 20.1 SAS Output for Example 20.1

Logistic Regression		
Example 20.1: Relapse Rate Adjusted for Remission Time in AML		
The LOGISTIC Procedure		
Model Information		
Data Set	WORK.AML	
Response Variable	relapse	
Number of Response Levels	2	
Model	binary logit	
Optimization Technique	Fisher's scoring	
Number of Observations Read	102	
Number of Observations Used	102	
Response Profile		
Ordered Value	relapse	Total Frequency
1	NO	49
2	YES	53
Probability modeled is relapse='YES'.		

OUTPUT 20.1 SAS Output for Example 20.1 (continued)

Logistic Regression
Example 20.1: Relapse Rate Adjusted for Remission Time in AML

Class Level Information

Class	Value	Design	
		Variables	②
group	ACT	1	
	PBO	0	

Model Convergence Status
Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Intercept		
	Intercept	and	Covariates
AIC	143.245		129.376
SC	145.870		137.251
-2 Log L	141.245		123.376

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	17.8687	2	0.0001
Score	16.4848	2	0.0003
Wald	14.0612	2	0.0009

Type 3 Analysis of Effects

Effect	DF	Chi-Square	Wald	Pr > Chisq
group	1	5.7446		0.0165
x	1	12.7187		0.0004

Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard		
			④	Error	Chi-Square
Intercept	1	2.6135	0.7149	13.3662	0.0003
group ACT	1	-1.1191	0.4669	⑨ 5.7446	0.0165
x	1	-0.1998	0.0560	12.7187	0.0004

Odds Ratio Estimates

Effect	Point Estimate	95% Wald Confidence Limits	
		Wald	Confidence Limits
Group ACT vs PBO	0.327 ⑩	0.131	0.815 ⑧
x	0.819	0.734	0.914

OUTPUT 20.1 SAS Output for Example 20.1 (continued)

Logistic Regression
Example 20.1: Relapse Rate Adjusted for Remission Time in AML

Association of Predicted Probabilities and Observed Responses

Percent Concordant	68.5	Somers' D	0.454
Percent Discordant	23.1	Gamma	0.496
Percent Tied	8.4	Tau-a	0.229
Pairs	2597	c	0.727

Wald Confidence Interval for Odds Ratios

Label	Estimate	95% Confidence Limits
group ACT vs PBO	0.327	0.131 0.815
x	0.819	0.734 0.914

Chi-Square Test Ignoring the Covariate

The FREQ Procedure

Table of group by relapse

group	relapse							
Frequency	NO	YES	Total					
Row Pct								
ACT	29	23	52					
	55.77	44.23						
-----+-----+-----+-----+-----+-----+-----+-----+								
PBO	20	30	50					
	40.00	60.00						
-----+-----+-----+-----+-----+-----+-----+-----+								
Total	49	53	102					

⑥

Statistics for Table of group by relapse

Statistic	DF	Value	Prob
Chi-Square	1	2.5394	0.1110
Likelihood Ratio Chi-Square	1	2.5505	0.1103
Continuity Adj. Chi-Square	1	1.9469	0.1629
Mantel-Haenszel Chi-Square	1	2.5145	0.1128
Phi Coefficient		0.1578	
Contingency Coefficient		0.1559	
Cramer's V		0.1578	

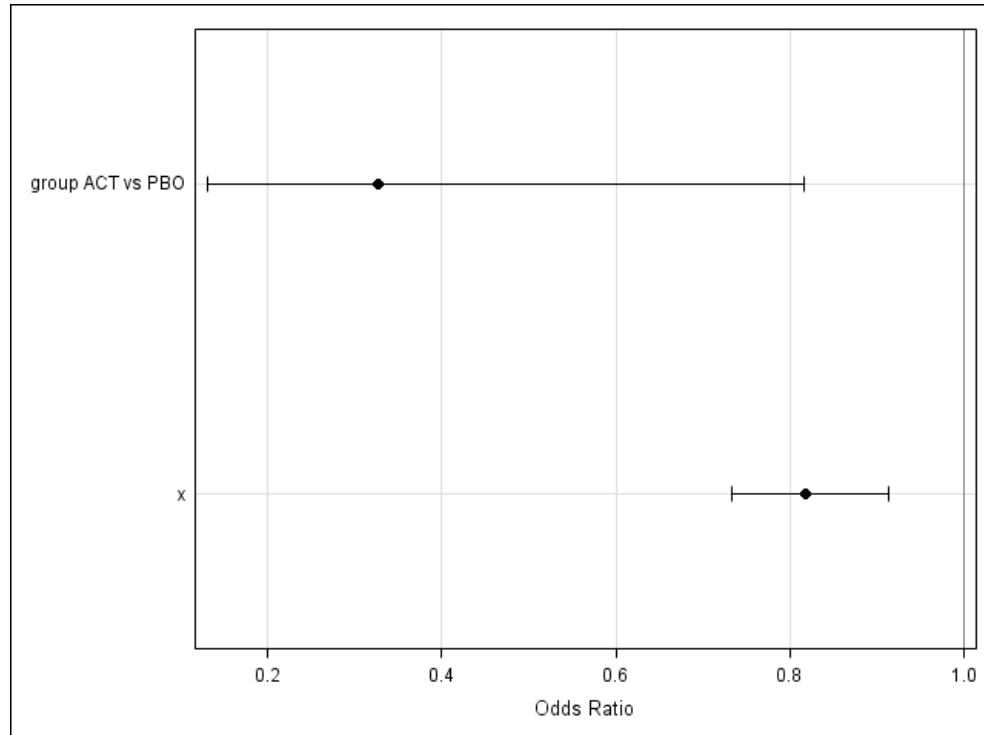
⑥

Fisher's Exact Test

Cell (1,1) Frequency (F)	29
Left-sided Pr <= F	0.9637
Right-sided Pr >= F	0.0813
Table Probability (P)	0.0450
Two-sided Pr <= P	0.1189

Sample Size = 102

FIGURE 20.3 Odds Ratios with 95% Wald Confidence Limits for Example 20.1



Multinomial Responses

In addition to dichotomous outcomes, *logistic regression* methods can be used in cases that involve ordinal categorical responses with more than two levels. The strategy uses a ‘proportional odds’ model, which simultaneously fits $m-1$ binary *logistic regression* models of the form just discussed, when there are m (>2) ordinal response levels.

Consider an example in which the response, Y , is measured on an ordinal improvement scale of ‘worse’, ‘no change’, ‘improved’, or ‘cured’. In this case, m is equal to 4, so you can define three ‘cumulative’ binary responses as follows:

1. $Y_1 = 0$ if $Y = \text{'worse'}$
 $= 1$ if $Y = \text{'no change', 'improved', or 'cured'}$
2. $Y_2 = 0$ if $Y = \text{'worse' or 'no change'}$
 $= 1$ if $Y = \text{'improved' or 'cured'}$
3. $Y_3 = 0$ if $Y = \text{'worse', 'no change', or 'improved'}$
 $= 1$ if $Y = \text{'cured'}$

The first dichotomous response variable, Y_1 , is measuring whether the patient's condition does not deteriorate. Similarly, Y_2 measures whether the patient improves, and Y_3 measures whether the patient is cured. Taken together, you can use Y_1 , Y_2 , and Y_3 as an overall measure of improvement.

You fit dichotomous *logistic regression* models for Y_j ($j = 1, 2$ and 3) of the form

$$\ln\left(\frac{P_j}{1-P_j}\right) = \alpha_j + \beta_{1j}X_1 + \beta_{2j}X_2 + \dots + \beta_{kj}X_k$$

with the constraint that the slopes must be equal (i.e., $\beta_{11}=\beta_{12}=\beta_{13}$, $\beta_{21}=\beta_{22}=\beta_{23}$, etc.), so you actually fit the model

$$\ln\left(\frac{P_j}{1-P_j}\right) = \alpha_j + \beta_1X_1 + \beta_2X_2 + \dots + \beta_kX_k$$

Requiring equal slopes among the $m-1$ levels for each covariate is called the 'proportional odds assumption'. Using *logistic regression* analysis, you fit the model by obtaining estimates of the coefficients of each covariate under this constraint. Assuming no interactions involving X_i , e^{β_i} is the odds ratio that represents the proportional increase in odds of improvement for each 1-unit increase in X_i ($i = 1, 2, \dots, k$).

In SAS, PROC LOGISTIC automatically fits a cumulative logit function for the proportional odds model when it detects more than two quantitative response levels, as shown in Example 20.2.

Example 20.2—Symptom Relief in Gastroparesis

Patients with severe symptoms of gastroparesis were randomized to receive an experimental therapy (A) or dietary changes with 'watchful waiting' (B). Response was measured after 7 days, using the following scale: 1=no response, 2=some response, 3=marked response, or 4=complete response, based on the degree of symptom relief. A patient's history of severe gastroparesis, thought to be an important covariate, was also recorded as 'no prior episodes' (0), 'one prior episode' (1), or 'more than one prior episode' (2). The data are shown in Table 20.5. Is there any difference in response between the patients who received the experimental therapy and the untreated patients?

TABLE 20.5 Raw Data for Example 20.2

New Treatment (A)			Untreated Group (B)		
Patient Number	History	Response	Patient Number	History	Response
101	1	3	103	1	3
102	2	3	105	1	3
104	1	2	107	1	2
106	0	4	110	2	3
108	2	1	111	2	1
109	0	4	112	0	4
113	1	3	118	0	3
114	1	4	119	2	2
115	0	4	120	1	1
116	2	2	122	2	1
117	1	3	123	1	4
121	2	1	126	0	2
124	1	4	129	2	2
125	1	2	131	1	3
127	2	4	134	1	2
128	0	3	136	2	3
130	1	4	138	2	1
132	1	3	139	0	4
133	1	1	141	1	3
135	1	4	142	2	4
137	0	3	146	1	2
140	2	4	148	1	1
143	1	3	150	1	2
144	2	3	152	1	3
145	2	2			
147	1	4			
149	2	1			
151	2	4			
153	1	2			
154	1	2			
155	1	4			

Solution

The response, Y, is ordinal with 4 levels, so the proportional odds approach is used by fitting three dichotomous *logistic regression* models as shown in Table 20.6. There are two categorical covariates: Study Group (X_1), a nominal effect with levels A and B, and History of severe gastroparesis (X_2), an ordinal effect with levels 0, 1, and 2. You will look at a main effects model, so there will be a total of 5 parameters to estimate (one ' α ' for each of the three models and one ' β ' for each of the two covariates).

TABLE 20.6 Dichotomous Sub-Models for the Proportional Odds Model

	Dichotomous Categories	Interpretation	Model (logit)
(i)	4 vs. 1,2,3	'complete' vs. 'incomplete response'	$\alpha_1 + \beta_1 X_1 + \beta_2 X_2$
(ii)	3,4 vs. 1,2	'marked or complete response' vs. 'some or no response'	$\alpha_2 + \beta_1 X_1 + \beta_2 X_2$
(iii)	2,3,4 vs. 1	'response' vs. 'no response'	$\alpha_3 + \beta_1 X_1 + \beta_2 X_2$

Maximum likelihood estimates of the model parameters are easily found by using SAS, as shown in the analysis that follows.

SAS Analysis of Example 20.2

In this case, the syntax used in PROC LOGISTIC is similar to that used in Example 20.1 for a dichotomous response. The Study Group effect, named rx in the input data set, can take character values (A and B). Here, rx is included in a CLASS statement in PROC LOGISTIC using B as the reference group (specified by the (REF='B') option for the class variable ⑩). In this case, the (REF='B') option is redundant since the default selection in SAS for the reference level is the last value (alphabetically or numerically). However, it's always a good idea to specify the reference level to avoid confusion. The PARAM=REF option is used to request 'reference' parameterization (see Appendix I). This method effectively codes rx values as A = 1, B = 0 (see "Class Level Information" in the output ⑪).

Notice also the use of the (DESCENDING) option for the response variable, resp, in the MODEL statement. This ensures that SAS models the response values as shown in the "Response Profile" section of Output 20.2. To avoid any confusion, SAS prints the note "Probabilities modeled are cumulated over the lower Ordered Values" at the bottom of that section. This means the cumulative logits will be (i) resp=4 corresponding to Ordered Value 1, (ii) resp=4 or 3 corresponding to Ordered Values 1 and 2, and (iii) resp=4, 3, or 2 corresponding to Ordered Values 1, 2, and 3, which agrees with the setup in Table 20.6.

Estimates of the model parameters are given under the heading "Analysis of Maximum Likelihood Estimates" ⑫ in Output 20.2. The slope parameters associated with X_1 and X_2 are the same for all 3 models. The intercepts, which change for each model, are identified by the suffices 4, 3, and 2, corresponding to the cumulative response values, (i) resp=4, (ii) resp=3,4, and (iii) resp=2,3,4. The fitted models, (i), (ii), and (iii) are

$$(i) \quad \ln\left(\frac{P_1}{1-P_1}\right) = -0.3890 + 0.8682X_1 - 0.9741X_2$$

$$(ii) \quad \ln\left(\frac{P_2}{1-P_2}\right) = 1.0756 + 0.8682X_1 - 0.9741X_2$$

$$(iii) \quad \ln\left(\frac{P_3}{1-P_3}\right) = 2.4430 + 0.8682X_1 - 0.9741X_2$$

The odds ratio for the Study Group effect is $e^{0.8682} = 2.383$ ⑭, with the interpretation that the odds of some degree of response with Group A (experimental treatment) is 138% greater than that of the untreated group (B), adjusted for history of prior experience with severe gastroparesis. The p-value is not quite significant ($p=0.0863$) ⑮. You also see that the hist covariate (X_2) is significant ($p=0.0112$) ⑯ with an odds ratio of $e^{-0.9741} = 0.378$ ⑰ (see Section 20.4.12 and 20.4.13 for additional details).

SAS Code for Example 20.2

```

data gi;
  input pat rx $ hist resp @@;
  /* A = new treatment, B = untreated */ 
  /* hist = 0 if no prior episodes, hist = 1 if one */ 
  /* prior episode, hist = 2 if >1 prior episodes */ 
  /* resp = 1 (none), 2 (some), 3 (marked), 4 (complete) */
  datalines;
  101 A 1 3 102 A 2 3 103 B 1 3 104 A 1 2
  105 B 1 3 106 A 0 4 107 B 1 2 108 A 2 1
  109 A 0 4 110 B 2 3 111 B 2 1 112 B 0 4
  113 A 1 3 114 A 1 4 115 A 0 4 116 A 2 2
  117 A 1 3 118 B 0 3 119 B 2 2 120 B 1 1
  121 A 2 1 122 B 2 1 123 B 1 4 124 A 1 4
  125 A 1 2 126 B 0 2 127 A 2 4 128 A 0 3
  129 B 2 2 130 A 1 4 131 B 1 3 132 A 1 3
  133 A 1 1 134 B 1 2 135 A 1 4 136 B 2 3
  137 A 0 3 138 B 2 1 139 B 0 4 140 A 2 4
  141 B 1 3 142 B 2 4 143 A 1 3 144 A 2 3
  145 A 2 2 146 B 1 2 147 A 1 4 148 B 1 1
  149 A 2 1 150 B 1 2 151 A 2 4 152 B 1 3
  153 A 1 2 154 A 1 2 155 A 1 4
;

proc logistic data = gi;
  class rx(ref='B') / param = ref;
  model resp(descending) = rx hist;
  title1 'Logistic Regression';
  title2 'Example 20.2: Symptom Relief in Severe
  Gastroparesis';
run;

```

OUTPUT 20.2 SAS Output for Example 20.2

Logistic Regression
Example 20.2: Symptom Relief in Severe Gastroparesis

The LOGISTIC Procedure

Model Information

Data Set	WORK.GI
Response Variable	resp
Number of Response Levels	4
Model	cumulative logit
Optimization Technique	Fisher's scoring
Number of Observations Read	55
Number of Observations Used	55

Response Profile

Ordered Value	resp	Total Frequency
1	4	16
2	3	17
3	2	13
4	1	9

Probabilities modeled are cumulated over the lower Ordered Values.

Class Level Information

Class	Value	Design Variables
rx	A	1
	B	0

⑫

Model Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Score Test for the Proportional Odds Assumption

Chi-Square	DF	Pr > ChiSq
2.5521	4	0.6353

⑯

Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	155.516	149.907
SC	161.538	159.944
-2 Log L	149.516	139.907

OUTPUT 20.2 SAS Output for Example 20.2 (continued)

Logistic Regression
Example 20.2: Symptom Relief in Severe Gastroparesis

The LOGISTIC Procedure

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	9.6084	2	0.0082
Score	9.0147	2	0.0110
Wald	8.7449	2	0.0126

Type 3 Analysis of Effects

Effect	DF	Chi-Square	Pr > ChiSq
rx	1	2.9426	0.0863
hist	1	6.4304	0.0112

Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Chi-Square	Pr > ChiSq
Intercept 4	1	-0.3890	0.5831	0.4449	0.5048
Intercept 3	1	1.0756	0.6001	3.2123	0.0731
Intercept 2	1	2.4430	0.6698	13.3018	0.0003
rx	A	0.8682	0.5061	2.9426	0.0863
hist	1	-0.9741	0.3841	6.4304	0.0112

Odds Ratio Estimates

Effect	Point Estimate	95% Wald Confidence Limits
rx A vs B	2.383	0.884 6.425
hist	0.378	0.178 0.802

Association of Predicted Probabilities and Observed Responses

Percent Concordant	57.6	Somers' D	0.339
Percent Discordant	23.7	Gamma	0.417
Percent Tied	18.7	Tau-a	0.255
Pairs	1115	c	0.670

Clustered Binomial Data

In Chapter 19, you saw how the *Cochran-Mantel-Haenszel test* can be used to analyze binomial responses from stratified groups. In that case, observations within strata are considered independent, generally arising from different patients.

Sometimes binary responses within strata are correlated, in which case the *CMH test* would not be appropriate. Such situations arise frequently in clinical trials when a number of binary outcomes are observed in each of several patients.

For example, suppose migraine patients are prescribed a new ‘as needed’ medication and are asked to keep a diary of the number of migraine headaches they experience and the number of those which are resolved within two hours of taking the medication. In this case, each migraine occurrence results in the binary outcome ‘resolved’ or ‘not resolved’ within two hours of medication. While independence can be assumed among patients, each patient can experience several occurrences, so there is a natural clustering of observations for each patient for whom you could reasonably assume responses are correlated.

Such data can be analyzed by using PROC LOGISTIC with a correction for overdispersion as shown in Example 20.3. This procedure essentially re-scales the correlation matrix that is used to estimate the standard errors of the parameter estimates. Without this adjustment, the within-cluster correlation might lead to underestimating the standard errors, which, in turn, leads to results that are overly significant.

Example 20.3—Intercourse Success Rate in Erectile Dysfunction

Male patients who experienced erectile dysfunction (ED) following prostate surgery were enrolled in a parallel study to compare a new ED treatment with a marketed drug. Patients were asked to keep a diary during the six week study to record the number of attempts at sexual intercourse after taking the study medication, and the number of those attempts that were ‘successful’. Data, including patient’s age, which is thought to be an important covariate, are shown in Table 20.7. Is there any difference in ‘success’ rates between the new medication and the product already on the market?

Solution

In this case, each patient represents a cluster of like binomial responses. It is natural to assume that within-patient responses might be correlated, certainly more so than among different patients. If you perform a *logistic regression* analysis of response rates with effects for treatment Group and Age that ignores this clustering, you might get inaccurate results. Proceeding with a *logistic regression* analysis that uses a correction for overdispersion would be more appropriate in this case.

TABLE 20.7 Raw Data for Example 20.3

Reference Control				New Drug			
Pat. No.	Age (yrs.)	Number of Successes	Number of Attempts	Pat. No.	Age (yrs.)	Number of Successes	Number of Attempts
1	41	3	6	2	57	3	8
3	44	5	15	4	54	10	12
5	62	0	4	7	65	0	0
6	44	1	2	9	51	5	8
8	70	3	8	10	53	8	10
11	35	4	8	12	44	17	22
13	72	1	6	14	66	2	3
15	34	5	15	16	55	9	11
18	61	1	7	17	37	6	8
22	35	5	5	19	40	2	4
24	52	6	8	20	44	9	16
25	66	1	7	21	64	5	9
27	35	4	10	23	78	1	3
30	61	4	8	26	51	6	12
31	55	2	5	28	67	5	11
34	41	7	9	29	44	3	3
37	53	2	4	32	65	7	18
39	72	4	6	33	69	0	2
40	68	0	0	35	53	4	14
41	56	12	17	36	49	5	8
44	53	8	15	38	74	10	15
45	45	3	4	42	39	4	9
48	40	14	20	43	35	8	10
				46	47	4	5
				47	46	6	7

SAS Analysis of Example 20.3

Two patients with no attempts (Pat. Nos. 7 and 40) are excluded from the analysis 19, which leaves 46 patients or ‘clusters’ of response data. The *logistic regression* model is specified in SAS in the usual way using PROC LOGISTIC, except that in this case, you use the ‘events/trials’ form, which is an alternative specification for the dependent variable in the MODEL statement. The ‘events’ are the number of successes (succ), and the ‘trials’ are the number of attempts (atpt). Include treatment group (trt) as a classification variable and age (age) as a continuous numeric covariate in the model 20.

Several different methods for re-scaling the covariance matrix are available in SAS. Williams’ method often works well with clustered data, especially when sample sizes differ among the clusters. This approach is implemented by specifying the SCALE=WILLIAMS option in the MODEL statement 20.

The results, shown in Output 20.3, indicate a significant treatment effect (p=0.0291) with an estimated odds ratio of 1.738. This means that the age-adjusted odds of success increase by 73.8% for the new drug relative to the control. Age is shown to be a significant covariate (p=0.0124). Its odds ratio is 0.973, indicating that the odds of success decrease by about 2.7% for each year the patient ages.

SAS Code for Example 20.3

```

data diary;
  input pat trt age succ attpt @@;
  /* trt = 1 for new drug, trt = 0 for reference control */
  if attpt = 0 then delete; 19
  datalines;
  1 0 41 3 6      3 0 44 5 15      5 0 62 0 4      6 0 44 1 2
  8 0 70 3 8      11 0 35 4 8      13 0 72 1 6      15 0 34 5 15
  18 0 61 1 7     22 0 35 5 5      24 0 52 6 8      25 0 66 1 7
  27 0 35 4 10    30 0 61 4 8      31 0 55 2 5      34 0 41 7 9
  37 0 53 2 4     39 0 72 4 6      40 0 68 0 0      41 0 56 12 17
  44 0 53 8 15    45 0 45 3 4      48 0 40 14 20    2 1 57 3 8
  4 1 54 10 12    7 1 65 0 0      9 1 51 5 8      10 1 53 8 10
  12 1 44 17 22   14 1 66 2 3      16 1 55 9 11    17 1 37 6 8
  19 1 40 2 4     20 1 44 9 16    21 1 64 5 9      23 1 78 1 3
  26 1 51 6 12    28 1 67 5 11    29 1 44 3 3      32 1 65 7 18
  33 1 69 0 2     35 1 53 4 14    36 1 49 5 8      38 1 74 10 15
  42 1 39 4 9     43 1 35 8 10    46 1 47 4 5      47 1 46 6 7
;

proc logistic data = diary;
  class trt(ref='0') / param = ref;
  model succ/attpt = trt age / scale = williams; 20
  title1 'Logistic Regression';
  title2 'Example 20.3: Intercourse Success Rate in Erectile
         Dysfunction';
run;

```

OUTPUT 20.3 SAS Output for Example 20.3

```

Logistic Regression
Example 20.3: Intercourse Success Rate in Erectile Dysfunction

The LOGISTIC Procedure

Model Information

Data Set                  WORK.DIARY
Response Variable (Events) succ
Response Variable (Trials) attpt
Weight Variable           1 / ( 1 + 0.056638 * (attpt - 1) ) 26
Model                     binary logit
Optimization Technique    Fisher's scoring

Number of Observations Read      46
Number of Observations Used     46
Sum of Frequencies Read        417
Sum of Frequencies Used        417
Sum of Weights Read            268.539
Sum of Weights Used            268.539

```

OUTPUT 20.3 SAS Output for Example 20.3 (continued)

Logistic Regression
Example 20.3: Intercourse Success Rate in Erectile Dysfunction

The LOGISTIC Procedure

Response Profile

Ordered Value	Binary Outcome	Total Frequency	Total Weight
1	Event	234	149.48211
2	Nonevent	183	119.05692

Class Level Information

Class	Value	Design Variables
trt	0	0
	1	1

Model Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Deviance and Pearson Goodness-of-Fit Statistics

Criterion	Value	DF	Value/DF	Pr > ChiSq
Deviance	48.2014	43	1.1210	0.2706
Pearson	42.9997	43	1.0000	0.4713

Number of events/trials observations: 46

NOTE: Since the Williams method was used to accommodate **25** overdispersion, the Pearson chi-squared statistic and the deviance can no longer be used to assess the goodness of fit of the model.

Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	370.820	364.792
SC	374.853	376.891
-2 Log L	368.820	358.792

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	10.0277	2	0.0066
Score	9.9254	2	0.0070
Wald	9.6295	2	0.0081

OUTPUT 20.3 SAS Output for Example 20.3 (continued)

Logistic Regression																													
Example 20.3: Intercourse Success Rate in Erectile Dysfunction																													
The LOGISTIC Procedure																													
Type 3 Analysis of Effects																													
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20.4 Details & Notes

- **20.4.1** In clinical trials, equality of response rates between groups is usually tested by the hypothesis of a 0 difference, as you have seen in previous chapters. You can also compare response rates by measuring how close their ratio is to 1. This ratio is called ‘relative risk’ and is often discussed in epidemiological studies along with the odds ratio. The odds ratio gets closer to the relative risk as the event becomes more rare.

In a modeling procedure such as *logistic regression*, any comparison of response rates between treatment groups depends on the values of other covariates in the model. With the logit function, the odds ratio is the ‘cleanest’ way of comparing levels of a model factor such as a treatment effect. The

treatment effect odds ratio is adjusted for other covariates but does not depend on their specific values if there are no interactions involving treatment.

- **20.4.2** In a multiple *logistic regression*, the importance of a numeric covariate, X_i , to another covariate in the same model, X_j , can be measured by comparing the relative sizes of the estimates β_i with β_j only when the two covariates are measured in the same units on the same scale. You can compare relative importance of covariates measured on different scales by standardizing the β estimates by dividing by their standard errors.
- **20.4.3** *Logistic regression* is a useful tool for conducting exploratory analyses to identify background factors that might help explain trends observed in treatment comparisons. The LOGISTIC procedure in SAS prints out two measures, Akaike's Information Criterion (AIC) and the Schwarz Criterion (SC), whose relative values can be used to assess the goodness of fit of the model. Covariates can be added or removed by sequentially fitting various models to obtain the most appropriate one by attempting to minimize the values of the AIC and SC. If you include the SELECTION option in the MODEL statement, SAS will automatically fit a series of *logistic regression* models with systematic inclusion or exclusion of effects in the model, based on their significance.

When you use the SCALE=NONE option in the MODEL statement in PROC LOGISTIC, two other goodness-of-fit tests, the *Pearson* and *Deviance tests*, are printed. These measures are useful only when the covariates have a small number of levels. The SCALE=NONE option prevents overdispersion adjustments to the goodness-of-fit tests. Non-significance of these tests, which have an approximate chi-square distribution with sufficiently large sample sizes relative to the number of covariates, provides an indication of adequate fit. As noted in Output 20.3 ㉙, these goodness-of-fit tests are not valid when the Williams' correction for overdispersion is made.

The AGGREGATE option in the MODEL statement can be used to identify subgroups defined by combinations of factor levels for assessing goodness-of-fit. These same subgroups are then used to compare relative goodness-of-fit measures among competing models. When one or more of the explanatory variables is measured on a continuous numeric scale, you might prefer to use the LACKFIT option to assess goodness of fit. This option performs a goodness-of-fit test based on a division of the range of the model-based predicted probabilities into intervals.

ODS Graphics under PROC LOGISTIC or PROC GENMOD can provide visual aids in model fitting diagnostics, including influence and leverage plots for locating outliers similar to those described in Chapter 10.

Assessing the fit of a *logistic regression* model can be a complex process, and the details are beyond the scope of this introduction to *logistic regression*. Examples that demonstrate goodness-of-fit methods and provide additional details can be

found in many excellent references, including two highly recommended SAS Press books: Allison (1999), and Stokes, Davis, and Koch (2000).

- **20.4.4** Model building by adding or removing explanatory variables in the logistic model should be performed under constant assumptions regarding the covariance structure of the response variable. When observations arise from different patients, the assumption of independent observations is usually valid, so this is not a problem. In the clustered binomial case, the variance-covariance matrix is weighted using a scale parameter that is estimated by SAS and included in the output. For the SCALE=WILLIAMS adjustment used in Example 20.3, this estimate is 0.056638, as shown in the “Model Information” section of Output 20.3 for Weight Variable ⑩. To maintain the same covariance structure for alternative models, you can include the scale parameter in parenthesis in the SCALE=WILLIAMS option to request that the same weightings be used when adding or removing model effects, e.g., SCALE = WILLIAMS(0.056638).

- **20.4.5** In Example 20.1, notice that the unadjusted odds (ignoring remission time) are

$$0.442 / 0.558 = 0.792 \text{ (Active group)}$$

$$0.600 / 0.400 = 1.500 \text{ (Control group)}$$

A comparison of these odds results in an odds ratio of $0.792 / 1.500 = 0.528$, which is considerably larger than the odds ratio (0.327) found by using the covariance adjustment.

- **20.4.6** An approximate 95% confidence interval can be constructed for the odds ratios by exponentiating the upper and lower confidence limits for the model parameter, β_1 . Because the MLEs of the model parameters have asymptotic normal distributions, confidence limits can be constructed in the usual manner. In Example 20.1, the confidence limits for the trt parameter, β_1 , is represented by

$$\mathbf{b}_1 \pm 1.96 \cdot s(\mathbf{b}_1)$$

where \mathbf{b}_1 and $s(\mathbf{b}_1)$ can be obtained from the SAS output ⑨. Output 20.1 shows $\mathbf{b}_1 = -1.1191$ and $s(\mathbf{b}_1) = 0.4669$, resulting in a 95% confidence interval for β_1 as

$$\begin{aligned} & -1.1191 \pm 1.96 \cdot (0.4669) \\ \text{or} \\ & (-2.034 \text{ to } -0.204) \end{aligned}$$

An approximate 95% confidence interval for the odds of response on active treatment relative to control is

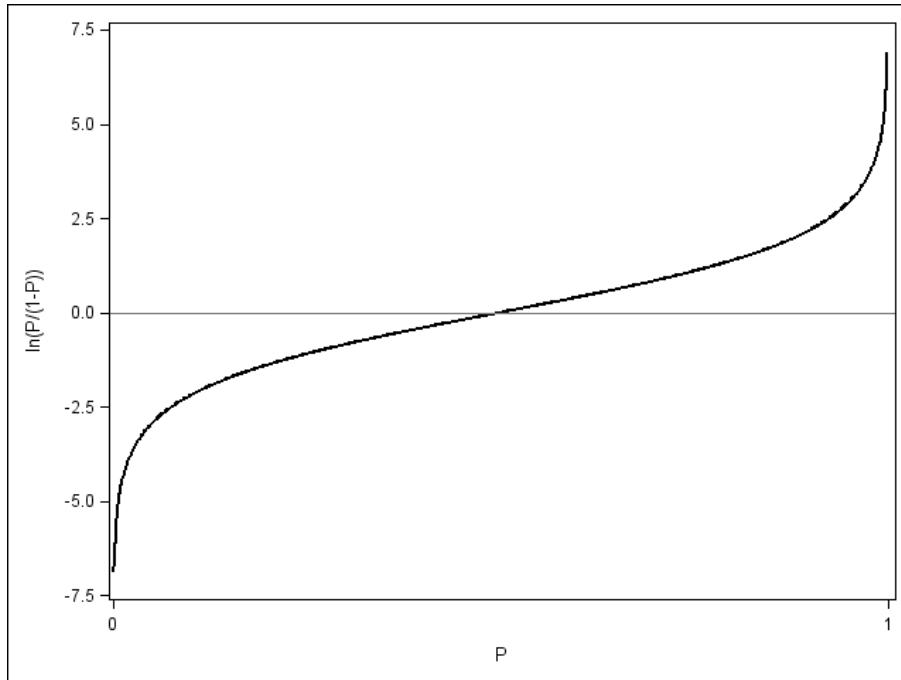
$$(e^{-2.034} \text{ to } e^{-0.204}) = (0.131 \text{ to } 0.815).$$

Estimates of the odds ratios and 95% confidence limits are automatically printed in the SAS output ⑧ when using PROC LOGISTIC.

- **20.4.7** The logistic transformation enjoys popular usage because of its application ease and appropriateness as a model for a wide range of natural phenomena. As illustrated in a plot of P vs. its logit function (Figure 20.4), $\ln(P/(1-P))$ is fairly linear in P over a large portion of the range of P (approximately 0.2 to 0.8). For very large or very small values of P, the logit of P is greatly magnified. This is consistent with a model based on intuition, in which a change in response rates, say from 45% to 50%, might not be as clinically relevant as a change from 5% to 10%. Other transformations, such as the probit (based on the inverse normal distribution) can be used in a manner similar to the logit.

Certain models based on a response, $P = \text{Prob}(\text{event})$, may result in estimates outside the observable range of 0 to 1. The logistic transformation has the advantage of mapping a limited range of observable values (0 to 1) onto an unlimited range ($-\infty$ to $+\infty$). Thus, the potential problem of obtaining estimates outside the observable range is eliminated by using the logit function.

FIGURE 20.4 Plot of Transformation from P to the Logit(P)



- **20.4.8** This chapter presents an introduction to *logistic regression* using covariates that have either continuous numeric or ordinal values. These methods include nominal dichotomous explanatory variables, because the dichotomous levels can always be coded as 0 or 1. In Examples 20.1–20.3, you

could use numeric (0-1) codes for the treatment or group effect and omit the CLASS statement with the same results.

Logistic regression can also be used with nominal-level, categorical explanatory variables that have more than two levels by defining dummy variables that correspond to the levels. For example, suppose geographic region is a covariate of interest, with levels A (North), B (South), C (Midwest), and D (West). One of the levels, e.g., A, is designated as a reference, and three dummy variables are created as follows:

$$\begin{aligned} X_1 &= 1 \text{ if from Region B} \\ X_1 &= 0 \text{ otherwise} \end{aligned}$$

$$\begin{aligned} X_2 &= 1 \text{ if from Region C} \\ X_2 &= 0 \text{ otherwise} \end{aligned}$$

$$\begin{aligned} X_3 &= 1 \text{ if from Region D} \\ X_3 &= 0 \text{ otherwise} \end{aligned}$$

Using a *logistic regression* model with these variables as covariates, the odds-ratios represent the odds of response relative to Region A. Specifying a model in this manner represents a ‘reference parameterization’ discussed in more detail in Appendix I.

While these dummy variables can easily be created by using a DATA step in SAS, this can become cumbersome for categorical variables that have large numbers of levels. PROC LOGISTIC automatically creates the dummy variables when the variable is included in a CLASS statement where, as shown in Examples 20.1–20.3, you can assign the reference level. This dummy coding also makes it easy to specify interaction terms in the MODEL statement by using GLM-type specifications (e.g., A*B). In Example 20.2, you can include the Study Group-by-History interaction simply by including rx*hist in the MODEL statement.

- **20.4.9** *Logistic regression* is a very important and widely used tool in the analysis of clinical research data. PROC LOGISTIC is capable of handling most *logistic regression* analyses encountered in clinical trials, as typified by the examples in this chapter, although most will include more covariates and larger sample sizes. One of the main advantages of using the LOGISTIC procedure is its extensive model building capabilities and diagnostics. You can also conduct *logistic regression* analyses using several other SAS procedures, including PROC CATMOD, PROC GENMOD, PROC GLIMMIX, and PROC PROBIT.

The CATMOD procedure is not appropriate for *logistic regression* with continuous numeric covariates but can be used effectively with nominal categorical explanatory variables. However, PROC LOGISTIC, which is easier to use and more flexible than PROC CATMOD, can perform the same analysis using the CLASS statement.

The GENMOD and GLIMMIX procedures are SAS procedures for handling generalized linear models. PROC GENMOD is used for fixed effects, and PROC GLIMMIX is used for random or mixed effects. The generalized linear model can model a wide array of different types of responses, such as those from a normal distribution, binomial or multinomial responses, log-linear responses, or Poisson data, as examples. When using these procedures, you need to specify the distribution of your response data with the DIST= option and an appropriate link function by using the LINK= option. By using the identity (ID) as the link function with normally distributed data, PROC GENMOD and PROC GLIMMIX will model GLM-type models discussed in earlier chapters for regression, ANOVA and ANCOVA. To obtain a *logistic regression* model with these procedures, specify LOGIT as the link function. For example, you could analyze Example 20.1 with the following SAS code:

```
proc genmod data=aml descending;
  class group;
  model relapse=group x / link=logit dist=binomial;
run;
```

The DIST=BINOMIAL option lets PROC GENMOD know the responses are binomial, and the LINK=LOGIT option specifies that the logit function is to be modeled, i.e., *logistic regression*. When using PROC GENMOD with a proportional odds model as in Example 20.2, you would specify LINK=CLOGIT for the cumulative logit. Note that LOGIT is the default LINK when using PROC LOGISTIC for a binary response, and CLOGIT is default when PROC LOGISTIC detects more than two response levels. You can print out the odds ratios and 95% confidence intervals by using the ODDSRATIOS option with PROC GLIMMIX. This option can include adjustments for multiple testing. The ODDSRATIOS option is not available with PROC GENMOD.

You can also use these SAS procedures to perform *logistic regression* analysis of more complex experimental layouts such as fitting generalized estimating equations (GEEs) to repeated measures designs, clustered layouts, and more complex proportional odds models. Details about using these alternative procedures in SAS for *logistic regression* can be found in a number of other excellent books, including Stokes, Davis, and Koch (2000), and Allison (1999), both published by SAS Press.

- **20.4.10** By making the proportional odds assumption, *logistic regression* techniques can be used to analyze ordinal categorical responses with more than two levels. A test for this assumption is included in the SAS output from PROC LOGISTIC. In Example 20.2, a ‘Score’ test is used based on the chi-square distribution, which shows a non-significant result ($p=0.6353$) ⑯. This result supports the assumption of equal slope parameters.

If this test is significant, caution should be used in the interpretation. If it is not possible to justify the proportional odds assumption, an alternative method is to use the LINK=GLOGIT option in PROC LOGISTIC to fit a

generalized logit model that treats the response as a nominal effect, ignoring the ordinality of the response levels (see Section 20.4.16).

- **20.4.11** In Output 20.1, you see that the odds ratio in Example 20.1 for the treatment effect is 0.327 ⑩, which means that the odds of relapse for active treatment is 67.3% less than that for the placebo. This estimate does not depend on the other covariate, prior remission time. However, in comparative clinical trials, it is often decided to report covariate-adjusted response rates for each treatment group. Estimates of these response rates do, however, depend on other covariates in the model. Table 20.4 shows the ‘naïve’ estimates of the covariate-adjusted response rates.

Substituting 1 and 0, respectively, for group in the estimated *logistic regression* model for Example 20.1, you obtain

$$\hat{P}_{(A)} = \frac{1}{1 + e^{-(1.4944 - 0.1998 \cdot X)}} \quad (\text{Active group})$$

$$\hat{P}_{(C)} = \frac{1}{1 + e^{-(2.6135 - 0.1998 \cdot X)}} \quad (\text{Control group})$$

Given a prior remission time of $X=4$ months, for example, the predicted relapse rates for the active and placebo groups are estimated from the preceding equations, namely, 67% for the active group and 86% for the control group. This method can be repeated for any other values of prior remission time in the range of observed X values (3 to 18 months), which includes unobserved values such as the overall mean remission time. Caution must be used when extrapolating beyond the experimental region.

These predicted values can be computed by SAS and written to an output data set by specifying the OUTPUT statement following the MODEL statement in PROC LOGISTIC. When the PREDICTED= option is used, SAS includes estimated probabilities of the event in the output data set for each combination of observed covariate values. If you want predicted probabilities for non-observed values, additional observations with missing values for the response variable and the covariate values that you choose can be included in the data set. This inclusion would not change the analysis since, if the response variable is missing for an observation, that observation is not used by PROC LOGISTIC in estimating the model. (Note: If the response is multinomial, the PREDPROBS=INDIVIDUAL option is recommended, since it provides predicted probabilities for each response level. See the *SAS/STAT User’s Guide* for PROC LOGISTIC for details.)

In Example 20.1, you can obtain the relapse rates for each group by creating a dummy data set of observations that contains the values of the covariate and concatenate this with the analysis data set. For example, to include estimated probabilities for $X = 3, 4, \dots, 18$, concatenate the data set ADDON (see below) with the AML data set.

```

data addon;
do i = 1 to 16;
  x = i + 2;
  trt = 0; output;
  trt = 1; output;
end;
run;

```

Note: Concatenation refers to the process of appending one data set with observations from another data set. This is done with the SET statement in the SAS DATA step. For more information, see *The Little SAS Book: a Primer, Third Edition* by Delwiche and Slaughter (page 172), which is published by SAS Press.

By adding the statement

```
output out = estim predicted = p_est;
```

following the MODEL statement in PROC LOGISTIC, the estimated probabilities are included in the output data set ESTIM, which can be rearranged to display the results, as shown in Table 20.8 and plotted as shown in Figure 20.5. Confidence intervals for these predicted values can also be easily obtained in the output data set (see PROC LOGISTIC in the *SAS/STAT User's Guide*).

Also note that you can avoid this data concatenation process by using the SCORE statement in PROC LOGISTIC. The following PROC LOGISTIC code produces a SCORES data set with the estimated relapse rates in Table 20.8 found in variable P_YES.

```

proc logistic data = all;
  class group(ref="PBO") / param=ref;
  model relapse(event="YES") = group x;
  score out=scores;
run;

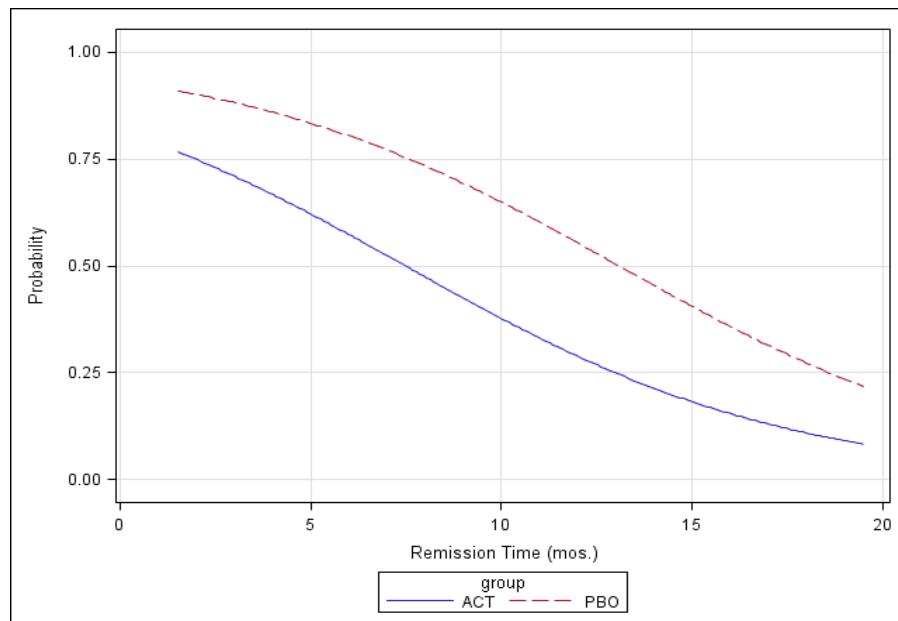
proc print data=scores;
  where pat=.;
run;

```

TABLE 20.8 Estimated Relapse Rates for Various Remission Times (X) in Example 20.1

X	Control	Active	X	Control	Active
3	0.88	0.71	11	0.60	0.33
4	0.86	0.67	12	0.55	0.29
5	0.83	0.62	13	0.50	0.25
6	0.80	0.57	14	0.45	0.21
7	0.77	0.52	15	0.41	0.18
8	0.73	0.47	16	0.36	0.15
9	0.69	0.42	17	0.31	0.13
10	0.65	0.38	18	0.27	0.11

FIGURE 20.5 Plot of Estimated Relapse Probabilities vs. Time since Prior Remission (X)



■ **20.4.12** Example 20.2 uses an ordinal response with 4 levels, so the estimated probabilities are provided for each of the three dichotomous models used (i, ii, and iii in Table 20.6). For $X_1=1$ (Group A) and $X_2=0$ (no previous episodes), these models result in the following estimated probabilities:

- (i) $\ln(P_1 / (1-P_1)) = -0.3890 + 0.8682 = 0.479$ or $P_1 = 0.6175$
- (ii) $\ln(P_2 / (1-P_2)) = 1.0756 + 0.8682 = 1.944$ or $P_2 = 0.8747$
- (iii) $\ln(P_3 / (1-P_3)) = 2.4430 + 0.8682 = 3.311$ or $P_3 = 0.9648$

P_1 represents the probability of ‘complete’ response, P_2 is the probability of ‘marked’ or ‘complete’ response, and P_3 is the probability of any response (‘some’, ‘marked’, or ‘complete’). You can obtain these estimated probabilities for each combination of covariate levels by using the OUTPUT statement as shown in Section 20.4.11. The results are shown below in Table 20.9.

TABLE 20.9 Estimated Probabilities for Example 20.2

Model	History (X ₂)	----- Group (X ₁) -----	
		A	B
(i)	0	0.618	0.404
	1	0.379	0.204
	2	0.187	0.088
(ii)	0	0.875	0.746
	1	0.725	0.525
	2	0.499	0.295
(iii)	0	0.965	0.920
	1	0.912	0.813
	2	0.796	0.621

- **20.4.13** The X₂ covariate in Example 20.2 (history of severe gastroparesis) is used as an ordinal covariate with 3 levels: ‘no prior episodes’, ‘one prior episode’, and ‘more than one prior episode’. The odds ratio seen in Output 20.2 is 0.378 $\textcircled{7}$, which means that the odds of improving response decreases by 62.2% for each incremental increase in the history category.

This analysis assumes that the log-odds is related to incremental changes in X₂ in a linear way. If there is reason to believe that the log-odds of ‘one prior episode’ vs. ‘no prior episodes’ should be different from the log-odds of ‘more than one prior episode’ vs. ‘one prior episode’, the slope cannot be assumed to be linear. In this case, a quadratic term for prior history (hist*hist) can be included in the model, or, alternatively, you can ignore the ordinality of the hist variable and include it in the CLASS statement. When used as a class variable, PROC LOGISTIC estimates β ’s and odds ratios separately for each level of hist relative to the reference value.

You can also include interaction effects in the MODEL statement with GLM-type specifications. When interactions are included, however, estimating the odds ratio for a main effect that is also included in an interaction is no longer a simple matter of exponentiating the main effect parameter estimate. The ODDSRATIO statement in PROC LOGISTIC can be used to produce the odds ratios in the presence of interactions.

- **20.4.14** Just as *analysis of covariance* assumes equal slopes among groups, *logistic regression* also assumes that the slope of the logit function is similar among groups. This assumption can be checked by a preliminary plot of the data along with the inclusion of the interaction terms between the appropriate X-variables in the *logistic regression* model. Caution must be used in the interpretation of treatment group differences in the presence of interactions.
- **20.4.15** The maximum likelihood equations might not always give a solution. In cases with small sample sizes, convergence to a unique solution based on the iterative calculations might not be attainable. Although conservative, a general guideline is that the sample size N should be at least 10 times the number of covariates when fitting a *logistic regression* model. When sample sizes are small, exact methods for fitting *logistic regression* models are available. However, other categorical methods might be more appropriate in clinical trials applications (see, e.g., Chapters 15–19) when sample sizes are very small.
- **20.4.16** In this chapter, you've seen how to fit a *logistic regression* model to binomial and ordered categorical response data. Most often, you think of logistic regression with continuous numeric or ordinal covariates. In addition, Section 20.4.8 discusses the use of nominal categorical covariates. *Logistic regression* can also be used when the response is a nominal categorical variable with more than two levels.

In SAS, you can fit nominal categorical responses to a logistic regression model using the generalized logit link function, GLOGIT. In PROC LOGISTIC, you would specify the LINK=GLOGIT option in the MODEL statement. See the *SAS/STAT User's Guide* for additional details.

CHAPTER 21

The Log-Rank Test

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21.1 Introduction

The *log-rank test* is a statistical method for comparing distributions of time until the occurrence of an *event* of interest among independent groups. The *event* is often death due to disease, but *event* might be any binomial outcome, such as cure, response, relapse, or failure. The elapsed time from initial treatment or observation until the *event* is the *event* time, often referred to as ‘survival time’, even when the *event* is not ‘death’.

The *log-rank test* provides a method for comparing ‘risk-adjusted’ *event* rates. This is useful when patients in a clinical study are subject to varying degrees of opportunity to experience the *event*. Such situations arise frequently in clinical trials due to the finite duration of the study, early termination of the patient from the study, or interruption of treatment before the *event* occurs.

Examples where use of the *log-rank test* might be appropriate include comparing survival times in cancer patients who are given a new treatment with patients who receive standard chemotherapy, or comparing times-to-cure among several doses of a topical antifungal preparation where the patient is treated for 10 weeks or until cured, whichever comes first.

Usually, *event* times are not well-modeled using the normal distribution. Survival analysis modeling techniques using specific distributional assumptions are discussed in Chapter 22. The *log-rank test* is a non-parametric test and, as such, does not require any distributional assumptions about the *event* times. If every patient were followed until the *event* occurrence, the *event* times could be compared between two groups using the *Wilcoxon rank-sum test* (Chapter 13). However, some patients might drop out or complete the study before the *event* occurs. In such cases, the actual time to *event* is unknown because the *event* does not occur while under study observation.

The *event* times for these patients are based on the last known time of study observation, and are called ‘censored’ observations because they represent the lower-bound of the true, unknown *event* times. The *Wilcoxon rank-sum test* can be highly biased in the presence of censored data. The *log-rank test* adjusts for censoring, thereby, accounting for the patient’s opportunity to experience the *event*.

21.2 Synopsis

The null hypothesis tested by the *log-rank test* is that of equal *event* time distributions among groups. Equality of the distributions of *event* times implies similar risk-adjusted *event* rates among groups not only for the clinical trial as a whole, but also for any arbitrary time point during the trial. Rejection of the null hypothesis indicates that the *event* rates differ among groups at one or more time points during the study.

Here, you will examine the case of two independent groups, although the same method is easily extended to more than two groups. A typical data-set layout has the following form, where Y represents the time from initial treatment to the *event* occurrence, and a † indicates a censored value.

TABLE 21.1 Layout for the Log-Rank Test

GROUP 1		GROUP 2	
Patient Number	Event Time	Patient Number	Event Time
101	Y_{11}	102	Y_{21} †
103	Y_{12} †	105	Y_{22}
104	Y_{13}	106	Y_{23}
:	:	:	:
:	:	:	:
N_1	Y_{1N_1} †	N_2	Y_{2N_2} †

† indicates censored time

Divide the study into k distinct time periods, t_1, t_2, \dots, t_k , where t_j ($j=1, 2, \dots, k$) represents the j^{th} time point when one or more patients in the combined samples becomes *event-positive*. Let d_{ij} represent the number of patients in Group i ($i=1, 2$) who first experience the *event* at time period t_j , and let n_{ij} represent the number of patients in Group i who are at risk at the beginning of time period t_j . At risk describes the patients who are ‘*event-negative*’ and still in the study. Let $d_j = d_{1j} + d_{2j}$ and let $n_j = n_{1j} + n_{2j}$. For $j=1, 2, \dots, k$, compute

$$e_{ij} = \frac{n_{ij} \cdot d_j}{n_j} \quad \text{and} \quad v_j = \frac{n_{1j} \cdot n_{2j} \cdot d_j \cdot (n_j - d_j)}{n_j^2 \cdot (n_j - 1)}$$

Finally, you obtain

$$O_1 = \sum_{j=1}^k d_{1j}$$

$$E_1 = \sum_{j=1}^k e_{1j}$$

and

$$V = \sum_{j=1}^k v_j$$

Denote by Y_i a random variable that represents the *event time* for Group i ($i=1, 2$), and let $S_i(t) = \text{Prob}(Y_i \geq t)$. The test summary for the *log-rank test* is as follows:

null hypothesis:	$H_0: S_1(t) = S_2(t)$ (for all times, t)
alt. hypothesis:	$H_A: S_1(t) \neq S_2(t)$ (for at least one time, t)

test statistic:	$\chi^2 = \frac{(O_1 - E_1)^2}{V}$
------------------------	------------------------------------

decision rule:	reject H_0 if $\chi^2 > \chi^2_1(\alpha)$ where, $\chi^2_1(\alpha)$ is the critical chi-square value with significance level, α , and 1 degree of freedom.
-----------------------	---

21.3 Examples

¶ Example 21.1—HSV-2 Episodes following gD2 Vaccine

Forty-eight patients with genital herpes (HSV-2) were enrolled in a study of a new recombinant herpes vaccine based on the antigen glycoprotein, gD2. Patients were required to have a history of at least 6 HSV-2 episodal outbreaks in the 12 months prior to enrollment in the study and be in remission at the time of vaccination. Patients were randomly assigned to receive either a single gD2 vaccine injection ($n=25$) or a placebo ($n=23$), and their conditions were followed for 1 year. The data in Table 21.2 show the time (in weeks) to first recurrence of HSV-2 following immunization for each patient (Y), along with the number of episodes during the 12-month period prior to enrollment (X). Is there evidence that the distributions of the times to recurrence differ between the groups?

TABLE 21.2 Raw Data for Example 21.1

----- gD2 Vaccine Group -----

Patient Number	X	Y	
1	12	8	
3	10	12	†
6	7	52	†
7	10	28	
8	6	44	
10	8	14	
12	8	3	
14	9	52	†
15	11	35	
18	13	6	
20	7	12	
23	13	7	†
24	9	52	†

Patient Number	X	Y	
26	12	52	†
28	13	36	
31	8	52	†
33	10	9	
34	16	11	†
36	6	52	†
39	14	15	
40	13	13	
42	13	21	
44	16	24	†
46	13	52	†
48	9	28	

----- Placebo Group -----

Patient Number	X	Y	
2	9	15	
4	10	44	†
5	12	2	†
9	7	8	
11	7	12	
13	7	52	†
16	7	21	
17	11	19	
19	16	6	
21	16	10	
22	6	15	†
25	15	4	

Patient Number	X	Y	
27	9	9	†
29	10	27	
30	17	1	
32	8	12	
35	8	20	
37	8	32	†
38	8	15	
41	14	5	
43	13	35	
45	9	28	
47	15	6	

X = number of episodes in prior 12 months

Y = time (weeks) to first recurrence

† indicates censored time (**Note:** Patients who had censored times of less than 52 weeks were study dropouts.)

Solution

This example is also used in Chapter 22, which discusses the comparison of survival distributions using covariate-adjustments with the *Cox proportional hazards model*. In this chapter, the X covariate will be ignored (except as illustrated in Section 21.4.9).

The first step in manual computation of the *log-rank test* is to construct a table for each *event* time as shown in Table 21.3. At each time period, t_j , the number of patients at risk, n_{ij} , is reduced by the number of *event*-positives (d_{ij}) and censored observations (w_{ij}) from the previous time period, t_{j-1} . Once the table is filled in, O_1 , E_1 and V can be found by simple arithmetic calculations, as shown at the bottom of the table.

Let $S_1(t)$ and $S_2(t)$ represent the cumulative probability distributions of the times from vaccine administration until the first HSV-2 recurrence for the gD2 and placebo groups, respectively. Using the calculations from Table 21.3, the hypothesis test summary is

null hypothesis: $H_0: S_1(t) = S_2(t)$ (for all times, t)

alt. hypothesis: $H_A: S_1(t) \neq S_2(t)$ (for at least one time, t)

test statistic:

$$\begin{aligned}\chi^2 &= \frac{(O_1 - E_1)^2}{V} \\ &= \frac{(14 - 19.1556)^2}{6.8197} = 3.8976\end{aligned}$$

decision rule: reject H_0 if $\chi^2 > \chi^2_1(0.05) = 3.841$.

conclusion: Because $3.897 > 3.841$, reject H_0 and conclude that the recurrence times are significantly different between the gD2 and placebo vaccine groups at a 0.05 level of significance.

**TABLE 21.3 Preliminary Summary Table for Log-Rank Computations
for Example 21.1**

WEEK	gD2 VACCINE GROUP			PLACEBO GROUP			TOTAL		COMPUTATIONS		
	t _j	d _{1j}	w _{1j}	n _{1j}	d _{2j}	w _{2j}	n _{2j}	d _j	n _j	e _{1j}	v _j
1		0	0	25	1	0	23	1	48	0.5208	0.2496
2		0	0	25	0	1	22	0	47	0	0
3		1	0	25	0	0	21	1	46	0.5435	0.2481
4		0	0	24	1	0	21	1	45	0.5333	0.2489
5		0	0	24	1	0	20	1	44	0.5455	0.2479
6		1	0	24	2	0	19	3	43	1.6744	0.7046
7		0	1	23	0	0	17	0	40	0	0
8		1	0	22	1	0	17	2	39	1.1282	0.4788
9		1	0	21	0	1	16	1	37	0.5676	0.2454
10		0	0	20	1	0	15	1	35	0.5714	0.2449
11		0	1	20	0	0	14	0	34	0	0
12		1	1	19	2	0	14	3	33	1.7273	0.6870
13		1	0	17	0	0	12	1	29	0.5862	0.2426
14		1	0	16	0	0	12	1	28	0.5714	0.2449
15		1	0	15	2	1	12	3	27	1.6667	0.6838
19		0	0	14	1	0	9	1	23	0.6087	0.2382
20		0	0	14	1	0	8	1	22	0.6364	0.2314
21		1	0	14	1	0	7	2	21	1.3333	0.4222
24		0	1	13	0	0	6	0	19	0	0
27		0	0	12	1	0	6	1	18	0.6667	0.2222
28		2	0	12	1	0	5	3	17	2.1176	0.5450
32		0	0	10	0	1	4	0	14	0	0
35		1	0	10	1	0	3	2	13	1.5385	0.3254
36		1	0	9	0	0	2	1	11	0.8182	0.1488
44		1	0	8	0	1	2	1	10	0.8000	0.1600
52		0	7	7	0	1	1	0	8	0	0
		14 (O ₁)			17			31		19.1556 (E ₁)	6.8197 (V)

(Numbers in shaded rows do not affect the calculations of E₁ and V. These are shown only to account for all censored observations.)

SAS Analysis of Example 21.1

As shown in the SAS code for Example 21.1, one way to identify censored values in SAS is to assign them a negative value as you read the *event* times (wks) into the SAS data set (hsv). The variable cens is defined as an indicator variable that takes the value 1 if the observation is censored ($wks < 1$), and 0 if the observation is not censored ❶. A partial listing of the data is displayed in the output from PROC PRINT ❷.

The SAS procedure LIFETEST is used to perform the *log-rank test*. The TIME statement designates the variable, wks, whose values are the *event* times, followed by the variable, cens, which identifies the censored observations. In this case, values of 1 for cens indicate a censored value (i.e., *cens(1)) ❸. The STRATA statement identifies the grouping or stratification variable, in this case, vaccine group (vac) ❹. The ODS GRAPHICS statement and PLOTS= option with the LIFETEST procedure are included to request survival plots and a plot of the negative log of the event times ❺. These are discussed further in Sections 21.4.5 and 21.4.6.

Output 21.1 shows the *log-rank test* resulting in a chi-square value of 3.8976 ❻, which confirms the manual calculations. This is a significant result with a p-value of 0.0484 ❼. Also shown in the output are the values of $O_1 - E_1 = -5.1556$ ➋ and $V = 6.81973$ ❼. The ODS plot of the survival function estimates over time is shown as well ⪻.

SAS Code for Example 21.1

```
data hsv;
    input vac $ pat wks x @@;
    cens = (wks < 1); ❶
    wks = abs(wks);
    datalines;
GD2 1 8 12 GD2 3 -12 10 GD2 6 -52 7
GD2 7 28 10 GD2 8 44 6 GD2 10 14 8
GD2 12 3 8 GD2 14 -52 9 GD2 15 35 11
GD2 18 6 13 GD2 20 12 7 GD2 23 -7 13
GD2 24 -52 9 GD2 26 -52 12 GD2 28 36 13
GD2 31 -52 8 GD2 33 9 10 GD2 34 -11 16
GD2 36 -52 6 GD2 39 15 14 GD2 40 13 13
GD2 42 21 13 GD2 44 -24 16 GD2 46 -52 13
GD2 48 28 9 PBO 2 15 9 PBO 4 -44 10
PBO 5 -2 12 PBO 9 8 7 PBO 11 12 7
PBO 13 -52 7 PBO 16 21 7 PBO 17 19 11
PBO 19 6 16 PBO 21 10 16 PBO 22 -15 6
PBO 25 4 15 PBO 27 -9 9 PBO 29 27 10
PBO 30 1 17 PBO 32 12 8 PBO 35 20 8
PBO 37 -32 8 PBO 38 15 8 PBO 41 5 14
PBO 43 35 13 PBO 45 28 9 PBO 47 6 15
;

proc sort data = hsv;
    by vac pat;
```

```

proc print data = hsv;
  var vac pat wks cens x;
  title1 'The Log-Rank Test';
  title2 'Example 21.1: HSV-2 Episodes with gD2 Vaccine';
run;

ods exclude
  productlimitestimates;
ods graphics on;
proc lifetest
  plots=(survival(atrisk=5 10 15 20 25 30 35 40 45 50 55 60) ls) ❸
  data = hsv;
    time wks*cens(1); ❹
    strata vac; ❺
run;
ods graphics off;

```

OUTPUT 21.1 SAS Output for Example 21.1

The Log-Rank Test Example 21.1: HSV-2 Episodes with gD2 Vaccine					
Obs	vac	pat	wks	cens	x
1	GD2	1	8	0	12
2	GD2	3	12	1	10
3	GD2	6	52	1	7
4	GD2	7	28	0	10
5	GD2	8	44	0	6
6	GD2	10	14	0	8
7	GD2	12	3	0	8
8	GD2	14	52	1	9
9	GD2	15	35	0	11
10	GD2	18	6	0	13
11	GD2	20	12	0	7
12	GD2	23	7	1	13
13	GD2	24	52	1	9
14	GD2	26	52	1	12
15	GD2	28	36	0	13
16	GD2	31	52	1	8
17	GD2	33	9	0	10
18	GD2	34	11	1	16
19	GD2	36	52	1	6
20	GD2	39	15	0	14
21	GD2	40	13	0	13
22	GD2	42	21	0	13
23	GD2	44	24	1	16
.					
.					
.					
43	PBO	37	32	1	8
44	PBO	38	15	0	8
45	PBO	41	5	0	14
46	PBO	43	35	0	13
47	PBO	45	28	0	9
48	PBO	47	6	0	15

❷

OUTPUT 2I.I SAS Output for Example 2I.I (continued)

The LIFETEST Procedure					
Stratum 1: vac = GD2					
Summary Statistics for Time Variable wks					
Quartile Estimates					
Point Estimate					
Percent	Transform	[Lower	Upper)		
75	LOGLOG	35.0000	.		
50	LOGLOG	14.0000	.	13	
25	LOGLOG	3.0000	28.0000		
Mean					
Standard Error					
		28.7214	3.2823	14	
NOTE: The mean survival time and its standard error were underestimated because the largest observation was censored and the estimation was restricted to the largest event time.					
Stratum 2: vac = PBO					
Summary Statistics for Time Variable wks					
Quartile Estimates					
Point Estimate					
Percent	Transform	[Lower	Upper)		
75	LOGLOG	19.0000	.		
50	LOGLOG	8.0000	27.0000	13	
25	LOGLOG	1.0000	15.0000		
Mean					
Standard Error					
		18.2397	2.5387	14	
NOTE: The mean survival time and its standard error were underestimated because the largest observation was censored and the estimation was restricted to the largest event time.					
Summary of the Number of Censored and Uncensored Values					
Percent					
Stratum	vac	Total	Failed	Censored	Censored
1	GD2	25	14	11	44.00
2	PBO	23	17	6	26.09

Total		48	31	17	35.42

OUTPUT 2I.I SAS Output for Example 2I.I (continued)

The Log-Rank Test
Example 2I.I: HSV-2 Episodes with gD2 Vaccine

Testing Homogeneity of Survival Curves for wks over Strata

Rank Statistics

vac	Log-Rank	Wilcoxon
GD2	-5.1556	❸ -160.00
PBO	5.1556	160.00

Covariance Matrix for the Log-Rank Statistics

vac	GD2	PBO
GD2	6.81973	❹ -6.81973
PBO	-6.81973	6.81973

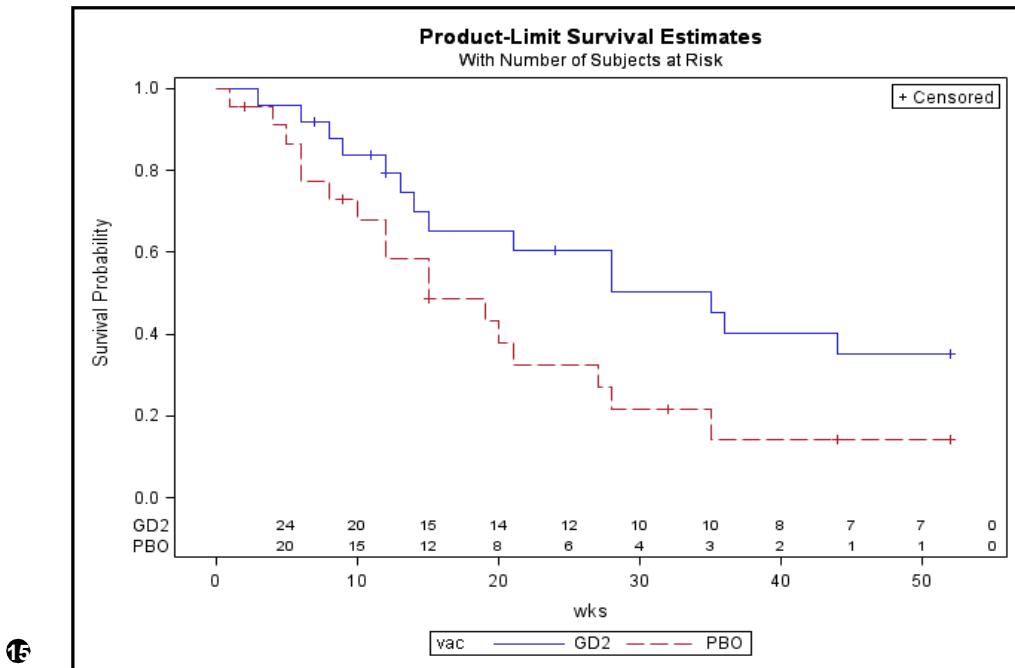
Covariance Matrix for the Wilcoxon Statistics

vac	GD2	PBO
GD2	7136.46	-7136.46
PBO	-7136.46	7136.46

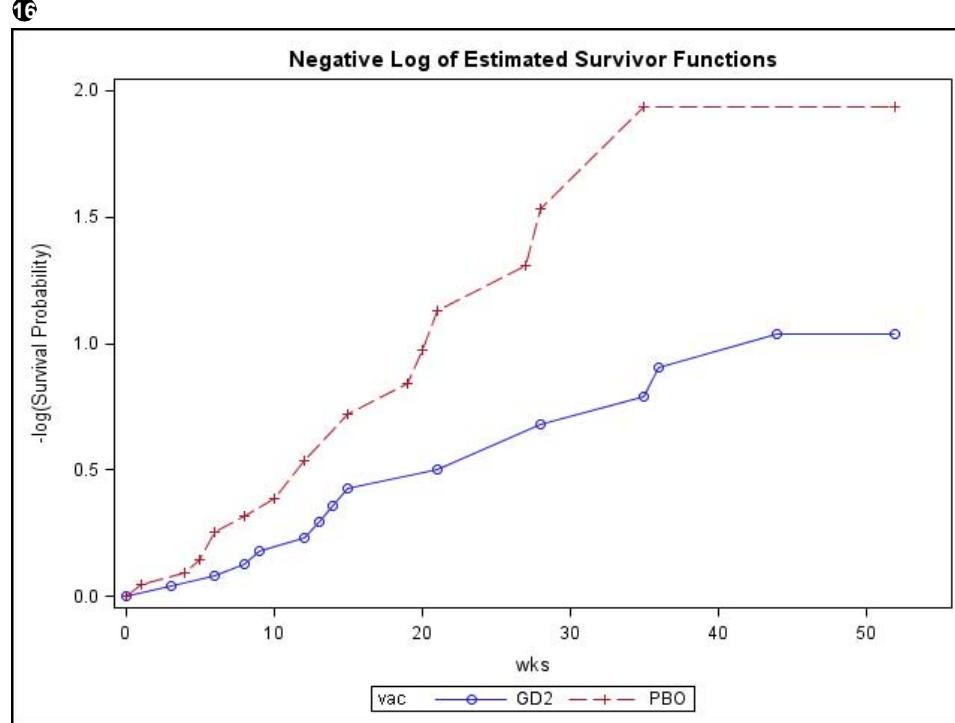
Test of Equality over Strata

Test	Chi-Square	DF	Chi-Square	Pr >
				❸
Log-Rank	3.8976	❶ 1	0.0484	❹
Wilcoxon	3.5872	1	0.0582	❺
-2Log (LR)	4.2589	1	0.0390	❻

OUTPUT 21.1 SAS Output for Example 21.1 (continued)



15



16

21.4 Details & Notes

- **21.4.1** Clinical study outcomes often have the goal of estimating response or *event* rates and comparing them among randomized groups. However, the selection of a time point during the clinical study at which to compare *event* rates is not always clear-cut. The last double-blind visit is often designated for such use, although drawbacks might exist for this choice. First, the duration of the study might be somewhat arbitrary or constrained by safety issues or other considerations unrelated to response. More importantly, the analysis ignores information prior to this evaluation time point that might have an important impact on the results.

If the data in Example 21.1 were analyzed with the *chi-square test* (Chapter 16) for comparing *event* rates at the final visit using a ‘last-observation-carried-forward’ method, you obtain the summary shown in Table 21.4.

TABLE 21.4 Unadjusted Recurrence Rates for Example 21.1

Response	gD2 Group	Placebo Group	TOTAL
HSV-2 Recurrence	14 (56.0%)	17 (73.9%)	31 (64.6%)
No Recurrence	11	6	17
Total Enrolled	25	23	48

The HSV-2 recurrence rate comparison (56.0% vs. 73.9%) is not significant ($\chi^2 = 1.680$, $p = 0.195$) based on the *chi-square test* (Chapter 16), which ignores the time that patients were at risk. By analyzing time-to-event occurrence and accounting for censoring, the *log-rank test* uses *event* rates over the whole trial, rather than relying on a single time point.

- **21.4.2** For the j^{th} time period (t_j), you can construct a 2×2 contingency table as follows:

	GROUP 1	GROUP 2	TOTAL
Event Positive	d_{1j}	d_{2j}	d_j
Event Negative	$n_{1j} - d_{1j}$	$n_{2j} - d_{2j}$	$n_j - d_j$
Total	n_{1j}	n_{2j}	n_j

The *log-rank test* is equivalent to applying the *Cochran-Mantel-Haenszel test* (Chapter 19) using these 2×2 tables at each time point as the strata.

- **21.4.3** Many variations of the *log-rank test* for comparing survival distributions exist. The most common variant has the form

$$\chi^2 = \frac{(O_1 - E_1)^2}{E_1} + \frac{(O_2 - E_2)^2}{E_2}$$

where O_i and E_i are computed for each group, as in the formulas given previously. This statistic also has an approximate chi-square distribution with 1 degree of freedom under H_0 .

In Example 21.1, you have (from Table 21.3) $O_1=14$, $O_2=17$, and $E_1=19.1556$. Using the relationship that $O_1+O_2=E_1+E_2$, you obtain $E_2=14+17-19.1556=11.8444$, which results in

$$\chi^2 = \frac{(14 - 19.1556)^2}{19.1556} + \frac{(17 - 11.8444)^2}{11.8444} = 3.632$$

with a p-value of 0.0567. Although computationally easier, this *chi-square test* gives a more conservative result than the version presented.

A continuity correction can also be used by reducing the numerators by 0.5 before squaring. Using this type of correction leads to even further conservatism and may be omitted when sample sizes are moderate or large.

- **21.4.4** Previously, it was noted that the *Wilcoxon rank-sum test* can be used to analyze the *event* times in the absence of censoring. A *generalized Wilcoxon test* (sometimes called the *Gehan test*) based on an approximate chi-square distribution has been developed for use in the presence of censored observations. Manual computations for this method can be tedious. (Dawson-Saunders and Trapp (1990, p. 196) provide an illustration of a worked example.) SAS automatically provides these results with PROC LIFETEST, as seen in Output 21.1. The *generalized Wilcoxon* chi-square for Example 21.1 is 3.587 with a p-value of 0.0582 ⑩, which is close to the results of the *log-rank test* for this example, but not quite significant.

Both the *log-rank* and the *generalized Wilcoxon* tests are non-parametric tests, and require no assumptions regarding the distribution of *event* times. When a greater *event* rate is seen early in the trial rather than toward the end of the trial, the *generalized Wilcoxon* test is the more appropriate test because it gives greater weight to the earlier differences.

- **21.4.5** Survival and failure times often follow the exponential distribution. If such a model can be assumed, a more powerful alternative to the *log-rank test* is the *likelihood ratio test*, the results of which are also printed in the SAS output as -2Log(LR) . Output 21.1 shows a likelihood ratio chi-square of 4.2589 with a p-value of 0.0390 ⑪.

This parametric test assumes that *event* probabilities are constant over time. That is, the chance that a patient becomes *event*-positive at time t given that he is *event*-negative up to time t does not depend on t . A plot of the negative log of the *event* times distribution that shows a linear trend through the origin is consistent with exponential *event* times. This can be depicted using SAS by specifying the PLOTS=(LS) option in PROC LIFETEST, as shown using ODS Graphics in Example 21.1 ⑫.

- **21.4.6** Life tables can be constructed to depict the estimates of the *event* time distributions. Estimates commonly used for data sets similar to that for Example 21.1 are known as the Kaplan-Meier estimates. These are the default estimates given by SAS (although the output can be suppressed by using the ODS EXCLUDE statement as is done in Example 21.1). A plot of the distribution function vs. time often makes for a good visual complement to the survival analyses. SAS will provide this plot by using the PLOTS=(SURVIVAL) option in PROC LIFETEST, as illustrated in the ODS graph of Output 21.1 ⑬. When you use ODS Graphics, you can include the ATRISK option with SURVIVAL to display the numbers of patients at risk at the times specified, in this case, at Weeks 5, 10, 15, 20, ..., 60 ⑭.
- **21.4.7** Additional summary statistics printed by SAS that might be useful are the estimated median and mean *event* times for each group. The median *event* times are shown in Output 21.1 as the 50% quartile, and, if possible to compute, they are accompanied by 95% confidence intervals. In Example 21.1, the median times until first recurrence are 35 and 15 weeks, respectively, for the gD2 and placebo vaccine groups ⑮, while the estimates of the means are 28.7 and 18.2 weeks, respectively ⑯. The estimates of the means will always be biased unless all patients become *event*-positive by study completion. As noted in the SAS output, these estimates are biased due to censored observations.
- **21.4.8** With g groups ($g > 2$), the hypothesis of equal *event*-time distributions can be tested with an extension of the chi-square statistic in Section 21.4.3, as follows:

$$\chi^2 = \sum_{i=1}^g \frac{(O_i - E_i)^2}{E_i}$$

This has an approximate chi-square distribution with $g-1$ degrees of freedom under H_0 . It is easy to see that the techniques of the *log-rank test* can be used to compare *event* times among any group factor with multiple levels.

When using SAS, PROC LIFETEST detects the number of levels of the STRATA variable and performs the appropriate test.

- **21.4.9** When you have data collected under a stratified sampling scheme, you can adjust treatment differences for this stratified factor. For example, suppose the data in Example 21.1 were stratified by patient gender. To compare the vaccine groups controlling for gender, you can include the gender variable name in the STRATA statement of PROC LIFETEST and use the GROUP= option to specify the randomized groups, as shown below. The output will provide the *log-rank* and Wilcoxon tests with the adjustment for gender.

```
proc lifetest data = hsv;
  time wks*cens(1);
  strata gender / group=vac;
run;
```

Extensions of the *log-rank test* enable the use of numeric covariates when comparing survival curves. However, it is usually more efficient to use Cox regression analyses (see Chapter 22) when including covariates in the analysis. You can also use the LIFETEST procedure to compare groups controlling for a numeric covariate by dividing the range of values of the covariate into intervals, which are then used as stratification groupings. For example, the STRATA statement in the SAS code below forms 4 intervals of the covariate X (number of HSV outbreaks in the prior 12 months) in Example 21.1, (i) $X < 8$, (ii) $8 \leq X < 10$, (iii) $10 \leq X < 12$, and (iv) $X \geq 12$.

```
proc lifetest data = hsv;
  time wks*cens(1);
  strata x(8,10,12) / group=vac;
run;
```

The *log-rank* test results in a p-value of 0.0217 for comparing the treatment groups controlling for these categories of pretreatment outbreak frequency. This test and the Wilcoxon tests are shown in an excerpt from the output below.

The Log-Rank Test			
Stratified Test of Equality over Group			
Test	Chi-Square	DF	Pr > Chi-Square
Log-Rank	4.3225	1	0.0217
Wilcoxon	5.2708	1	0.0054

Note that this adjustment does not take into account the ordinal structure of the categories of X. In addition, the results are highly dependent on the way the intervals of X values are established. Furthermore, while you can conduct tests of importance about the stratification covariate using the TEST statement in PROC LIFETEST, no estimates of the covariate effects are available. PROC PHREG is more useful than PROC LIFETEST when comparing survival times in the presence of categorical cofactors or numeric covariates. This procedure is presented in Chapter 22.

CHAPTER 22

The Cox Proportional Hazards Model

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22.1 Introduction

Like the *log-rank test* discussed in the previous chapter, the *Cox proportional hazards model* (sometimes referred to as ‘*Cox regression*’) is used to analyze event or ‘survival’ times. This is a modeling procedure that adjusts for censoring, may include numeric covariates, one or more of which may be time-dependent, and requires no assumption about the distribution of event times. Categorical factors may also be included as covariates in the model.

Examples of where the Cox proportional hazards model might be useful in clinical trials is a comparison of time to death among treatment groups in a cancer trial with adjustments for age, duration of disease, and family disease history, or comparison of various dose groups of a new dermatologic treatment on time to heal a skin ulcer, adjusted for skin type, lesion size, and smoking history.

Informally, the inverse of the time to event occurrence is called the ‘hazard’, given a suitable time unit. For example, if an event is expected to occur in 6 months, the hazard is 1/6 (‘events per month’), assuming the unit of time is ‘months’. Of chief concern when using the Cox proportional hazards model is the way in which the hazard changes over time. The hazard function is discussed in more detail later in this chapter (see Section 22.4.2).

The hazard of some events might be likely to increase with the passage of time, such as death due to certain types of cancer. Other types of events might become less frequent over longer periods of time. For example, reinfarction following coronary artery bypass can have the greatest risk in the weeks immediately following surgery, then risk might decrease as time passes. Still other types of events might be subject to fluctuating risks, both increasing and decreasing, over time.

Theoretically, survival analysis would be pretty easy if you could assume that the hazard remains constant over time. Recognizing the impracticalities of such an assumption, the *Cox proportional hazards model* adopts the more reasonable assumption that the *event* hazard rate can change over time, but that the ratio of *event* hazards between two individuals is constant. This is known as the ‘proportional hazards’ assumption.

22.2 Synopsis

The *Cox proportional hazards model* is used to test the effect of a specified set of k covariates, X_1, X_2, \dots, X_k on the *event* times. The X_i ’s can be numeric-valued covariates or numerically coded categorical responses that have some natural ordering. The X_i ’s can also be dummy variables that represent treatment groups in a comparative trial or levels of a nominal categorical factor as described for *logistic regression* (see Section 20.4.8).

The model for the hazard function of the *Cox proportional hazards* method has the form

$$h(t) = \lambda(t) \cdot e^{(\beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k)}$$

where $h(t)$ represents the hazard function, the X_i ’s represent the covariates, the β_i ’s represent the parameter coefficients of the X_i ’s, and $\lambda(t)$ represents an unspecified initial hazard function. As with regression analysis, the magnitudes of the β_i ’s reflect the importance of the covariates in the model, and inference about these parameters is the focus here.

D. R. Cox, after whom the model is named, developed a method for estimating β_i ($i=1,2,\dots,k$) by b_i based on a ‘maximum partial likelihood’ approach, which is a modification of the maximum likelihood method mentioned in Chapter 20. Manual computations are impractical, and solutions generally require numerical techniques using high-speed computers. When using SAS, the PHREG procedure is recommended for fitting the *Cox proportional hazards model*.

For large samples, these estimates (b_i) have an approximate normal distribution. If s_{bi} represents the standard error of the estimate b_i , then b_i/s_{bi} has an approximate standard normal distribution under the null hypothesis that $\beta_i = 0$, and its square has the chi-square distribution with 1 degree of freedom. The test summary for each model parameter, β_i , can be summarized as follows:

null hypothesis:	$H_0: \beta_i = 0$
alternative hypothesis:	$H_A: \beta_i \neq 0$
test statistic:	$\chi^2_w = \left(\frac{b_i}{S_{b_i}} \right)^2$
decision rule:	reject H_0 if $\chi^2_w > \chi^2_{\alpha}$

The magnitude of the effect of a covariate is often expressed as a hazard ratio, similar to the odds ratio discussed in Chapter 20. Because the hazard at any time, t , for a specified set of covariate values, x_1, x_2, \dots, x_k , is given by

$$h(t) = \lambda(t) \cdot e^{(\beta_1 x_1 + \beta_2 x_2 + \dots + \beta_k x_k)}$$

the ratio of hazards for a 1-unit increase in the numeric covariate X_i (all other covariates held constant) is e^{β_i} . The estimated hazard ratio associated with the X_i is found by simply exponentiating the parameter estimate, b_i .

Example 22.1, which follows, illustrates the *Cox proportional hazards model* with a simple example using one dichotomous group variable and one numeric covariate.

22.3 Examples

¶ Example 22.1—HSV-2 Episodes with gD2 Vaccine (*continued*) (See Example 21.1)

The data in Example 21.1 include a history of HSV-2 lesional episodes during the year prior to the study (X). Does the covariate X have any impact on the comparison of HSV-2 recurrence times between treatment groups?

Solution

It is not very efficient to include covariates in a non-parametric test like the *log-rank test*, as discussed in Chapter 21. Cox regression, using PROC PHREG in SAS can easily incorporate numeric and categorical covariates to fit the *Cox proportional hazards model*, as shown below for this example.

SAS Analysis of Example 22.1

Prior to SAS 9.2, PROC PHREG required all covariates to be numeric-valued covariates. In this example, the number of episodes in the prior 12 months (x) is a numeric variable you will be using as a covariate. For the vaccine group (vac), you can code it in the data step as a new variable, say trt, which takes numeric values 0 or 1. With the current version of SAS, you can simply use a CLASS statement to include nominal categorical covariates, much in the same way as with PROC LOGISTIC (see Chapter 20). In this case, we use a reference parameterization (see Appendix I) with the PARAM=REF option in the CLASS statement and designate the placebo group as the reference group using the (REF='PBO') CLASS variable option.

With PROC PHREG, the MODEL statement is used with the time variable and censoring indicator on the left side of the equal sign (=) and the covariates on the right side of the equal sign ❶. Recall from Example 21.1 that the indicator variable CENS takes a value of 1 if the observation is censored, 0 if it is not censored. The left side of the MODEL equality is specified in a similar format as the TIME statement in PROC LIFETEST for identifying censored values, i.e., wks*cens(1). You specify the vaccine group factor, vac, and the HSV-2 lesion frequency during the prior year, x, as the two covariates. The TIES option is used in the event that more than one observation has tied *event* times. This is discussed further in Section 22.4.9.

The results are shown in Output 22.1. The estimate of the parameter associated with the covariate X is 0.176 ❷, which is significant ($p = 0.0073$) ❸ based on the *chi-square* value of 7.1896. This means that prior lesional frequency is an important consideration when analyzing the *event* times. The test for the group effect (vac) is also significant ($p = 0.0160$) ❹ and indicates a difference in the distributions of *event* times between vaccine groups after adjusting for the covariate, x. The hazard ratio, 0.404 ❺, indicates that the covariate-adjusted ‘hazard’ of HSV-2 recurrence for the active (gD2) group is only about 40% of that for the placebo group.

Note that the *log-rank test* using the same data set (ignoring the covariate, x) resulted in a significant finding with a p-value close to 0.05 (Example 21.1). The *Cox proportional hazards model* resulted in an even greater significance ($p = 0.016$) after adjusting for the important covariate, x.

SAS Code for Example 22.1

... (continued from Example 21.1)

```
proc phreg data = hsv;
  class vac(ref='PBO') / param = ref;
  model wks*cens(1) = vac x / ties = exact;      ❶
  title1 "Cox Proportional Hazards Model";
  title2 "Example 22.1: HSV-2 Episodes with gD2 Vaccine -
  cont'd." ;
run;
```

OUTPUT 22.1 SAS Output for Example 22.1

Cox Proportional Hazards Model
Example 22.1: HSV-2 Episodes with gD2 Vaccine - cont'd.

The PHREG Procedure

Model Information

Data Set	WORK.HSV
Dependent Variable	wks
Censoring Variable	cens
Censoring Value(s)	1
Ties Handling	EXACT

Number of Observations Read	48
Number of Observations Used	48

Class Level Information

Class	Value	Design Variables
vac	GD2	1
	PBO	0

Summary of the Number of Event and Censored Values

Total	Event	Censored	Percent Censored
48	31	17	35.42

Convergence Status
Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	183.628	172.792
AIC	183.628	176.792
SBC	183.628	179.660

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	10.8364	2	0.0044
Score	11.0159	2	0.0041
Wald	10.7236	2	0.0047

Type 3 Tests

Effect	DF	Chi-Square	Pr > ChiSq
vac	1	5.8033	0.0160
x	1	7.1896	0.0073

Analysis of Maximum Likelihood Estimates

Variable	Parameter DF	Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
vac	GD2 1	-0.90578	0.37600	5.8033	0.0160	④ 0.404 ⑤
x	1	0.17627 ②	0.06574	7.1896	0.0073	③ 1.193

The next example illustrates the use of multiple covariates in *Cox regression* along with the concept of time-dependent covariates.

Example 22.2—Hyalurise in Vitreous Hemorrhage

A new treatment, Hyalurise, is being tested to facilitate the clearance of vitreous hemorrhage, a condition that results in severe vision impairment. Patients were randomly assigned to receive a single injection of either Hyalurise ($n = 83$) or saline ($n = 60$) in the affected eye and were followed for 1 year. Time, in weeks, to ‘response’ is shown in Table 22.1. (‘Response’ is defined as sufficient clearance of the hemorrhage to permit diagnosis of the underlying cause and appropriate intervention.) The time for patients who discontinued or completed the trial before achieving ‘response’ is considered censored (†). Covariates include age (years), a measure of baseline hemorrhage density (Grade 3 or 4), study center (A, B, or C), and whether the patient developed certain infectious complications (ocular infection, inflammation, or herpetic lesion) during the study (based on ‘Infect. Time’ in Table 22.1). Does the time to response differ between the patients treated with Hyalurise and the control group treated with saline?

Solution

The response variable is time, in weeks, from randomization to ‘response’, some of whose values are censored. Because this analysis clearly calls for ‘survival’ methodology that incorporates covariates, you use PROC PHREG in SAS to fit a *Cox regression* model under the assumption of proportional hazards.

In addition to the treatment group effect, you want to include as covariates: age, baseline hemorrhage density, study center, and the occurrence of infectious complications. Notice that the last covariate is a bit unusual. The occurrence of complications can take the values ‘yes’ or ‘no’, which can be coded 1 and 0, respectively. However, these values can change during the study and depend on the time of the response. Clearly, the degree to which complications affect ‘response’ is only relevant if the complication develops before ‘response’ occurs. For this reason, this is called a ‘time-dependent’ covariate. Such time-dependent covariates can be included in the *Cox proportional hazards model*, as illustrated in the SAS analysis that follows.

SAS Analysis of Example 22.2

In the SAS program for Example 22.2, the variables are coded as:

rsptim = time (weeks) from randomization to ‘response’
trt = treatment group: HYA (Hyalurise), SAL (Saline)
age = patient age at enrollment (years)
dens = baseline hemorrhage density grade (3 or 4)
center = study center (A, B, or C)
inftim = time (weeks) from randomization to onset of complication.

TABLE 22.1 Raw Data for Example 22.2

----- Hyalurise Injection -----										
Center	Pat. No.	Age	Density	Infect. Time	Resp. Time	Pat. No.	Age	Density	Infect. Time	Resp. Time
A	101	72	3	.	32	132	72	4	18	46
	102	55	4	10	20	133	75	4	.	21
	105	72	4	52	24	134	65	3	.	14
	106	77	3	.	41	136	72	3	.	24 †
	108	77	4	.	32	139	48	3	.	19
	110	59	3	.	10 †	140	59	4	20	24
	112	68	4	.	32	141	69	3	12	31
	114	82	3	36	45	144	71	3	.	52 †
	116	49	3	.	6	146	80	4	.	40
	117	69	4	.	52 †	148	71	4	24	7
	119	54	3	.	13	150	49	4	.	16
	121	72	4	.	6	151	69	3	4	36
	123	77	3	28	44	153	58	3	.	28
	125	72	4	.	23	155	66	3	.	38 †
	126	59	3	.	30	156	51	3	.	9
	128	58	3	.	2	159	63	4	.	30
	130	60	4	.	26	160	50	4	.	6
B	202	52	3	.	4 †	220	68	4	.	25
	203	53	4	.	10	223	62	4	.	20
	206	58	3	.	11	224	63	3	24	9
	207	67	4	.	52 †	225	74	3	.	22
	208	61	3	.	12	226	55	3	.	23
	210	77	4	.	46	228	71	3	.	13
	212	61	3	.	10	230	59	4	.	8
	214	78	4	30	31	231	69	4	.	37
	215	62	4	.	4	233	77	3	10	24
	217	67	3	33	20	234	70	4	.	52 †
	219	57	3	.	2	237	55	3	.	27
C	301	48	3	.	10	324	47	4	.	22
	303	75	4	.	32	327	59	3	30	15
	304	65	4	20	14	329	64	3	.	20
	306	64	4	18	14	330	79	3	30	52 †
	309	67	3	.	7	332	57	3	.	10
	311	65	3	.	5	334	60	4	.	2 †
	312	63	3	.	9	337	63	3	.	16
	313	66	4	.	12	338	54	3	20	33
	315	69	3	.	10	340	55	3	.	12
	317	80	4	.	52 †	341	63	4	18	27
	318	52	3	.	10	342	60	4	.	2
	320	70	3	.	5	344	66	4	.	20 †
	321	88	3	16	34	346	59	3	.	3
	323	69	3	.	6					

† indicates censored observation

TABLE 22.1 Raw Data for Example 22.2 (continued)

----- Saline Injection -----										
Center	Pat. No.	Age	Density	Infect. Time	Resp. Time	Pat. No.	Age	Density	Infect. Time	Resp. Time
A	103	80	3	.	52 †	131	60	3	.	8
	104	46	4	.	4	135	73	3	.	52 †
	107	68	4	12	32	137	78	3	.	22 †
	109	66	3	20	25	138	66	3	.	35 †
	111	64	3	.	8	142	52	4	.	28
	113	65	4	38	52 †	143	67	4	34	52 †
	115	59	4	.	14 †	145	59	3	.	35
	118	61	4	28	9	147	43	3	.	4
	120	69	4	.	36	149	46	4	.	2 †
	122	68	4	.	42 †	152	54	3	.	12
	124	67	3	.	52 †	154	77	3	42	48
	127	61	3	.	16	157	60	3	.	11
	129	61	3	6	21	158	76	4	.	20
B	201	61	3	.	15 †	221	68	4	.	11
	204	57	4	9	10	222	75	4	40	52 †
	205	57	3	.	8	227	47	3	.	11
	209	67	3	.	20	229	51	4	.	7 †
	211	72	4	44	32	232	54	4	26	32
	213	71	3	.	42	235	76	4	.	20
	216	67	3	.	24 †	236	82	4	.	34
	218	53	3	.	16					
C	302	74	4	8	10 †	326	67	3	.	16
	305	71	3	.	24	328	73	4	22	33
	307	71	3	.	36	331	50	4	.	5
	308	69	3	.	52 †	333	62	3	15	16
	310	62	4	.	6	335	59	4	.	17
	314	59	3	.	21	336	56	4	.	16
	316	57	4	36	26	339	63	3	11	24
	319	64	4	12	27	343	49	4	.	6 †
	322	52	4	.	14	345	58	3	.	14
	325	77	4	12	45					

† indicates censored observation

In this example, censored observations are read into the data set as negative values, and the variable cens is created to allow SAS to identify the uncensored observations as those associated with a value of 0 **7**. Thus, the left side in the MODEL statement in PROC PHREG is ‘rsptim*cens(0)=’ **8**.

The age and baseline hemorrhage density (dens) are included in the MODEL statement as numeric covariates. Treatment group (trt) and study center (center) are nominal categorical factors included in a CLASS statement. Reference parameterization is used (see Appendix I) for these. The reference levels are ‘SAL’ for treatment group and ‘C’ for study center **9**.

To include the possible effect of occurrence of complications in the analysis, you must create a new, time-dependent covariate (infctn in SAS code) using a special feature in PROC PHREG that allows SAS DATA step type programming statements to be used within the procedure. This ensures that the covariate takes the correct values (0 or 1) at the appropriate time points. The variable infctn is defined as 1 if a complication occurs prior to response, and 0, if a complication occurs after response or not at all **10**.

Output 22.2 shows the analysis of the maximum likelihood estimates of the model parameters, including p-values based on the *Wald chi-square tests*. The p-value for treatment effect (trt), which controls for the covariates, is 0.0653 **11**. The hazard ratio of 1.431**12** indicates that the ‘hazard’ of responding at any arbitrary time point is 43.1% higher with Hyalurise injection compared with saline.

Age is a highly significant covariate ($p<0.0001$), with a hazard ratio of 0.926. This means the response ‘hazard’ decreases by 7.4% ($100\times(1-0.926)$) for each year the patient ages. Center appears to be marginally significant due to Center A relative to Center C. Neither baseline hemorrhage density (dens) nor the time-dependent covariate (infctn) is significant ($p=0.281$, $p=0.584$, respectively).

SAS Code for Example 22.2

```

data vitclear;
  input pat age rsptim trt center $ dens inftim @@;
  cens = (rsptim ge 0); 7
  rsptim = abs(rsptim);
  /* rsptim = time (wks) from randomization to response
     (RSPTIM is censored if negative) */
  /* trt      = 1 for Hyalurise, TRT = 0 for Saline */
  /* center   = study center (A, B, or C) */
  /* dens     = 3, 4 for Grade 3 or 4, respectively */
  /* inftim   = time (wks) from randomization to onset of
     infection or other complications */
  datalines;
101 72 32 HYA A 3 . 102 55 20 HYA A 4 10 103 80 -52 SAL A 3 .
104 46 4 SAL A 4 . 105 72 24 HYA A 4 52 106 77 41 HYA A 3 .
107 68 32 SAL A 4 12 108 77 32 HYA A 4 . 109 66 25 SAL A 3 .
110 59 -10 HYA A 3 . 111 64 8 SAL A 3 . 112 68 32 HYA A 4 .
113 65 -52 SAL A 4 38 114 82 45 HYA A 3 36 115 59 -14 SAL A 4 .

```

SAS Code for Example 22.2 (continued)

```

116 49   6 HYA A 3 . 117 69 -52 HYA A 4 . 118 61   9 SAL A 4 28
119 54   13 HYA A 3 . 120 69  36 SAL A 4 . 121 72   6 HYA A 4 .
122 68 -42 SAL A 4 . 123 77  44 HYA A 3 28 124 67 -52 SAL A 3 .
125 72   23 HYA A 4 . 126 59  30 HYA A 3 . 127 61   16 SAL A 3 .
128 58   2 HYA A 3 . 129 61  21 SAL A 3  6 130 60   26 HYA A 4 .
131 60   8 SAL A 3 . 132 72  46 HYA A 4 18 133 75   21 HYA A 4 .
134 65   14 HYA A 3 . 135 73 -52 SAL A 3 . 136 72 -24 HYA A 3 .
137 78 -22 SAL A 3 . 138 66 -35 SAL A 3 . 139 48   19 HYA A 3 .
140 59   24 HYA A 4 20 141 69  31 HYA A 3 12 142 52   28 SAL A 4 .
143 67 -52 SAL A 4 34 144 71 -52 HYA A 3 . 145 59   35 SAL A 3 .
146 80   40 HYA A 4 . 147 43   4 SAL A 3 . 148 71   7 HYA A 4 24
149 46   -2 SAL A 4 . 150 49  16 HYA A 4 . 151 69   36 HYA A 3  4
152 54   12 SAL A 3 . 153 58  28 HYA A 3 . 154 77   48 SAL A 3 42
155 66 -38 HYA A 3 . 156 51   9 HYA A 3 . 157 60   11 SAL A 3 .
158 76   20 SAL A 4 . 159 63  30 HYA A 4 . 160 50   6 HYA A 4 .
201 61 -15 SAL B 3 . 202 52 -4 HYA B 3 . 203 53   10 HYA B 4 .
204 57   10 SAL B 4 . 205 57   8 SAL B 3 . 206 58   11 HYA B 3 .
207 67 -52 HYA B 4 . 208 61  12 HYA B 3 . 209 67   20 SAL B 3 .
210 77   46 HYA B 4 . 211 72  32 SAL B 4 44 212 61   10 HYA B 3 .
213 71   42 SAL B 3 . 214 78  31 HYA B 4 20 215 62   4 HYA B 4 .
216 67 -24 SAL B 3 . 217 67  20 HYA B 3 33 218 53   16 SAL B 3 .
219 57   2 HYA B 3 . 220 68  25 HYA B 4 . 221 68   11 SAL B 4 .
222 75 -52 SAL B 4 . 223 62  20 HYA B 4 . 224 63   9 HYA B 3 .
225 74   22 HYA B 3 . 226 55  23 HYA B 3 . 227 47   11 SAL B 3 .
228 71   13 HYA B 3 . 229 51 -7 SAL B 4 . 230 59   8 HYA B 4 .
231 69   37 HYA B 4 . 232 54  32 SAL B 4 26 233 77   24 HYA B 3 10
234 70 -52 HYA B 4 . 235 76  20 SAL B 4 . 236 82   34 SAL B 4 .
237 55   27 HYA B 3 . 301 48  10 HYA C 3 . 302 74 -10 SAL C 4  8
303 75   32 HYA C 4 . 304 65  14 HYA C 4 20 305 71   24 SAL C 3 .
306 64   14 HYA C 4 . 307 71  36 SAL C 3 . 308 69 -52 SAL C 3 .
309 67   7 HYA C 3 . 310 62   6 SAL C 4 . 311 65   5 HYA C 3 .
312 63   9 HYA C 3 . 313 66  12 HYA C 4 . 314 59   21 SAL C 3 .
315 69   10 HYA C 3 . 316 57  26 SAL C 4 36 317 80 -52 HYA C 4 .
318 52   10 HYA C 3 . 319 64  27 SAL C 4 12 320 70   5 HYA C 3 .
321 88   34 HYA C 3 16 322 52  14 SAL C 4 . 323 69   6 HYA C 3 .
324 47   22 HYA C 4 . 325 77  45 SAL C 4 12 326 67   16 SAL C 3 .
327 59   15 HYA C 3 30 328 73  33 SAL C 4 22 329 64   20 HYA C 3 .
330 79 -52 HYA C 3 30 331 50   5 SAL C 4 . 332 57   10 HYA C 3 .
333 62   16 SAL C 3 . 334 60 -2 HYA C 4 . 335 59   17 SAL C 4 .
336 56   16 SAL C 4 . 337 63  16 HYA C 3 . 338 54   33 HYA C 3 20
339 63   24 SAL C 3 11 340 55  12 HYA C 3 . 341 63   27 HYA C 4 18
342 60   2 HYA C 4 . 343 49 -6 SAL C 4 . 344 66 -20 HYA C 4 .
345 58   14 SAL C 3 . 346 59   3 HYA C 3 .

;

proc phreg data = vitclear;
  class trt(ref='SAL') center(ref='C') / param = ref;          ⑨
  model rsptim*cens(0) = trt center age dens infctn /
    ties = exact; ⑧
  if inftim gt rsptim or inftim = . then infctn = 0;          ⑩
  else infctn = 1;
  title1 'Cox Proportional Hazards Model';
  title2 'Example 22.2: Hyalurise in Vitreous Hemorrhage' ;
run;

```

OUTPUT 22.2 SAS Output for Example 22.2

Cox Proportional Hazards Model
Example 22.2: Hyalurise in Vitreous Hemorrhage

The PHREG Procedure

Model Information

Data Set	WORK.VITCLEAR
Dependent Variable	rsptim
Censoring Variable	cens
Censoring Value(s)	0
Ties Handling	EXACT

Number of Observations Read 143
Number of Observations Used 143

Class Level Information

Class	Value	Design Variables
trt	HYA	1
	SAL	0
center	A	1 0
	B	0 1
	C	0 0

Summary of the Number of Event and Censored Values

Total	Event	Censored	Percent Censored
143	114	29	20.28

Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	795.879	739.985
AIC	795.879	751.985
SBC	795.879	768.403

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	55.8933	6	<.0001
Score	59.8640	6	<.0001
Wald	57.7918	6	<.0001

OUTPUT 22.2 SAS Output for Example 22.2 (continued)

Cox Proportional Hazards Model Example 22.2: Hyalurise in Vitreous Hemorrhage					
The PHREG Procedure					
Type 3 Tests					
Effect	DF		Chi-Square	Pr > ChiSq	
trt	1		3.3963	0.0653	
center	2		5.5593	0.0621	
age	1		43.0906	<.0001	
dens	1		1.1611	0.2812	
infctn	1		0.2997	0.5841	

Analysis of Maximum Likelihood Estimates						
Parameter	DF	Parameter	Standard	Hazard		
		Estimate	Error	Chi-Square	Pr>ChiSq	Ratio
trt HYA	1	0.35863	0.19460	3.3963	0.0653	⑪ 1.431 ⑫ trt HYA
center A	1	-0.50499	0.22547	5.0162	0.0251	0.604 center A
center B	1	-0.12266	0.25297	0.2351	0.6277	0.885 center B
age	1	-0.07666	0.01168	43.0906	<.0001	0.926
dens	1	-0.20629	0.19145	1.1611	0.2812	0.814
infctn	1	0.14918	0.27252	0.2997	0.5841	1.161

22.4 Details & Notes

- **22.4.1** The *Cox proportional hazards model* is a ‘semi-parametric’ approach that does not require the assumption of any specific hazard function or distribution of survival times. In this sense, it takes the character of a non-parametric test. However, as in regression analysis, this method models the survival times as a function of the covariates with the goal of obtaining estimates of the model parameters (β_i 's). Various types of functions can be used and different models can be specified for the distribution of the error variation. In this sense, the *Cox proportional hazards model* is parametric.
- **22.4.2** The hazard is a function of time, t. Formally, the hazard represents the probability that the *event* of interest occurs at a specified time (t) given that it has not occurred prior to time (t).

If the hazard is constant over time, *event* times can be modeled with the exponential distribution. The natural log of the hazard function is

$$\ln h(t) = \ln \lambda(t) + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k$$

If the event times have an exponential distribution, $\lambda(t)$ is a constant,

independent of t , say α , and the model becomes

$$\ln h(t) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k$$

which means the hazard is constant with respect to time and is a simple linear function of the covariates, X_i 's, on the log-scale.

Section 21.4.5 describes how to test for exponential *event* times by checking whether a plot of the log of the survival probabilities is linear through the origin. This test is a direct result of the fact that the exponential distribution is associated with a constant hazard function (i.e., risk is independent of time).

In general, if a model for the hazard function can be assumed, then the survival distributions can be specified. In fact, the cumulative survival distribution is a function of the hazard function integrated over time. Therefore, the survival distribution can be described by the hazard function, and, conversely, the survival distribution uniquely defines a hazard function.

When the survival distribution is known or can be accurately assumed, a parametric analysis can be applied, often with greater efficiency than the non-parametric *log-rank test* or the ‘semi-parametric’ *Cox proportional hazards model*. The parametric approach with a known survival distribution can easily incorporate covariates into the analysis. In addition to exponential *event* times, widely used models include the log-normal, Weibull, gamma, and log-logistic distributions. The SAS procedure LIFEREG can be used to fit any of these parametric models with any number of covariates.

The main disadvantage of the parametric approach is that, in many cases, model assumptions might be incorrect and there might not be enough data to adequately test the assumptions or, perhaps, the actual hazard function is not well represented by any of those in common usage. Often, the results are highly dependent on the appropriate distributional assumptions.

- **22.4.3** The strength of the *Cox proportional hazards model* is its ability to incorporate background factors as covariates. When many covariates are being considered, this method can be used in an exploratory fashion to build a model that includes significant covariates and excludes the covariates that have little apparent effect on the *event* times. This stepwise procedure is demonstrated in the SAS documentation for PROC PHREG (see the *SAS/STAT User’s Guide*).
- **22.4.4** PROC PHREG prints the results of a global test that determines whether any of the covariates included in the model are significant after adjusting for all the other covariates. As shown in the output for Example 22.1⑥, three different methods are used: "Likelihood Ratio", "Score" and "Wald". Each of these is a goodness-of-fit test with an approximate chi-square distribution with 2 degrees of freedom under the global null hypothesis of ‘no covariate effects’. With the significant results of $p < 0.005$ for all three methods

in the example, you conclude that fluctuations in *event* times are associated with either vaccine group (trt) or prior lesional frequency (x) or both.

The global tests in Example 22.2 are also all significant with p-values of <0.0001 ⑬.

- **22.4.5** A positive parameter estimate indicates that the hazard increases with increasing values of the covariate. A negative value indicates that the hazard decreases with increasing values of the covariate. In Example 22.1, the negative parameter estimate for trt (-0.906) indicates that the hazard of HSV-2 recurrence decreases with active treatment (trt=1) compared with a placebo (trt=0). The hazard increases with larger prior lesional frequencies (x), as seen by the positive parameter estimate for x (0.176).

The term ‘hazard’ is often associated with the event of death or disease. However, in survival analysis, ‘hazard’ does not always connote a danger or an undesirable event. In Example 22.2, the event of interest is ‘response,’ which is a desirable result. Although it sounds awkward, an increasing ‘hazard’ in the desirable response with active treatment compared with saline injection is seen. Notice that the parameter estimate for trt is positive, and the hazard ratio for the treatment effect is greater than 1 (1.431⑭), just the opposite of Example 22.1.

- **22.4.6** The model for the hazard function using *Cox proportional hazards* with only one covariate ($k=1$) is

$$h(t) = \lambda(t) \cdot e^{\beta X}$$

For two patients with different values of X, such as X_u and X_v , the ratio of hazards at time t is

$$\frac{h_u(t)}{h_v(t)} = \frac{\lambda(t) \cdot e^{X_u}}{\lambda(t) \cdot e^{X_v}} = e^{\beta(X_u - X_v)}$$

which is constant with respect to time, t. This is the proportional hazards assumption.

If X is a dummy variable with $X_u = 1$ (the active group) and $X_v = 0$ (the placebo group), $X_u - X_v = 1$ so that e^β represents the ratio of the hazard that is associated with the active treatment to the hazard that is associated with the placebo. This is the hazard ratio in the SAS printout for Example 22.1 ⑮ and Example 22.2⑯.

In Example 22.1, the hazard ratio for TRT is $e^{-0.906} = 0.404$. This can be interpreted as the hazard at any time t for the active group is only 40.4% of the placebo group's hazard. The hazard ratio for X is 1.193. Because X is a numeric variable (rather than a numerically coded categorical variable), you interpret this as a 19.3% increase in the hazard of an HSV-2 recurrence for each additional prior lesion that was experienced.

- **22.4.7** Example 22.2 illustrates the inclusion of a dichotomous time-dependent covariate. Continuous numeric covariates can also be time-dependent. For example, time to a certain response in a nutritional study might depend on a subject's protein intake, which might change during the course of the study. This type of continuous numeric time-dependent covariates can also be included in PROC PHREG. Details and further discussion of time-dependent covariates using PROC PHREG can be found in *Survival Analysis Using SAS: A Practical Guide, Second Edition*, by Paul D. Allison (2010), and *SAS Survival Analysis Techniques for Medical Research, Second Edition*, by Alan Cantor (2003), both of which are published by SAS Press.
- **22.4.8** ‘Non-proportional hazards’ implies an increasing or decreasing hazard ratio over time. In PROC PHREG, this can be represented by a covariate-by-time ‘interaction’. The proportional hazards assumption for any covariate can be checked by including the interaction of that covariate with time as a new time-dependent covariate in the MODEL statement in PROC PHREG. For example, to check the proportional hazards assumption for the covariate DENS in Example 22.2, you can include the time-dependent covariate, TDENS, by specifying the following SAS statement

```
tdens = dens*rsptim;
```

after the MODEL statement. A significant test for the parameter that is associated with TDENS would indicate that the hazard changes over time.

This test is also illustrated in the SAS Help and Documentation for PROC PHREG, which recommends using the natural logarithm of the response times decreased by the average of the log response times to obtain greater stability. Use of this stabilizing transformation in Example 22.2 would require the statement

```
tdens = dens*(log(rsptim) - 2.85);
```

in place of the preceding statement (2.85 is the mean of the $\ln(\text{RSPTIM})$ over all observations).

A visual aid in determining conformance with the assumption of proportional hazards is a plot of the $\log(-\log(S(t)))$ versus time, where $S(t)$ is the estimated survival distribution. This can be done in SAS using the PLOTS=(LLS) option in the LIFETEST procedure. The graphs of these functions for each group should be parallel under the proportional hazards assumption. Lack of parallelism suggests deviations from the proportional

hazards assumption. You can also use ODS plots generated with the ASSESS statement in PROC PHREG to visually check the proportional hazards assumption for numeric covariates that are not time dependant (see PROC GLIMMIX in the *SAS/STAT User's Guide*).

When the proportional hazards assumption does not seem to hold for a specific covariate, the *Cox proportional hazards model* might still be used by performing the analysis within each of a series of sub-intervals of the range of the covariate's values, and then combining the results. This is done in SAS by using the STRATA statement. If the covariate has continuous numeric values, the strata can be defined by numeric intervals in the STRATA statement (for details, see the SAS Help and Documentation for PROC PHREG). Use of the STRATA statement is primarily advantageous for groups whose levels may not adhere to the proportional hazards property. For example, if you believe the different study centers in Example 22.2 might have different hazard functions, you would include center in the STRATA statement instead of the MODEL statement.

- **22.4.9** An adjustment might be necessary when ties occur in *event* times. SAS has 4 adjustment options for handling ties: BRESLOW, EFRON, EXACT, and DISCRETE. BRESLOW is the default, although it might not be the best option to use. With small data sets or a small number of tied values, the EXACT option is usually preferable. Because of computational resources, the EXACT option is not efficient for larger data sets. The EFRON option provides a good approximation and is computationally faster when large amounts of data are being analyzed. To perform the analysis in SAS, you would specify the method (BRESLOW, EFRON, EXACT, or DISCRETE) in the TIES= option of the MODEL statement in PROC PHREG. The EXACT method is illustrated in Example 22.1 ①.

CHAPTER 23

Exercises

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23.1 Introduction

The data set, TRIAL, introduced in Chapter 3, is based on a contrived, simplified clinical study that measures three types of response variables for 100 patients. The exercises in this section provide an opportunity to practice applying some of the basic methods discussed in this book using this data set. Section 23.2 lists the exercises and requests an analysis that uses a specific method referenced in an earlier chapter. The SAS code for performing these exercises is shown in Appendix G. Section 23.3 provides a critique of the methods used and a discussion of their appropriateness, based on the assumptions the analyst is willing to make.

23.2 Exercises

Write a SAS program to perform the following analyses using the ‘TRIAL’ data set in Chapter 3. Analyses 1 to 11 use the continuous numeric response SCORE, Analyses 12 to 17 use the dichotomous response RESP, and Analyses 18 to 27 use the ordinal categorical response SEV.

■ **23.2.1** Using the SCORE response variable:

Analysis 1: Compare mean responses between treatment groups by using the *two-sample t-test* (Chapter 5).

Analysis 2: Apply the *Wilcoxon rank-sum test* to test for any shifts in the distribution between Treatment Groups A and B (Chapter 13).

Analysis 3: Using a *one-way ANOVA* model (Chapter 6), test for a difference in mean response between treatment groups.

- Analysis 4:** Determine if there is an interaction between treatment group and study center by using the *two-way ANOVA* (Chapter 7).
- Analysis 5:** Re-run the *two-way ANOVA* without the interaction effect to determine the significance of treatment group differences.
- Analysis 6:** Compare treatment group responses by using *ANOVA* accounting for any effects due to Study Center and Sex. Include all interactions.
- Analysis 7:** Check for a significant treatment group effect adjusted for Study Center and Sex by using a main effects *ANOVA* model.
- Analysis 8:** Ignoring all group factors, determine if the response variable SCORE is linearly related to Age (Chapter 10). Plot the data.
- Analysis 9:** Determine if the slope of the *linear regression* in Analysis 8 differs between treatment groups (Chapter 11).
- Analysis 10:** Estimate the mean response for each treatment group using Age as a covariate. Assuming the answer to 9 is ‘no’, determine the p-value for comparing these adjusted means (Chapter 11). Plot the data by treatment group.
- Analysis 11:** Compare treatment group means adjusted for Age and Study Center (Chapter 11). Assume there are no interactions.

■ **23.2.2** Using the RESP response variable:

- Analysis 12:** Compare response rates between treatment groups by using the *chi-square test* (Chapter 16).
- Analysis 13:** Compare response rates between treatment groups by using *Fisher’s exact test* (Chapter 17).
- Analysis 14:** Compare response rates between treatment groups controlling for Study Center by using the *Cochran-Mantel-Haenszel test* (Chapter 19).
- Analysis 15:** Compare response rates between treatment groups by using *logistic regression* (Chapter 20).
- Analysis 16:** Compare response rates between treatment groups adjusted for Age assuming a logistic model (Chapter 20).
- Analysis 17:** Using a *logistic regression* model, test for a significant treatment group effect adjusted for Age, Sex, Study Center, and Treatment-by-Center interaction (Chapter 20).

■ **23.2.3** Using the SEV response variable:

- Analysis 18:** Test for general association between severity of response and treatment group by using the *chi-square test* (Chapter 16).
- Analysis 19:** Apply the generalized *Fisher's exact test* to determine if the distributions of response by severity differ between treatment groups (Chapter 17).
- Analysis 20:** Use the *chi-square test* to determine if the row mean scores differ between treatment groups (Chapter 16). Use 'table' scores for coding severity.
- Analysis 21:** Repeat Analysis 20 using modified ridit scores in place of 'table' scores.
- Analysis 22:** Test for a shift in the distribution of severity between treatment groups by using the *Wilcoxon rank-sum test* (Chapter 13).
- Analysis 23:** Test for a difference in mean severity scores between treatment groups controlling for Study Center (Chapter 19). Use 'table' scores.
- Analysis 24:** Repeat Analysis 23 using modified ridit scores in place of 'table' scores.
- Analysis 25:** Determine the significance of the treatment group effect by using a *logistic regression* model under the assumption of proportional odds (Chapter 20).
- Analysis 26:** Using a proportional odds model, compare response rates between treatment groups adjusted for Age (Chapter 20).
- Analysis 27:** Adjusting for Age, Sex, and Study Center, determine the significance of the treatment group effect by using the proportional odds model (Chapter 20).

23.3 Appropriateness of the Methods

SAS code for conducting the analyses in the previous section is given in Appendix G. Table 23.1 summarizes the p-values for the treatment group effect for each of these analyses. Although the exercises are assigned for practice in using SAS to perform commonly used statistical analyses, some of these methods would clearly be inappropriate to use in practice. Let's take a look at the results.

TABLE 23.1 Summary of Significance of Treatment Group Effects from Exercises

Analysis	Method	p-Value for Treatment Group effect
Analyses Using the SCORE Variable		
1	Two-sample t-test	0.0422
2	Wilcoxon rank-sum test	0.0464
3	One-way ANOVA	0.0422
4	Two-way ANOVA with interaction	0.0702
5	Two-way ANOVA without interaction	0.0378
6	Three-way ANOVA with all interactions	0.1036
7	Three-way ANOVA main effects model	0.0493
8	Linear regression	--
9	ANCOVA / unequal slopes	0.5663
10	ANCOVA / equal slopes	0.0257
11	Stratified ANCOVA / equal slopes	0.0252
Analyses Using the RESP Variable		
12	Chi-square test	0.4510
13	Fisher's exact test	0.4794
14	Cochran-Mantel-Haenszel test	0.4113
15	Logistic regression, trt effect only	0.4523
16	Logistic regression, trt and age effects	0.3576
17	Logistic regression, trt, age, sex, center, trtxcenter effects	0.6984
Analyses Using the SEV Variable		
18	Chi-square test	0.1216
19	Generalized Fisher's exact test	0.1277
20	Mantel-Haenszel, row mean scores differ / table scores	0.0514
21	Mantel-Haenszel, row mean scores differ / mod. ridit scores	0.0468
22	Wilcoxon rank-sum test	0.0472
23	CMH, row mean scores differ / table scores	0.0445
24	CMH, row mean scores differ / mod. ridit scores	0.0471
25	Proportional odds model, trt effect only	0.0447
26	Proportional odds model, trt and age effects	0.0244
27	Proportional odds model, trt, age, sex, center	0.0313

The SCORE variable is considered a continuous numeric response. As such, you can apply a variety of *ANOVA*, *ANCOVA*, and non-parametric methods to test for equality of treatment groups.

- **23.3.1** The *two-sample t-test* and *one-way ANOVA* produce the same p-values (Analyses 1 and 3). This will always be the case when there are only two groups because the *ANOVA F-test* is functionally equivalent to the *t-test* in this situation. Whether using the *t-test* or *ANOVA*, you can test for variance homogeneity in SAS and apply an appropriate (Satterthwaite's or Welch's) adjustment in the case of unequal variances (see Sections 5.4.2, 6.4.3).
- **23.3.2** The p-values differ considerably for the *two-way ANOVA* with (Analysis 4) and without the interaction (Analysis 5). In an unbalanced design, the inclusion of an interaction term in the model, when, in fact, no interaction exists, can produce conservative main effects comparisons based on the SAS Type III sums of squares in the *ANOVA*. For this reason, many analysts prefer to use the interaction model only as a preliminary test, rather than as a final model.
- **23.3.3** Notice that inclusion of the Study Center effect using the two-way main effects model (Analysis 5) provides sufficient blocking effect to bring out a slightly more significant treatment effect than the *one-way ANOVA* (Analysis 3), even though it is not a significant effect. When a third main effect (Sex) is added (Analysis 7), the treatment comparison actually becomes less significant than either the one-way or two-way main effects models. Although such a scenario can occur for a number of reasons, in this case, it appears to be an inconsequential difference based on the sample size imbalance, which affects the SAS Type III sums of squares. The treatment effect can be masked, as in Analysis 6, when exploring interaction effects.
- **23.3.4** *ANCOVA* (Analysis 10) reveals that Age might be an important covariate, and the adjusted Treatment Group differences appear more significant than those unadjusted for Age. This comes at the expense of having to assume equal slopes between the two groups, an assumption that is supported by Analysis 9. As you've seen previously, the Treatment Group effect is masked when testing for the interaction (equal slope assumption) as in Analysis 9.
- **23.3.5** Addition of the Study Center effect to the *ANCOVA* (Analysis 11) does not appreciably alter the significance of the Treatment effect noted in Analysis 10. Overall, it is clear that the inclusion of the Age effect in the model removes more background noise than the inclusion of either Study Center or Sex.
- **23.3.6** Although the *t-test* and *ANOVA* methods assume a normal distribution of the response data, these methods are fairly robust against departures from this assumption, especially with larger sample sizes. A histogram of the data (Output 3.5) does not support the normality assumption in this case. However, treatment comparisons based on ranked data (Analysis 2) result in similar p-values with this size sample ($N=100$). Therefore, any of the following analyses: 1, 2, 3, 5, 7, 10, or 11, would be appropriate. The analyses intended to explore interactions (4, 6, and

9) would not be the best tests to use for treatment differences when interactions do not exist. In the presence of an interaction that involves Treatment Group, alternative approaches, such as subgroup analyses, might be more appropriate.

Notice that the treatment group comparisons adjusted for Age result in the greatest significance. This might be due to Age differences for this sample, or it might be a repeatable effect. In any case, to select appropriate statistical methods for pivotal studies prior to examination of the data, you must rely on trends found from previous studies. If a specific covariate (such as Age) was consistently found to be an important factor in Phase II studies, for example, the pre-specified statistical model to be used for the Phase III program should include that covariate. Usually, the simplest models that contain all factors known to have an important effect on the treatment comparisons should be used in pivotal studies.

The RESP variable is a dichotomous response. For this, you can apply multiple categorical methods to analyze differences between treatment groups.

- **23.3.7** Comparisons of response rates between treatment groups are not significant. Similar p-values for the treatment group comparisons, which range between 0.41 and 0.48, are found by using the *chi-square test* (Analysis 12), *Fisher's exact test* (Analysis 13), the *CMH test* stratified by Study Center (Analysis 14), and a *logistic regression* model with no covariates (Analysis 15) (a finding that might be expected in studies of moderate sample sizes, such as this one). With very small sample sizes or response rates close to 0 or 1, *Fisher's exact test* would be more appropriate than the other methods. Exact methods, useful in the case of small samples, are also available in *logistic regression* analysis.
- **23.3.8** The inclusion of Age as a covariate in the *logistic regression* model results in a smaller p-value for the treatment effect (Analysis 16). As shown in the *ANCOVA* (Analysis 10), Age appears to be an important factor when modeling treatment differences.
- **23.3.9** The inclusion of Study Center, Sex, and the Treatment-by-Center interaction in the *logistic regression* model results in even less significant treatment comparisons (Analysis 17), further supporting the recommendation to use parsimonious models. This can often be done by using *logistic regression* in a stepwise fashion to eliminate non-significant effects. Stepwise procedures are most often used in an exploratory role or in earlier phase studies.

The SEV variable is an ordinal categorical response with 4 levels. Here, you can apply a *rank test*, a *logistic regression* analysis using the proportional odds model, or a number of other categorical procedures.

- **23.3.10** Both the *chi-square test* (Analysis 18) and the *generalized Fisher's exact test* (Analysis 19) are tests for general association. Notice that the p-values for treatment comparisons are much larger than those of the other tests (Analyses 20 to 27) for the SEV response variable. As explained in Chapter 16, these methods are testing a less-specific hypothesis.

- **23.3.11** The *Mantel-Haenszel chi-square test* (Analysis 20) for ‘row mean scores differ’ is more appropriate for ordinal data than the test for ‘general association’. The *Mantel-Haenszel chi-square* tests for equality of mean scores between treatment groups, when the scores are assigned to the categorical levels in integer increments (‘table scores’). In this case, greater significance is found by using the modified ridit scores (Analysis 21) compared with the ‘table scores’ (Chapter 16).

This will not always be the case, as seen in Analyses 23 and 24. In these analyses, the *CMH test* controlling for Study Center is used for comparing mean scores between treatment groups. The p-value using the ‘table scores’ (Analysis 23) is slightly smaller than that based on the modified ridit scores (Analysis 24).

- **23.3.12** The *Wilcoxon rank-sum test* (Analysis 22) is actually a special version of the *Kruskal-Wallis test*. Recall that the *Mantel-Haenszel chi-square* using modified ridit scores is identical to the *Kruskal-Wallis test* (Chapter 18). The difference in p-values (0.0472 in Analysis 22 vs. 0.0468 in Analysis 21) is due only to a correction factor used by SAS.
- **23.3.13** *Logistic regression* modeling, similar to the *ANCOVA* method, shows the most significant treatment comparison (Analysis 26) when adjusting only for Age. Interestingly, this p-value (0.0244) is slightly less than that obtained by using the *ANCOVA* method (Analysis 10), perhaps due to the deviation from normality of the SCORE variable.

23.4 Summary

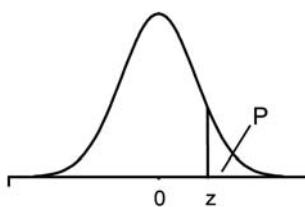
When you can quantify degree of response, it is usually easier to discriminate between treatment groups because more powerful statistical methods are available. With only the knowledge of whether or not a patient responded, you see that treatment group differences are non-significant for all of the applicable methods (Analyses 12 to 17). When the response is categorized by severity, you obtain significant treatment group discrimination. Notably, when using a more precise response (continuous numeric representation of severity), the significance of treatment comparisons was not improved. This might be due, in part, to the non-normality of the score results, but it also points out the power of categorical analyses when using ordinal response categories relative to *ANOVA* methods.

Comparison of these results points out the need to know how closely the underlying assumptions are satisfied before selecting an appropriate statistical method.

APPENDIX A

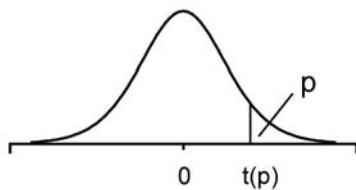
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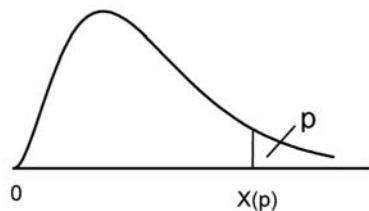
APPENDIX A.I Probabilities of the Standard Normal Distribution

z	P								
0.00	0.5000								
0.01	0.4960	0.51	0.3050	1.01	0.1562	1.51	0.0655	2.01	0.0222
0.02	0.4920	0.52	0.3015	1.02	0.1539	1.52	0.0643	2.02	0.0217
0.03	0.4880	0.53	0.2981	1.03	0.1515	1.53	0.0630	2.03	0.0212
0.04	0.4840	0.54	0.2946	1.04	0.1492	1.54	0.0618	2.04	0.0207
0.05	0.4801	0.55	0.2912	1.05	0.1469	1.55	0.0606	2.05	0.0202
0.06	0.4761	0.56	0.2877	1.06	0.1446	1.56	0.0594	2.06	0.0197
0.07	0.4721	0.57	0.2843	1.07	0.1423	1.57	0.0582	2.07	0.0192
0.08	0.4681	0.58	0.2810	1.08	0.1401	1.58	0.0571	2.08	0.0188
0.09	0.4641	0.59	0.2776	1.09	0.1379	1.59	0.0559	2.09	0.0183
0.10	0.4602	0.60	0.2743	1.10	0.1357	1.60	0.0548	2.10	0.0179
0.11	0.4562	0.61	0.2709	1.11	0.1335	1.61	0.0537	2.11	0.0174
0.12	0.4522	0.62	0.2676	1.12	0.1314	1.62	0.0526	2.12	0.0170
0.13	0.4483	0.63	0.2643	1.13	0.1292	1.63	0.0516	2.13	0.0166
0.14	0.4443	0.64	0.2611	1.14	0.1271	1.64	0.0505	2.14	0.0162
0.15	0.4404	0.65	0.2578	1.15	0.1251	1.65	0.0495	2.15	0.0158
0.16	0.4364	0.66	0.2546	1.16	0.1230	1.66	0.0485	2.16	0.0154
0.17	0.4325	0.67	0.2514	1.17	0.1210	1.67	0.0475	2.17	0.0150
0.18	0.4286	0.68	0.2483	1.18	0.1190	1.68	0.0465	2.18	0.0146
0.19	0.4247	0.69	0.2451	1.19	0.1170	1.69	0.0455	2.19	0.0143
0.20	0.4207	0.70	0.2420	1.20	0.1151	1.70	0.0446	2.20	0.0139
0.21	0.4168	0.71	0.2389	1.21	0.1131	1.71	0.0436	2.21	0.0136
0.22	0.4129	0.72	0.2358	1.22	0.1112	1.72	0.0427	2.22	0.0132
0.23	0.4090	0.73	0.2327	1.23	0.1093	1.73	0.0418	2.23	0.0129
0.24	0.4052	0.74	0.2296	1.24	0.1075	1.74	0.0409	2.24	0.0125
0.25	0.4013	0.75	0.2266	1.25	0.1056	1.75	0.0401	2.25	0.0122
0.26	0.3974	0.76	0.2236	1.26	0.1038	1.76	0.0392	2.26	0.0119
0.27	0.3936	0.77	0.2206	1.27	0.1020	1.77	0.0384	2.27	0.0116
0.28	0.3897	0.78	0.2177	1.28	0.1003	1.78	0.0375	2.28	0.0113
0.29	0.3859	0.79	0.2148	1.29	0.0985	1.79	0.0367	2.29	0.0110
0.30	0.3821	0.80	0.2119	1.30	0.0968	1.80	0.0359	2.30	0.0107
0.31	0.3783	0.81	0.2090	1.31	0.0951	1.81	0.0351	2.31	0.0104
0.32	0.3745	0.82	0.2061	1.32	0.0934	1.82	0.0344	2.32	0.0102
0.33	0.3707	0.83	0.2033	1.33	0.0918	1.83	0.0336	2.33	0.0099
0.34	0.3669	0.84	0.2005	1.34	0.0901	1.84	0.0329	2.34	0.0096
0.35	0.3632	0.85	0.1977	1.35	0.0885	1.85	0.0322	2.35	0.0094
0.36	0.3594	0.86	0.1949	1.36	0.0869	1.86	0.0314	2.36	0.0091
0.37	0.3557	0.87	0.1922	1.37	0.0853	1.87	0.0307	2.37	0.0089
0.38	0.3520	0.88	0.1894	1.38	0.0838	1.88	0.0301	2.38	0.0087
0.39	0.3483	0.89	0.1867	1.39	0.0823	1.89	0.0294	2.39	0.0084
0.40	0.3446	0.90	0.1841	1.40	0.0808	1.90	0.0287	2.40	0.0082
0.41	0.3409	0.91	0.1814	1.41	0.0793	1.91	0.0281	2.41	0.0080
0.42	0.3372	0.92	0.1788	1.42	0.0778	1.92	0.0274	2.42	0.0078
0.43	0.3336	0.93	0.1762	1.43	0.0764	1.93	0.0268	2.43	0.0075
0.44	0.3300	0.94	0.1736	1.44	0.0749	1.94	0.0262	2.44	0.0073
0.45	0.3264	0.95	0.1711	1.45	0.0735	1.95	0.0256	2.45	0.0071
0.46	0.3228	0.96	0.1685	1.46	0.0721	1.96	0.0250	2.46	0.0069
0.47	0.3192	0.97	0.1660	1.47	0.0708	1.97	0.0244	2.47	0.0068
0.48	0.3156	0.98	0.1635	1.48	0.0694	1.98	0.0239	2.48	0.0066
0.49	0.3121	0.99	0.1611	1.49	0.0681	1.99	0.0233	2.49	0.0064
0.50	0.3085	1.00	0.1587	1.50	0.0668	2.00	0.0228	2.50	0.0062
								3.00	0.0013



APPENDIX A.2 Critical Values of the Student t-Distribution

df	t(0.100)	t(0.050)	t(0.025)	t(0.010)
1	3.078	6.314	12.706	31.821
2	1.886	2.920	4.303	6.965
3	1.638	2.353	3.182	4.541
4	1.533	2.132	2.776	3.747
5	1.476	2.015	2.571	3.365
6	1.440	1.943	2.447	3.143
7	1.415	1.895	2.365	2.998
8	1.397	1.860	2.306	2.896
9	1.383	1.833	2.262	2.821
10	1.372	1.812	2.228	2.764
11	1.363	1.796	2.201	2.718
12	1.356	1.782	2.179	2.681
13	1.350	1.771	2.160	2.650
14	1.345	1.761	2.145	2.624
15	1.341	1.753	2.131	2.602
16	1.337	1.746	2.120	2.583
17	1.333	1.740	2.110	2.567
18	1.330	1.734	2.101	2.552
19	1.328	1.729	2.093	2.539
20	1.325	1.725	2.086	2.528
21	1.323	1.721	2.080	2.518
22	1.321	1.717	2.074	2.508
23	1.319	1.714	2.069	2.500
24	1.318	1.711	2.064	2.492
25	1.316	1.708	2.060	2.485
26	1.315	1.706	2.056	2.479
27	1.314	1.703	2.052	2.473
28	1.313	1.701	2.048	2.467
29	1.311	1.699	2.045	2.462
30	1.310	1.697	2.042	2.457
31	1.309	1.696	2.040	2.453
32	1.309	1.694	2.037	2.449
33	1.308	1.692	2.035	2.445
34	1.307	1.691	2.032	2.441
35	1.306	1.690	2.030	2.438



APPENDIX A.3 Critical Values of the Chi-Square Distribution

df	X(0.100)	X(0.050)	X(0.025)	X(0.010)
1	2.706	3.841	5.024	6.635
2	4.605	5.991	7.378	9.210
3	6.251	7.815	9.348	11.345
4	7.779	9.488	11.143	13.277
5	9.236	11.070	12.832	15.086
6	10.645	12.592	14.449	16.812
7	12.017	14.067	16.013	18.475
8	13.362	15.507	17.535	20.090
9	14.684	16.919	19.023	21.666
10	15.987	18.307	20.483	23.209
11	17.275	19.675	21.920	24.725
12	18.549	21.026	23.337	26.217
13	19.812	22.362	24.736	27.688
14	21.064	23.685	26.119	29.141
15	22.307	24.996	27.488	30.578
16	23.542	26.296	28.845	32.000
17	24.769	27.587	30.191	33.409
18	25.989	28.869	31.526	34.805
19	27.204	30.144	32.852	36.191
20	28.412	31.410	34.170	37.566
21	29.615	32.671	35.479	38.932
22	30.813	33.924	36.781	40.289
23	32.007	35.172	38.076	41.638
24	33.196	36.415	39.364	42.980
25	34.382	37.652	40.646	44.314
26	35.563	38.885	41.923	45.642
27	36.741	40.113	43.195	46.963
28	37.916	41.337	44.461	48.278
29	39.087	42.557	45.722	49.588
30	40.256	43.773	46.979	50.892
31	41.422	44.985	48.232	52.191
32	42.585	46.194	49.480	53.486
33	43.745	47.400	50.725	54.775
34	44.903	48.602	51.966	56.061
35	46.059	49.802	53.203	57.342

APPENDIX B

Common Distributions Used in Statistical Inference

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B.1 Notation

$$X \sim N(\mu, \sigma^2)$$

means that the random variable, X, is normally distributed with mean μ and variance σ^2 .

$$X \sim \chi^2_v$$

means that the random variable, X, has the chi-square distribution with v degrees of freedom.

$$X \sim F_{v_2}^{v_1}$$

means that the random variable, X, has the F-distribution with v_1 upper and v_2 lower degrees of freedom.

$$X \sim t_v$$

means that the random variable, X, has the Student-t distribution with v degrees of freedom.

$$X \sim U_{[0-1]}$$

means that the random variable, X, has the Uniform distribution over the interval (0–1).

B.2 Properties

1. If $X \sim N(\mu, \sigma^2)$ and $Z = (X - \mu)/\sigma$, then $Z \sim N(0, 1)$.
Z is called a standard normal random variable.
2. If $X_1 \sim N(\mu_1, \sigma^2_1)$ and $X_2 \sim N(\mu_2, \sigma^2_2)$, X_1 and X_2 are independent, and $Y = X_1 + X_2$, then $Y \sim N(\mu_1 + \mu_2, \sigma^2_1 + \sigma^2_2)$.

More generally, for any constants, a_1, a_2, \dots, a_k , if $X_i \sim N(\mu_i, \sigma^2_i)$ independently for $i = 1, 2, \dots, k$, and $Y = a_1 X_1 + a_2 X_2 + \dots + a_k X_k$, then

$$Y \sim N\left(\sum_{i=1}^k a_i \mu_i, \sum_{i=1}^k a_i^2 \sigma_i^2\right)$$

3. If $Z \sim N(0, 1)$, then $Z^2 \sim \chi^2_1$
4. If $Z_i \sim N(0, 1)$ independently for $i = 1, 2, \dots, n$, and $Y = Z_1^2 + Z_2^2 + \dots + Z_n^2$, then $Y \sim \chi^2_n$
5. If $X \sim \chi^2_m$ and $Y \sim \chi^2_n$ independently, and $W = X + Y$, then $W \sim \chi^2_{m+n}$
6. If $Z \sim N(0, 1)$ and $Y \sim \chi^2_v$ independently, and $T = Z/(Y/v)^{1/2}$, then $T \sim t_v$
7. If $X \sim \chi^2_m$ and $Y \sim \chi^2_n$, independently, and $F = (n/m)(X/Y)$, then $F \sim F_{m,n}$
8. If $X \sim U_{[0,1]}$, then $-2 \cdot \ln(X) \sim \chi^2_2$

B.3 Results

Let $X_i \sim N(\mu, \sigma^2)$ independently for $i = 1, 2, \dots, n$, and let

$$\bar{X} = \frac{\sum_{i=1}^n X_i}{n}$$

represent the sample mean.

Then, Property 2 implies that

$$\bar{X} \sim N\left(\mu, \sigma^2/n\right) \quad (\text{i})$$

Property 1 and Equation (i) imply that

$$Z = \frac{\sqrt{n}(\bar{X} - \mu)}{\sigma} \sim N(0, 1) \quad (\text{ii})$$

Property 3 and Equation (ii) imply that

$$Z^2 = \frac{n(\bar{X} - \mu)^2}{\sigma^2} \sim \chi^2_1 \quad (\text{iii})$$

Also, Property 1 implies that

$$\frac{X_i - \mu}{\sigma} \sim N(0, 1) \quad (\text{iv})$$

Property 4 and Equation (iv) imply that

$$\sum_{i=1}^n \frac{(X_i - \mu)^2}{\sigma^2} \sim \chi^2_n \quad (\text{v})$$

It can be shown that

$$\sum(X_i - \mu)^2 = \sum(X_i - \bar{X})^2 + n(\bar{X} - \mu)^2$$

and that the summations on the right side of the equal sign are independent, so that,

$$\frac{(n-1)s^2}{\sigma^2} = \frac{\sum(X_i - \mu)^2}{\sigma^2} - \frac{n(\bar{X} - \mu)^2}{\sigma^2} \quad (\text{vi})$$

where s^2 is the sample variance given by

$$s^2 = \frac{\sum(X_i - \bar{X})^2}{n - 1}$$

Property 5 and Equations (iii), (v), and (vi) imply that

$$\frac{(n-1) \cdot s^2}{\sigma^2} \sim \chi^2_{n-1} \quad (\text{vii})$$

Property 6 and Equations (ii) and (vii) imply that

$$t = \frac{\left(\frac{(\bar{X} - \mu)}{\sigma/\sqrt{n}} \right)}{\left(\frac{s}{\sigma} \right)} = \frac{(\bar{X} - \mu)}{s/\sqrt{n}} \sim t_{n-1} \quad (\text{viii})$$

Property 7 and Equations (iii) and (vii) imply that

$$F = t^2 \sim F_{n-1}^1 \quad (\text{ix})$$

With application to *ANOVA*, let MS_1 and MS_2 represent two independent, unbiased estimates of the error variance, σ^2 , based on v_1 and v_2 degrees of freedom, respectively, then, by (vii),

$$\frac{v_1 MS_1}{\sigma^2} \sim \chi^2_{v_1} \quad \text{and} \quad \frac{v_2 MS_2}{\sigma^2} \sim \chi^2_{v_2} \quad (\text{x})$$

which, together with Property 7, imply that

$$\frac{MS_1}{MS_2} \sim F_{v_2}^{v_1} \quad (\text{xi})$$

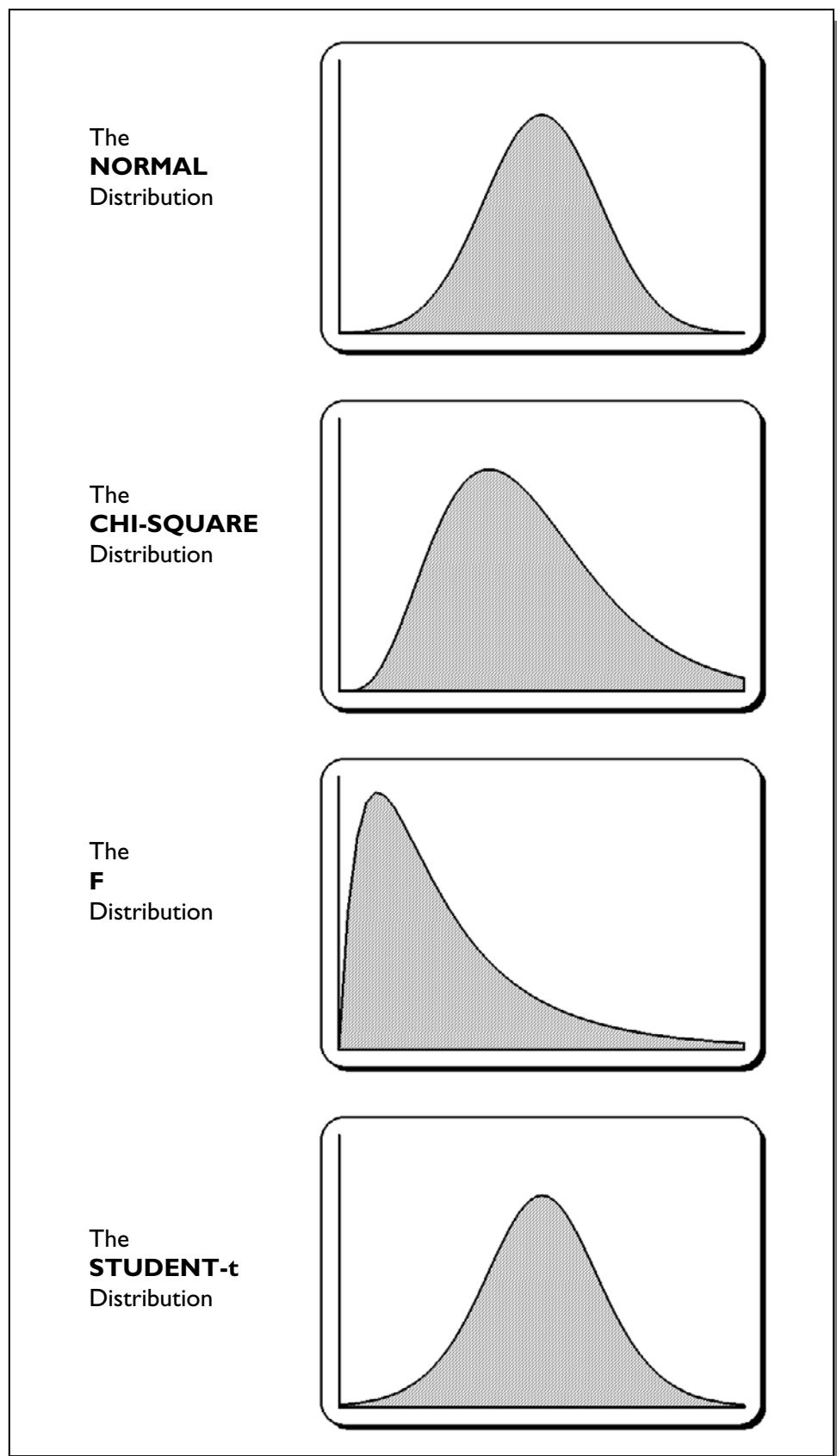
With application to *meta-analysis*, let p_i represent the p-value for the treatment comparison for the i^{th} of k studies. Under the null hypothesis of no treatment differences, $p_i \sim U_{(0-1)}$. By Property 8, $-2 \cdot \ln(p_i) \sim \chi^2_2$ and, together with Property 5,

$$W = -2 \cdot (\ln(p_1) + \ln(p_2) + \dots + \ln(p_k)) \sim \chi^2_{2k} \quad (\text{xii})$$

B.4 Distributional Shapes

Figure B.1 illustrates the shapes of some common probability distributions. The value of the random variable is shown on the horizontal axis, and the probability is shown on the vertical axis. These shapes vary with changes in the distributional parameters, such as the variance or degrees of freedom. Notice the symmetry of the normal and the t-distributions and the skew associated with the chi-square and F-distributions.

FIGURE B.1 Distributional Shapes



APPENDIX C

Basic ANOVA Concepts

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C.1 Within- vs. Between-Group Variation

Suppose 12 recent college graduates are assigned to three groups: 4 subjects to an exercise group (I), 4 subjects to a drug treatment group (II), and 4 subjects to a control group (III). Ages, pulse rates, diastolic blood pressures, and triglyceride measurements are taken after 8 weeks in the study, with the following results:

--- GROUP ---			
	I	II	III
1. <u>Age (years)</u>	22	22	22
	22	22	22
	22	22	22
	22	22	22
2. <u>Pulse Rates (beats/min.)</u>	I	II	III
	64	59	70
	64	59	70
	64	59	70
	64	59	70

The age measurements are the same for all 12 subjects. Clearly, there is no variation among the group means and, therefore, no difference among groups with respect to age.

The pulse rate measurements are the same for all 4 subjects within each group, but the measurements vary across groups. Observation of these data without further analysis would likely lead to the conclusion of a difference in mean pulse rates among the three groups.

3. Diastolic BP (mm Hg)

Diastolic blood pressure measurements show a variation both within and among groups. Within-group measurements vary by no more than 2 mm Hg, while the variation among the group means is as much as 14 mm. Because the among-group variation is large compared to the within-group variation, you would likely think that there is a difference among groups in mean diastolic blood pressure.

	I	II	III
74	74	81	89
76	76	80	89
75	75	79	88
75	75	80	90

4. Triglycerides (mg/dl)

The triglyceride measurements show considerable variation both within and among groups. Because the variation within groups no longer allows our intuition to distinguish among groups, it is not clear whether a group effect exists.

	I	II	III
85	85	72	141
101	101	130	78
68	68	91	91
121	121	99	121

For the triglyceride results, there might not be a real difference that can be attributed to the groups, but you need an analytic method to determine this. The ANOVA methods are used for exactly this purpose, i.e., to analyze the variability among groups relative to the variability within groups to determine if differences among groups are meaningful or significant.

The ANOVA methods seek to identify sources of variation for each measurement, and separate the total variability into components associated with each source. The total variability can generally be described as the sum of squared deviations of each measurement from the overall mean. This is comprised of a sum of squares due to suspected sources of variation (often called the ‘model sum of squares’) and a sum of squares (SS) due to error (SSE):

$$SS(\text{Total}) = SS(\text{Model}) + SS(\text{Error})$$

The error variability includes such things as measurement error, normal variation due to repeated sampling, and variation among ‘like’ experimental units from other unknown factors. Such sources might be difficult to control even in a well-designed study. The sources that can be identified and controlled are included in the model SS.

An ANOVA is conducted using *F-tests* that are constructed from the ratio of between-group to within-group variance estimates. Under the hypothesis of no group effect, the variation among groups is just another measure of patient-to-patient variability, so their ratio should be about 1. Assumptions for these *F-tests* usually entail independent samples from normally distributed populations with equal variances.

In the example given in the beginning of this Appendix, the three groups (I, II, III) represent levels of a factor named GROUP. A ‘significant GROUP effect’ indicates that there are significant differences in mean responses among the levels of the factor GROUP.

The *one-way ANOVA* has one main effect or grouping factor with two or more levels. In analyzing clinical trials, the main effect will often be a treatment effect. The levels of the factor Treatment might be ‘low dose’, ‘middle dose’, ‘high dose’, and ‘placebo’. The *two-way ANOVA* has two main effects, usually a grouping or treatment factor and a blocking factor (such as Gender, Study Center, Diagnosis Group, etc.).

Statistical interactions among main effects can also be analyzed when two or more factors are used. An interaction occurs when differences among the levels of one factor change with different levels of another factor. A *two-way ANOVA* can have only one two-way interaction. A *three-way ANOVA* has three main effects (for example, A, B, and C), 3 two-way interactions (A-by-B, A-by-C, and B-by-C), and 1 three-way interaction (A-by-B-by-C). Multi-way ANOVA setups can have any number of factors, each at any number of levels. The *two-way ANOVA* (Chapter 7) is one of the most commonly used analyses for multi-center clinical studies, usually with Treatment (or Dose group) and Study Center as the two main effects.

Other types of commonly used ANOVAs include the *repeated-measures ANOVA* (Chapter 8), *nested* or *random-effects ANOVA*, and an *ANOVA of mixed models*. *Analysis of Covariance* (Chapter 11) is a special type of ANOVA that includes adjustments of treatment effects for numeric covariates.

In most types of ANOVA used in clinical trials, the primary question the researcher wants to answer is whether there are any differences among the group population means based on the sample data. The null hypothesis to be tested is ‘there is no Group effect’ or, equivalently, ‘the mean responses are the same for all groups’. The alternative hypothesis is that ‘the Group effect is important’ or, equivalently, ‘the Group means differ for at least one pair of groups’.

The *one-way ANOVA* involves a straightforward comparison of the between-group variation to the within-group variation. An ANOVA that involves blocking factors or covariates seeks to refine treatment comparisons by factoring out extraneous variation due to *known* sources.

C.2 Noise Reduction by Blocking

The more extraneous variability or ‘background noise’ that you can account for, the more precise the Group comparisons become. Accounting for known blocking factors is one means of reducing background noise.

Suppose you collect body weight data (in pounds) for 31 patients randomized to two groups (as shown in Table C.1). You want to determine whether the groups, Treatment A or Treatment B, have different mean weights. Initially, you would use the *one-way ANOVA* methods shown in Chapter 6.

TABLE C.1 Body Weight Data for Two Treatment Groups

Treatment Group A		Treatment Group B	
Patient Number	Weight	Patient Number	Weight
10	110	11	121
12	101	13	116
14	124	15	144
17	120	16	125
18	111	19	115
21	117	20	118
22	120	23	127
24	131	30	205
31	185	33	193
35	181	34	196
37	173	36	189
40	190	38	180
42	181	39	193
44	202	41	210
45	175	43	189
		46	179

Mean weights are computed to be 148.1 and 162.5 for Treatment Groups A and B, respectively. *One-way ANOVA* (with $k = 2$ groups) produces an *F-test* statistic of 1.23 with 1 upper and 29 lower degrees of freedom, as shown in Table C.2. This results in a p-value of 0.277, leading to the conclusion that there's no significant difference in mean weights between the groups.

TABLE C.2 One-Way ANOVA Summary

Source	df	SS	MS	F
Treatment	1	1612.8	1612.8	F = 1.23
Error	29	38154.9	1315.7	
Total	30	39767.7		

F = 1.23 is not significant (p=0.277)

Now, suppose you know that patients with numbers less than 30 are females, and patients with numbers of 30 or above are males. It is clear from scanning the data that the males weigh more than the females. The error variance, σ^2 , is a within-group (patient-to-patient) variability. The MSE of 1315.7 overestimates σ^2 because it includes a component due to variation between genders. The inflated estimate using this MSE leads to an F-value for treatment group differences that is smaller and less significant than if a more precise estimate of σ^2 were obtained. Removing the gender variation from the MSE might result in a more precise comparison of the groups.

After Gender is identified as a source of variation, you can include that factor in the ANOVA by computing a sum of squares, a mean square, and an F-value for this factor similar to the methods used for the Group effect in a *one-way ANOVA* (Chapter 6). In the *two-way ANOVA* (Chapter 7), you can test whether Gender is a significant factor, and remove its variation from the MSE to get a more precise test for the Treatment effect.

As seen in Table C.3, Gender is not only significant, but the estimate of error variance, $MSE = 93.9$, is substantially reduced from that of the *one-way ANOVA* ($MSE = 1315.7$). This leads to greater precision in testing for the Treatment group effect, which is now seen to be significant.

TABLE C.3 Two-Way ANOVA Summary

Source	df	SS	MS	F
Treatment	1	480.1	480.1	$F_t = 5.1^*$
Gender	1	35526.6	35526.6	$F_b = 378.5^{**}$
Error	28	2628.3	93.9	
Total	30	39767.7		

* Significant ($p = 0.032$)

** Significant ($p < 0.001$)

The general setup of a two-way layout with g levels of the ‘treatment’ factor, Group, and b levels of the ‘blocking’ factor, Block, is shown in Table C.4. From each Group-by-Block combination or ‘cell’, you independently sample a number of observations, letting y_{ijk} represent the k^{th} data value from Group i and Block j . The number of data values within Cell $i-j$ is represented by n_{ij} .

TABLE C.4 Two-Way ANOVA Layout (Randomized Block Design)

Group	----- Block -----			
	1	2	...	b
1	n_{11}	n_{12}	...	n_{1b}
2	n_{21}	n_{22}	...	n_{2b}
...
g	n_{g1}	n_{g2}	...	n_{gb}

In the weight example discussed earlier, you have $g = 2$, $b = 2$, and n_{ij} as follows:

Treatment (Group)	Gender (Block)	
	Females	Males
A	$n_{11} = 8$	$n_{12} = 7$
B	$n_{21} = 7$	$n_{22} = 9$

If each cell in the two-way layout has the same number of observations ($n_{ij} = n$ for all i,j), you have a ‘balanced’ design. Computing formulas for balanced designs are straightforward and similar to those given for the one-way layout (Chapter 6). With imbalance, there are a number of different ways to compute the sums of squares. In SAS, there are four types of sums of squares computations available: Type I, Type II, Type III, and Type IV. The differences among these types are discussed in Appendix D. Analysis of clinical research data using an ANOVA often focuses on the Type III sums of squares from PROC GLM in SAS.

In the previous weight example, notice the p-value for Gender of <0.001 , which means that there is a highly significant difference in mean weights between the males and the females. Furthermore, the p-value of 0.032 for Treatment tells you that a significant (<0.05) difference in mean weights *does* exist between the two treatment groups, A and B. This difference was masked by the large variability when the Gender factor was ignored.

The ANOVA results given in Table C.3 do not include the interaction between treatment group and gender as a source of variation. A statistical interaction between two effects suggests that differences in response means among the levels of one effect are not consistent across the levels of the other effect. A significant Treatment-by-Gender interaction in the weight example would indicate that the difference in mean weight between Treatment A and Treatment B depends on whether we’re talking about males or females.

Different response patterns, which indicate interactions and no interactions, are shown in Chapter 7. Cell means from the weight example would depict a pattern as shown in Figure C.1, which suggests no interaction. The ANOVA that includes the interaction effect confirms this, as shown in Table C.5.

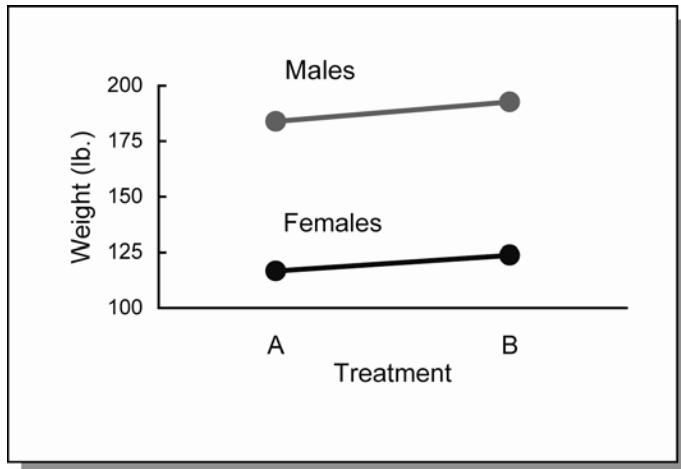
TABLE C.5 ANOVA Summary – Interaction Effect

Source	df	SS	MS	F
Treatment	1	476.8	476.8	$F_t = 4.9^*$
Gender	1	35475.8	35475.8	$F_b = 365.3^{**}$
Treatment-by-Gender	1	6.5	6.5	$F_{tg} = 0.07$
Error	28	2621.8	97.1	
Total	30	39767.7		

* Significant ($p = 0.035$)

** Significant ($p < 0.001$)

Figure C.1 Plot of Mean Weights Shows No Treatment-by-Gender Interaction



C.3 Least Squares Mean (LS-mean)

In the development of a new recumbent exercise bicycle, the designers needed to estimate the average height of potential users. They took the height of 100 members of a local health club chain and computed the mean height as

$$\bar{X} = \frac{\sum_{i=1}^{100} x_i}{100}$$

where x_i represents the height of the i^{th} member. Is this a good estimate? Suppose it is known that the club selected has mostly male members, and that 90 of the 100 members selected were male. The sample mean above can then be represented as

$$\bar{X} = \frac{(90 \cdot \bar{X}_m) + (10 \cdot \bar{X}_f)}{100} = 0.9 \cdot \bar{X}_m + 0.1 \cdot \bar{X}_f$$

where \bar{X}_m and \bar{X}_f represent the mean heights of the males and females, respectively. This estimate is a good one if the population is composed of approximately 90% males, i.e., if 90% of the potential users of the new exercise equipment are male. However, if the sample that was taken is not in proportion to the true composition of the population, this simple average might be a poor estimate.

Suppose it is known that users of the recumbent cycle are, in general, equally distributed between males and females. Under such an assumption, the mean that was computed for this example would tend to overestimate the population mean because it is highly weighted by the males, who are known to be taller in the general population than females.

A mean height for males of 70 inches and a mean height for females of 62 inches would produce an overall mean height estimate of 69.2 inches when using the weighted approach just presented. A better estimate might be $(70 + 62)/2 = 66$ inches or, in general,

$$\bar{X} = \frac{(\bar{X}_m + \bar{X}_f)}{2} = 0.5 \cdot \bar{X}_m + 0.5 \cdot \bar{X}_f$$

This estimate is called a least squares mean or LS-mean. This is the estimate computed by SAS when using a ‘saturated’ model (i.e., inclusion of main effects and all possible interactions).

In clinical trials, the LS-mean is often used for estimating treatment effects when blocking by study center. A typical trial might involve a larger number of patients from one study center than from another. The LS-mean of the treatment effect would be equivalent to a combined average over study centers as if the same number of patients were studied within each center.

If the sample sizes from each study center are proportional to the population sizes, the usual arithmetic mean would be appropriate. When the population sizes are considered ‘infinite’, the LS-mean is used to give equal weighting to the effect sizes from each study center. Estimates based on the LS-mean can differ markedly from that of the usual arithmetic mean if either the cell means or the cell sample sizes within any dose group differ substantially among study centers. In such instances, the analyst must examine the assumptions of population sizes and sample representation to decide which estimate is more appropriate.

In Example 7.2 (Chapter 7), the means and LS-means of the number of items correctly remembered by patients in the three Dose groups in the memory study are shown in Table C.6.

TABLE C.6 Arithmetic and Least Squares Mean for Example 7.2

CENTER	DOSE GROUP			
	Placebo	30 mg	60 mg	
Dr. Abel	Mean (N)	5.67 (12)	8.38 (8)	10.11 (9)
Dr. Best	Mean (N)	6.82 (11)	6.40 (10)	12.11 (9)
Combined	Mean LS-Mean	6.22 6.24	7.28 7.39	11.11 11.11

Notice that the two estimates are very close to each other. This might not be the case if, for example, there are 25 patients in one study center and only 5 patients in another. When such a degree of imbalance occurs in the sampling, it is important to consider the sampling assumptions before deciding which estimate of treatment effects is more appropriate.

APPENDIX D

SS Types I, II, III, and IV Methods for an Unbalanced Two-Way Layout

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D.I SS Types Computed by SAS

This section uses the randomized block design to illustrate the differences among the various *ANOVA* types of sums of squares (SS) computed by SAS: Type I, Type II, Type III, and Type IV.

Consider the unbalanced two-way layout that has two treatment groups (A and B) and 3 study centers (1, 2, and 3). Let μ_{ij} and n_{ij} represent the mean and sample size, respectively, for Treatment i, Center j. For this example, 8 patients receive Treatment A and 6 patients receive Treatment B. The patients are distributed among study centers as shown in Table D.1.

TABLE D.1 Sample Configuration for an Unbalanced Two-Way Layout

Center	Treatment	
	A	B
1	μ_{11} $n_{11} = 2$	μ_{21} $n_{21} = 1$
2	μ_{12} $n_{12} = 2$	μ_{22} $n_{22} = 3$
3	μ_{13} $n_{13} = 4$	μ_{23} $n_{23} = 2$
overall	μ_A $n_A = 8$	μ_B $n_B = 6$

Denote each treatment mean as a linear combination of the cell means for that treatment.

$$\mu_A = a_1\mu_{11} + a_2\mu_{12} + a_3\mu_{13}$$

and

$$\mu_B = b_1\mu_{21} + b_2\mu_{22} + b_3\mu_{23}$$

The goal is to test the hypothesis of equality of treatment means

$$H_0: \mu_A = \mu_B$$

The method used to perform this test depends on how you define the treatment means in terms of the cell means. Consider the following three cases:

Case (i): Let $a_j = n_{1j}/n_A, b_j = n_{2j}/n_B (j = 1, 2, 3)$

The hypothesis of equal treatment means becomes

$$\begin{aligned} H_0: & (2/8)\mu_{11} + (2/8)\mu_{12} + (4/8)\mu_{13} = \\ & (1/6)\mu_{21} + (3/6)\mu_{22} + (2/6)\mu_{23} \end{aligned}$$

The treatment means are the weighted averages of the cell means for that treatment, weighted by the cell sample sizes. This is the hypothesis tested by the SAS Type I sum of squares for Treatment if the Treatment factor is specified first in the MODEL statement.

Case (ii): Let $a_j = b_j = u_j/U (j = 1, 2, 3)$
 where $u_1 = n_{11} n_{21} (n_{12} + n_{22}) (n_{13} + n_{23})$
 $u_2 = n_{12} n_{22} (n_{11} + n_{21}) (n_{13} + n_{23})$
 $u_3 = n_{13} n_{23} (n_{11} + n_{21}) (n_{12} + n_{22})$
 and $U = u_1 + u_2 + u_3$

The hypothesis of equal treatment means becomes

$$\begin{aligned} H_0: & (5/24)\mu_{11} + (9/24)\mu_{12} + (10/24)\mu_{13} = \\ & (5/24)\mu_{21} + (9/24)\mu_{22} + (10/24)\mu_{23} \end{aligned}$$

The treatment mean is the weighted average of the cell means, weighted by what seems to be a complex function of the cell sample sizes. This is the hypothesis tested by the SAS Type II sum of squares for Treatment.

The weights are actually inversely related to the variance of the estimates of the means for each center. This can be seen when the coefficients u_j/U (in Case ii) are rewritten in a more familiar form.

For Center j ($j=1, 2$, or 3), let $w_j = \sigma^2 \cdot (1/n_{1j} + 1/n_{2j})$. Under the usual *ANOVA* assumptions of independent groups and variance homogeneity, this represents the variance of $\bar{X}_{1j} + \bar{X}_{2j}$. Letting $W = w_1 + w_2 + w_3$, the weights $(w_j)^{-1} / W^{-1}$ are proportional to the u_j/U shown in Case ii. Written this way, you see that each treatment mean is a weighted average of the center means for that treatment, where the weights are proportional to the inverse of the variances within the center.

Case (iii): Let $a_1 = a_2 = a_3 = b_1 = b_2 = b_3 (= 1/3)$

The hypothesis of equal treatment means becomes

$$H_0: (1/3)\mu_{11} + (1/3)\mu_{12} + (1/3)\mu_{13} = \\ (1/3)\mu_{21} + (1/3)\mu_{22} + (1/3)\mu_{23}$$

which does not depend on the sample sizes. This is the hypothesis tested by the SAS Type III and Type IV sums of squares for Treatment.

Note: The SAS Type III and Type IV tests are identical in a two-way layout with no empty cells. If $n_{ij} = 0$ for at least one cell, the Type III and IV sums of squares for Treatment will differ if there are more than two levels of the Treatment factor. If there are empty cells, it is recommended that the Type IV tests be used (see Section D.3).

D.2 How to Determine the Hypotheses Tested

Note: This section assumes that the reader is familiar with vector notation.

You can express the mean response for cell $i-j$ in terms of model parameters,

$$\mu_{ij} = \mu + \alpha_i + \beta_j + \gamma_{ij}$$

where μ represents the overall mean response, α_i represents the average additive effect of Treatment i , β_j is the average additive effect of Center j , and γ_{ij} is the average additive effect of the Treatment i -by-Center j combination (interaction). SAS uses vectors of the parameter coefficients and matrices in PROC GLM to compute sums of squares, and it is also convenient to express hypotheses in terms of

matrix algebra. With two treatment groups, the hypotheses tested by SAS are of the form

$$H_0: \underline{\mathbf{a}}' \underline{\mathbf{B}} = 0$$

where $\underline{\mathbf{B}}$ is the vector of parameters and $\underline{\mathbf{a}}$ is a vector of parameter coefficients. In the example with two Treatments and three Centers, the parameter vector is

$$\underline{\mathbf{B}}' = (\mu \ \alpha_1 \ \alpha_2 \ \beta_1 \ \beta_2 \ \beta_3 \ \gamma_{11} \ \gamma_{12} \ \gamma_{13} \ \gamma_{21} \ \gamma_{22} \ \gamma_{23})$$

Use the following SAS statements:

```
proc glm;
  class trt center;
  model y = trt center trt*center / e1 e2 e3 e4;
```

to generate the ANOVA for the configuration in Table D.1, and print the form of the parameter coefficient vectors for each of the four types of sums of squares. The next section shows how these vectors can be used to convert the hypothesis from a parametric hypothesis ($\underline{\mathbf{a}}' \underline{\mathbf{B}} = 0$) to a hypothesis based on the cell means.

Type I Hypotheses

The output of the vector of parameter coefficients ($\underline{\mathbf{a}}$) for the Type I estimable functions for Treatment is shown in Output D.1.

L2 refers to any constant that is chosen by the user. Setting L2 = 12, the vector $\underline{\mathbf{a}}'$ is

$$(0 \ 12 \ -12 \ 1 \ -3 \ 2 \ 3 \ 3 \ 6 \ -2 \ -6 \ -4),$$

and the hypothesis becomes

$$\begin{aligned} \underline{\mathbf{a}}' \underline{\mathbf{B}} = & 0\mu + 12\alpha_1 - 12\alpha_2 + 1\beta_1 - 3\beta_2 + 2\beta_3 + \\ & 3\gamma_{11} + 3\gamma_{12} + 6\gamma_{13} - 2\gamma_{21} - 6\gamma_{22} - 4\gamma_{23} = 0 \end{aligned}$$

Algebraic manipulation of this equation yields

$$\begin{aligned} (3\mu + 3\alpha_1 + 3\beta_1 + 3\gamma_{11}) + (3\mu + 3\alpha_1 + 3\beta_2 + 3\gamma_{12}) + (6\mu + 6\alpha_1 + 6\beta_3 + 6\gamma_{13}) = \\ (2\mu + 2\alpha_2 + 2\beta_1 + 2\gamma_{21}) + (6\mu + 6\alpha_2 + 6\beta_2 + 6\gamma_{22}) + (4\mu + 4\alpha_2 + 4\beta_3 + 4\gamma_{23}) \end{aligned}$$

which can be expressed in terms of the cell means as the SAS Type I hypothesis:

$$3\mu_{11} + 3\mu_{12} + 6\mu_{13} = 2\mu_{21} + 6\mu_{22} + 4\mu_{23}$$

or

$$(2/8)\mu_{11} + (2/8)\mu_{12} + (4/8)\mu_{13} = (1/6)\mu_{21} + (3/6)\mu_{22} + (2/6)\mu_{23}$$

OUTPUT D.1 Form of the SAS Type I Estimable Functions

Type I Estimable Functions for: trt		
Effect		Coefficients
Intercept		0
trt	A	L2
	B	-L2
cntr	1	0.0833*L2
	2	-0.25*L2
	3	0.1667*L2
trt*cntr	A 1	0.25*L2
	A 2	0.25*L2
	A 3	0.5*L2
	B 1	-0.1667*L2
	B 2	-0.5*L2
	B 3	-0.3333*L2

The form of the Type I hypothesis for Treatment depends on the order in which the factors are listed in the MODEL statement. If Treatment is listed before Center, the Type I hypothesis is as given in the preceding example. If Center is listed before Treatment, the Type I hypothesis for Treatment is the same as the Type II hypothesis for Treatment. Types II, III, and IV do not depend on the order in which the factors are listed in the MODEL statement.

Type II Hypotheses

The output of the vector of parameter coefficients for the Type II estimable functions for Treatment is shown in Output D.2.

By substituting 24 for L2, the hypothesis becomes

$$\mathbf{a}^T \boldsymbol{\beta} = 0\mu + 24\alpha_1 - 24\alpha_2 + 0\beta_1 + 0\beta_2 + 0\beta_3 + \\ 5\gamma_{11} + 9\gamma_{12} + 10\gamma_{13} - 5\gamma_{21} - 9\gamma_{22} - 10\gamma_{23} = 0$$

Algebraic manipulation of this equation results in

$$(5\mu + 5\alpha_1 + 5\beta_1 + 5\gamma_{11}) + (9\mu + 9\alpha_1 + 9\beta_2 + 9\gamma_{12}) + (10\mu + 10\alpha_1 + 10\beta_3 + 10\gamma_{13}) = \\ (5\mu + 5\alpha_2 + 5\beta_1 + 5\gamma_{21}) + (9\mu + 9\alpha_2 + 9\beta_2 + 9\gamma_{22}) + (10\mu + 10\alpha_2 + 10\beta_3 + 10\gamma_{23})$$

which is expressed in terms of the cell means as the SAS Type II hypothesis:

$$(5/24)\mu_{11} + (9/24)\mu_{12} + (10/24)\mu_{13} = (5/24)\mu_{21} + (9/24)\mu_{22} + (10/24)\mu_{23}$$

OUTPUT D.2 Form of the SAS Type II Estimable Functions

Type II Estimable Functions for: trt		
Effect	Coefficients	
Intercept	0	
trt	A	L2
	B	-L2
cntr	1	0
	2	0
	3	0
trt*cntr	A 1	0.2083*L2
	A 2	0.375*L2
	A 3	0.4167*L2
	B 1	-0.2083*L2
	B 2	-0.375*L2
	B 3	-0.4167*L2

Type III Hypotheses

The form of the Type III hypotheses for Treatment is a vector of parameter coefficients shown in Output D.3.

By using L2= 3 the hypothesis becomes

$$\mathbf{a}'\boldsymbol{\beta} = 0\mu + 3\alpha_1 - 3\alpha_2 + 0\beta_1 - 0\beta_2 + 0\beta_3 + \\ 1\gamma_{11} + 1\gamma_{12} + 1\gamma_{13} - 1\gamma_{21} - 1\gamma_{22} - 1\gamma_{23} = 0$$

Algebraic manipulation of this equation yields

$$(\mu + \alpha_1 + \beta_1 + \gamma_{11}) + (\mu + \alpha_1 + \beta_2 + \gamma_{12}) + (\mu + \alpha_1 + \beta_3 + \gamma_{13}) = \\ (\mu + \alpha_2 + \beta_1 + \gamma_{21}) + (\mu + \alpha_2 + \beta_2 + \gamma_{22}) + (\mu + \alpha_2 + \beta_3 + \gamma_{23})$$

which can be re-expressed in terms of the cell means as the SAS Type III hypothesis:

$$(1/3)(\mu_{11} + \mu_{12} + \mu_{13}) = (1/3)(\mu_{21} + \mu_{22} + \mu_{23})$$

OUTPUT D.3 Form of the SAS Type III Estimable Functions

```
Type III Estimable Functions for: trt

Effect          Coefficients

Intercept       0

trt            A      L2
                B      -L2

cntr           1      0
                2      0
                3      0

trt*cntr     A 1    0.3333*L2
                A 2    0.3333*L2
                A 3    0.3333*L2
                B 1    -0.3333*L2
                B 2    -0.3333*L2
                B 3    -0.3333*L2
```

Type IV Hypotheses

As previously mentioned, the Type IV hypotheses for Treatment are identical to the Type III hypotheses when there are no empty cells.

D.3 Empty Cells

The SAS Type III and Type IV sums of squares (SS) for Treatment are identical when each cell in the two-way layout has at least one observation. If there is an empty cell ($n_{ij} = 0$ for some $i-j$ combination), SS for Types III and IV will be the same if there are two treatment groups but different if there are more than two treatment groups (see next section). By printing the form of the Type IV parameter coefficient vector for Treatment when there is one empty cell, you see that the treatment means are unweighted averages of the cell means excluding the block that contains the empty cell.

For example, in the 2×3 example introduced in Section D.1, suppose that the $i=2$, $j=2$ cell has no observations ($n_{22}=0$). The SAS Type IV sum of squares for Treatment tests the cell means hypothesis $H_0: (\mu_{11} + \mu_{13}) = (\mu_{21} + \mu_{23})$. Notice that Center 2 does not enter into the hypothesis at all because of its incomplete data.

When there are many empty cells, the *two-way ANOVA* can result in testing hypotheses that exclude many of the cell means. Such an analysis might not be beneficial due to the differences in the hypotheses actually tested versus those planned in the design phase of the study.

One method of circumventing these difficulties is to ignore the Treatment-by-Center interaction effect as a source of variation if it can be assumed that no interaction exists, or if preliminary tests suggest that such an assumption is credible. The SAS Type I, II, III, and IV tests for the interaction effect are the same regardless of the empty cell pattern. This test for interaction, while not including all cells, can provide an indication of whether an interaction is present. If this test is not significant, the analysis might be re-run using just the main effects Treatment and Center. With no interaction in the MODEL statement, the SAS Types II, III, and IV sums of squares are identical, and each Type tests the hypothesis of equality of treatment means when the treatment means are represented by an unweighted average of the cell means for that treatment.

Sometimes, creative ways can be found to combine blocks to eliminate empty cells while retaining the advantages of blocking and ease of interpretation. For example, suppose a study that includes 10 study centers is conducted using 2 treatment groups and targets 5 patients per cell. At the end of the study, perhaps a number of empty cells exist due to attrition of patients or protocol violations leading to exclusion of data. It might be possible to combine centers by specialty, region, or some other common factor. By combining centers from the same geographic regions, the analyst can ignore Center and use Geographic Region (e.g., North, South, West, Midwest) as a new blocking factor. This technique can help eliminate the problem of empty cells by combining blocks that are alike.

If a preliminary test for interaction is significant, you might proceed immediately to treatment comparisons *within* each level of the blocking factor (e.g., Center or Geographic Region) using a *two-sample t-test* (Chapter 5) or a *one-way ANOVA* (Chapter 6). This can often be helpful in revealing subgroups of study centers (or regions) that have similar response patterns within each subgroup. In general, caution must be used in the analysis and interpretation of results when there are empty cells.

D.4 More Than Two Treatment Groups

The preceding examples apply to the often-used case of two treatment groups. When there are g ($g > 2$) treatment groups, the hypothesis of ‘no Treatment effect’ is tested by SAS using simultaneous statements of the form

$$H_0: \underline{a}_2' \underline{\beta} = \underline{a}_3' \underline{\beta} = \dots = \underline{a}_g' \underline{\beta} = 0$$

By requesting the form for the parameter coefficient vectors using the E1, E2, E3, or E4 options in the MODEL statement in PROC GLM, values denoted by L2, L3, ..., Lg appear in the SAS output. The analyst may select $g-1$ sets of values for L2 through Lg to obtain the actual coefficients that determine the simultaneous statements that comprise the hypothesis. This is demonstrated below for $g = 3$ in the unbalanced layout shown in Table D.2.

TABLE D.2 Sample Configuration for Two-Way Layout with >2 Treatments

Center	Treatment		
	A	B	C
1	μ_{11} $n_{11} = 3$	μ_{21} $n_{21} = 5$	μ_{31} $n_{31} = 4$
2	μ_{12} $n_{12} = 1$	μ_{22} $n_{22} = 2$	μ_{32} $n_{32} = 3$
3	μ_{13} $n_{13} = 3$	μ_{23} $n_{23} = 4$	μ_{33} $n_{33} = 2$
4	μ_{14} $n_{14} = 4$	μ_{24} $n_{24} = 1$	μ_{34} $n_{34} = 2$
5	μ_{15} $n_{15} = 3$	μ_{25} $n_{25} = 3$	μ_{35} $n_{35} = 2$
Overall	μ_A $n_A = 14$	μ_B $n_B = 15$	μ_C $n_C = 13$

A commonly used set of selections for the (L_2, L_3, \dots, L_g) values is $((1, 0, \dots, 0), (0, 1, \dots, 0), \dots, (0, 0, \dots, 1))$. However, that is not always the most convenient set to use, as shown for the Type I functions which follow.

For the cell sizes of Table D.2, the parameter coefficient vectors for the Types I and III hypotheses for Treatment generated by SAS are shown in Output D.4. This printout can be used to state the hypotheses in terms of cell means using a similar procedure as shown in Section D.2.

For the Type I hypotheses, two vectors (\underline{a}_2 and \underline{a}_3) are required. To get \underline{a}_2 , let $(L_2, L_3) = (182, 0)$ and for \underline{a}_3 , let $(L_2, L_3) = (0, 195)$. You can use other choices, but as you’ll see, this choice facilitates using integers (182 is the common denominator based on the sample sizes for Treatments A and C, $182=14\times13$, and 195 is based on Treatments B and C, $195=15\times13$).

**OUTPUT D.4 Forms of the Types I and III Estimable Functions in SAS
for >2 Treatment Groups**

Type I Estimable Functions for trt		
Effect	Coefficients	
Intercept	0	
trt	A	L2
trt	B	L3
trt	C	-L2-L3
center	1	-0.0934*L2+0.0256*L3
center	2	-0.1593*L2-0.0974*L3
center	3	0.0604*L2+0.1128*L3
center	4	0.1319*L2-0.0872*L3
center	5	0.0604*L2+0.0462*L3
trt*center	A 1	0.2143*L2
trt*center	A 2	0.0714*L2
trt*center	A 3	0.2143*L2
trt*center	A 4	0.2857*L2
trt*center	A 5	0.2143*L2
trt*center	B 1	0.3333*L3
trt*center	B 2	0.1333*L3
trt*center	B 3	0.2667*L3
trt*center	B 4	0.0667*L3
trt*center	B 5	0.2*L3
trt*center	C 1	-0.3077*L2-0.3077*L3
trt*center	C 2	-0.2308*L2-0.2308*L3
trt*center	C 3	-0.1538*L2-0.1538*L3
trt*center	C 4	-0.1538*L2-0.1538*L3
trt*center	C 5	-0.1538*L2-0.1538*L3

Type III Estimable Functions for: trt		
Effect	Coefficients	
Intercept	0	

trt	A	L2
	B	L3
	C	-L2-L3
cntr	1	0
	2	0
	3	0
	4	0
	5	0
trt*cntr	A 1	0.2*L2
	A 2	0.2*L2
	A 3	0.2*L2
	A 4	0.2*L2
	A 5	0.2*L2
	B 1	0.2*L3
	B 2	0.2*L3
	B 3	0.2*L3
	B 4	0.2*L3
	B 5	0.2*L3
	C 1	-0.2*L2-0.2*L3
	C 2	-0.2*L2-0.2*L3
	C 3	-0.2*L2-0.2*L3
	C 4	-0.2*L2-0.2*L3
	C 5	-0.2*L2-0.2*L3

Using Output D.4, the \underline{a}_2' vector becomes:

$$(0 \ 182 \ 0 \ -182 \ -17 \ -29 \ 11 \ 24 \ 11 \\ 39 \ 13 \ 39 \ 52 \ 39 \ 0 \ 0 \ 0 \ 0 \ -56 \ -42 \ -28 \ -28).$$

In this example, the $\underline{\beta}$ vector is:

$$\underline{\beta}' = (\mu \ \alpha_1 \ \alpha_2 \ \alpha_3 \ \beta_1 \ \beta_2 \ \beta_3 \ \beta_4 \ \beta_5 \\ \gamma_{11} \ \gamma_{12} \ \gamma_{13} \ \gamma_{14} \ \gamma_{15} \ \gamma_{21} \ \gamma_{22} \ \gamma_{23} \ \gamma_{24} \ \gamma_{25} \ \gamma_{31} \ \gamma_{32} \ \gamma_{33} \ \gamma_{34} \ \gamma_{35})$$

so that the hypothesis of $\underline{a}_2' \underline{\beta} = 0$ becomes:

$$39\alpha_1 + 13\alpha_1 + 39\alpha_1 + 52\alpha_1 + 39\alpha_1 + \\ 39\beta_1 + 13\beta_2 + 39\beta_3 + 52\beta_4 + 39\beta_5 + \\ 39\gamma_{11} + 13\gamma_{12} + 39\gamma_{13} + 52\gamma_{14} + 39\gamma_{15} = \\ 56\alpha_3 + 42\alpha_3 + 28\alpha_3 + 28\alpha_3 + 28\alpha_3 + \\ 56\beta_1 + 42\beta_2 + 28\beta_3 + 28\beta_4 + 28\beta_5 + \\ 56\gamma_{31} + 42\gamma_{32} + 28\gamma_{33} + 28\gamma_{34} + 28\gamma_{35}).$$

This can be rewritten in the form:

$$(3/14)\mu_{11} + (1/14)\mu_{12} + (3/14)\mu_{13} + (4/14)\mu_{14} + (3/14)\mu_{15} = \\ (4/13)\mu_{31} + (3/13)\mu_{32} + (2/13)\mu_{33} + (2/13)\mu_{34} + (2/13)\mu_{35}.$$

Similarly, the hypothesis of $\underline{a}_3' \underline{\beta} = 0$ becomes:

$$(5/15)\mu_{21} + (2/15)\mu_{22} + (4/15)\mu_{23} + (1/15)\mu_{24} + (3/15)\mu_{25} = \\ (4/13)\mu_{31} + (3/13)\mu_{32} + (2/13)\mu_{33} + (2/13)\mu_{34} + (2/13)\mu_{35}$$

The hypotheses being tested, therefore, are:

$$H_0: \mu_A = \mu_C \quad \text{and} \quad H_0: \mu_B = \mu_C$$

where the treatment means are the weighted averages of the cell means for that treatment, weighted by the cell sample sizes.

For the Type III hypotheses, you can start with the selection set ((1,0), (0,1)) for the (L2,L3) values, which results in the vectors \underline{a}_2 and \underline{a}_3 as follows:

$$H_0: \underline{a}_2' \underline{\beta} = 0 \Rightarrow$$

$$H_0: (1/5)\mu_{11} + (1/5)\mu_{12} + (1/5)\mu_{13} + (1/5)\mu_{14} + (1/5)\mu_{15} = \\ (1/5)\mu_{31} + (1/5)\mu_{32} + (1/5)\mu_{33} + (1/5)\mu_{34} + (1/5)\mu_{35}$$

$$H_0: \underline{\alpha}_3' \underline{\beta} = 0 \Rightarrow$$

$$H_0: (1/5)\mu_{21} + (1/5)\mu_{22} + (1/5)\mu_{23} + (1/5)\mu_{24} + (1/5)\mu_{25} = \\ (1/5)\mu_{31} + (1/5)\mu_{32} + (1/5)\mu_{33} + (1/5)\mu_{34} + (1/5)\mu_{35}$$

Taken together, the hypothesis tested by the Type III sum of squares for Treatment is the equality of treatment means, $H_0: \mu_A = \mu_B = \mu_C$, when each treatment mean is the unweighted average of the cell means for that treatment.

When there are no empty cells, the Type IV hypothesis for Treatment is the same as the Type III. However, suppose a number of empty cells exist, for example, cells 1-1, 2-4, and 3-5 (that is, $n_{11} = n_{24} = n_{35} = 0$), as shown in Table D.3.

TABLE D.3 Sample Layout with Empty Cells

Center	Treatment		
	A	B	C
1	0	5	4
2	1	2	3
3	3	4	2
4	4	0	2
5	3	3	0

The SAS Type IV sum of squares simultaneously tests the equality of pairs of treatment means when each treatment mean is an unweighted average of the cell means for that treatment, excluding any cell in a block that has one or more empty cells. The hypothesis is framed in terms of statements about the cell means as

$$H_0: \mu_{12} + \mu_{13} + \mu_{14} = \mu_{32} + \mu_{33} + \mu_{34}$$

and

$$\mu_{21} + \mu_{22} + \mu_{23} = \mu_{31} + \mu_{32} + \mu_{33}$$

The Type III hypothesis for the same configuration defines a treatment mean in terms of its cell means plus extraneous effects from other treatments, making interpretation very difficult. Therefore, the Type IV results seem to be more useful in the empty cell case with more than two treatment groups. As previously discussed, if the interaction is omitted as a source of variation from the ANOVA, the Types II, III, and IV results are the same, each testing for pure Treatment effects that are free of block effects and are not a function of the cell sizes.

D.5 Summary

Knowing the sampling plan and the reasons for design imbalance help the analyst determine the most appropriate SAS SS type to use when performing *analysis of variance*. The first step in the analysis is to request a printout from SAS of the form of the estimable functions by using the option E1 E2 E3 E4 in the MODEL statement in PROC GLM. It is then possible to specify the hypotheses tested by

each SS type. Selection of the most appropriate type can be made by most closely matching the hypothesis that is actually tested with the intended hypothesis. The Type III sum of squares, which ignores the cell sample sizes, is often used in the analysis of clinical study data under the assumption of ‘infinite’ or very large population sizes within each Block level.

The Type I tests weight the cell means in proportion to the amount of information they contribute, i.e., the cell sample sizes. The Type II tests also weight the amount of information contributed to the treatment means, but they do this by block and cell rather than just individual cells. The Type II tests for Treatment are appealing because they will always be free of block effects, regardless of the degree of imbalance. This can be seen in the example shown under “Type II Hypotheses”, where the coefficients of the block effects ($\beta_1, \beta_2, \beta_3$) are 0. The same is true of the Type III methods, which are also simple to interpret and do not depend on the sample sizes in each cell. As mentioned in Chapter 6, the Type III tests are generally the choice of clinical data analysts and should be used when the LS-means are used for estimation (LS-means are discussed in Appendix C). However, Type I or II tests are preferable if it is known that the sample sizes are proportional to the population sizes. In each analysis, the assumptions should be reviewed before automatically selecting one of the SAS Types of sum of squares.

The method for determining the hypotheses is demonstrated here for the simple 2-way layout. However, a similar technique can be used for more complex designs.

APPENDIX E

Multiple Comparison Methods

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E.1 Introduction

When you obtain a significant F-test in *ANOVA* for comparing responses among several (>2) treatment groups, your next step is to try to determine where the differences lie. Chapter 6 (“*One-Way ANOVA*”) introduces the concept of multiple comparisons by demonstrating a method of all pairwise comparisons and a method to compare each treatment with a control. These comparisons are easily carried out using the T and DUNNETT options, respectively, in the GLM procedure. Chapter 6 also shows how you can test the significance of customized linear contrasts of the treatment means using CONTRAST statements. For example, looking back at Example 6.1, you could add CONTRAST statements to the SAS code to test all pairwise comparisons as follows:

```
contrast 'Hi Dose vs. Lo Dose' dosegrp 1 -1 0;
contrast 'Hi Dose vs. Placebo' dosegrp 1 0 -1;
contrast 'Lo Dose vs. Placebo' dosegrp 0 1 -1;
```

These statements (inserted following the MODEL statement in PROC GLM) would provide the same results as if you use the LSMEANS statement:

```
lsmeans dosegrp / pdiff;
```

The CONTRAST statement, introduced with PROC GLM in Chapter 6, can also be used with other SAS procedures in a similar way, such as with PROC MIXED, PROC GENMOD or PROC GLIMMIX. The concept of pairwise testing using contrasts, regardless of whether you’re working with normal or non-normal data, is the same. Because multiple testing affects the significance levels, the use of contrasts and all pairwise comparisons via t-tests are not always the best way to go about multiple comparisons, especially as the number of treatment levels increases. In this appendix, you’ll see some examples of multiple comparison methods that are

easy to use, provide protection of the overall significance level, and offer an introduction to some of the more extensive techniques available to the statistical analyst.

E.2 Multiple Comparisons of Means

The Treatment effect is of primary concern in comparative clinical trials. When there are more than two treatment groups, say K ($K > 2$), an ‘omnibus’ approach, such as *ANOVA*, can be applied to test the overall null hypothesis of ‘no difference among the treatment groups’. If rejected, the next step is to determine which pairs of groups differ, resulting in as many as $K \cdot (K-1)/2$ possible pairwise comparisons.

The following example illustrates a number of approaches to multiple comparisons of treatment means associated with *ANOVA*.

Example E.1

Consider a parallel study that includes a placebo group, a reference group, and 3 different dose levels of an experimental treatment. The response, Y , is measured for each patient in each of the 5 groups shown in Table E.1.

TABLE E.1 Raw Data for Example E.1

Treatment Group				
A Lo-Dose	B Mid-Dose	C Hi-Dose	D Reference	E Placebo
21	27	25	23	14
13	28	19	18	17
25	31	22	22	21
18	24	24	19	20
17	20	34	24	13
23	19	26	14	23
16	18	29	20	27
12	24	28	29	12
	27	32		16
	29			

Treatment group summaries are shown in Table E.2, with a pooled standard deviation of 4.671 (see Chapter 6 for calculating formula).

TABLE E.2 Summary Statistics by Treatment Group for Example E.1

	A	B	C	D	E
Mean	18.125	24.700	26.556	21.125	18.111
SD	4.612	4.473	4.746	4.486	5.011
N	8	10	9	8	9

With 5 treatment groups, there are $(5) \cdot (4)/2 = 10$ possible pairwise comparisons. You can proceed by conducting a series of 10 *two-sample t-tests* (Chapter 5) to obtain the ‘raw’ (unadjusted) p-values for these comparisons. With the assumption of homogeneous variability across groups, the estimate of the standard deviation pooled over the 5 groups (4.671) is used in the calculation of the *t-tests*, as demonstrated in Chapter 5. Alternatively, you could use the *one-way ANOVA*, as shown next.

In SAS, pairwise *t-tests* can be performed as part of the *ANOVA* by using PROC GLM and specifying the PDIFF option in the LSMEANS statement as shown in the SAS code and output that follow. You can omit the ADJUST=T option ❶, which requests pairwise *t-tests*, because that is the default.

SAS Code for Example E.1

```

data mc;
  input trt $ y @@;
  datalines;
A 21  A 13  A 25  A 18  A 17  A 23  A 16  A 12  B 27  B 28
B 31  B 24  B 20  B 19  B 18  B 24  B 27  B 29  C 25  C 19
C 22  C 24  C 34  C 26  C 29  C 28  C 32  D 23  D 18  D 22
D 19  D 24  D 14  D 20  D 29  E 14  E 17  E 21  E 20  E 13
E 23  E 27  E 12  E 16
;
proc glm data = mc;
  class trt;
  model y = trt / ss3;
  lsmeans trt / pdiff adjust=t;           ❶
  title1 'EXAMPLE E.1: Multiple Comparisons';
  title2 'All Pairwise Comparisons of Means';
run;
quit;

```

The p-values associated with the 10 pairwise comparisons are summarized in Table E.3 based on the results shown in Output E.1.❷ (Note: The LS-means are the same as the arithmetic means for the *one-way ANOVA*). The values that are flagged with an asterisk (*) in Table E.3 indicate a value less than 0.05, the nominal significance level of each test. Because the ‘raw’ p-values are not adjusted for multiple testing, the overall ‘experimentwise’ error rate is not controlled. To maintain an overall significance level at the 0.05 level, you can use one of the methods described in the sections that follow Table E.3.

OUTPUT E.1 SAS Output with Pairwise t-Tests for Example E.1

EXAMPLE E.1: Multiple Comparisons All Pairwise Comparisons of Means							
The GLM Procedure Class Level Information							
Class		Levels	Values				
trt		5	A B C D E				
		Number of observations read 44					
		Number of observations used 44					
Dependent Variable: y							
Source		Sum of Squares		Mean Square	F Value		
Model	4	521.470707	130.367677	5.97	0.0008		
Error	39	850.961111	21.819516				
Corrected Total	43	1372.431818					
R-Square		Coeff Var	Root MSE	y Mean			
0.379961		21.34268	4.671136	21.88636			
Source		Type III SS	Mean Square	F Value	Pr > F		
trt	4	521.4707071	130.3676768	5.97	0.0008		
Least Squares Means							
trt		y	LSMEAN	Number			
2							
A		18.1250000		1			
B		24.7000000		2			
C		26.5555556		3			
D		21.1250000		4			
E		18.1111111		5			
Least Squares Means for effect trt Pr > t for H0: LSMean(i)=LSMean(j)							
Dependent Variable: y							
i/j	1	2	3	4	5		
1		0.0051	0.0006	0.2066	0.9951		
2	0.0051		0.3926	0.1147	0.0039		
3	0.0006	0.3926		0.0216	0.0004		
4	0.2066	0.1147	0.0216		0.1919		
5	0.9951	0.0039	0.0004	0.1919			
NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.							

TABLE E.3 p-Values from t-Tests for Pairwise Treatment Group Comparisons in Example E.1

Comparison	Mean Difference	'Raw' p-value
C vs. E	8.444	0.0004*
A vs. C	8.431	0.0006*
B vs. E	6.589	0.0039*
A vs. B	6.575	0.0051*
C vs. D	5.431	0.0216*
B vs. D	3.575	0.1147
D vs. E	3.014	0.1919
A vs. D	3.000	0.2066
B vs. C	1.856	0.3926
A vs. E	0.014	0.9951

The Tukey-Kramer Method

Tukey developed a test statistic for simultaneous comparisons of means, which is related to the maximum mean difference over all pairwise comparisons. This statistic has the so-called ‘studentized range’ distribution when sample sizes are the same for all groups. Probabilities associated with this distribution can be found by using numerical techniques and are tabulated in many statistical texts.

The *Tukey-Kramer* method is based on an approximation to the studentized range distribution when sample sizes differ among groups, as is usually the case in clinical trials. The SAS function PROBMC with the “RANGE” distribution can be used to obtain the adjusted p-values for all pairwise comparisons associated with the *Tukey-Kramer* approach.

Let T_{ij} represent the two-sample t-statistic for comparing the means of Groups i and j.

$$T_{ij} = \frac{\bar{x}_i - \bar{x}_j}{s \cdot \sqrt{\frac{1}{n_i} + \frac{1}{n_j}}}$$

where s is the square root of the ANOVA mean square error (MSE). The *Tukey-Kramer* p-value can be found by using a SAS statement in the form

$$P_{ij} = 1 - \text{PROBMC}(\text{"RANGE"}, \text{ABS}(\text{SQRT}(2)*T_{ij}), ., df, K)$$

where df is the number of ANOVA error degrees of freedom, and K is the number of treatment groups.

For example, to compute the *Tukey-Kramer* adjusted p-value for comparing Groups A (Lo-Dose) and B (Mid-Dose) in Example E.1, find

$$T_{AB} = (18.125 - 24.700) / 4.671((1/8) + (1/10))^{1/2} = -2.9675$$

then use SAS to obtain the adjusted p-value P_{AB} ,

```
p_ab = 1 - probmc("range", sqrt(2)*2.9675, ., 39, 5);
```

for which SAS returns a value of 0.0386.

These probabilities can also be obtained directly by using PROC GLM for all comparisons. Simply specify the ADJUST=TUKEY option in the LSMEANS statement, as follows.

```
lsmeans trt / pdiff adjust=tukey;
```

The results from this statement are shown in Output E.2.

OUTPUT E.2 Tukey-Kramer Results for Example E.1

EXAMPLE E.1: Multiple Comparisons All Pairwise Comparisons of Means					
The GLM Procedure Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer					
trt	y	LSMEAN	Number		
A		18.1250000	1		
B		24.7000000	2		
C		26.5555556	3		
D		21.1250000	4		
E		18.1111111	5		
Least Squares Means for effect TRT Pr > t for H0: LSMean(i)=LSMean(j)					
Dependent Variable: y					
i/j	1	2	3	4	5
1		0.0386	0.0054	0.7021	1.0000
2	0.0386		0.9080	0.4980	0.0300
3	0.0054	0.9080		0.1389	0.0039
4	0.7021	0.4980	0.1389		0.6759
5	1.0000	0.0300	0.0039	0.6759	

Dunnett's Test

Although the *Tukey-Kramer* method has wide appeal for all pairwise comparisons, *Dunnett's test* is the preferred method if the goal is to maintain the overall significance level when performing multiple tests to compare a set of treatment means with a control group. With K groups, you now have only K–1 comparisons.

Similar to the *Tukey-Kramer* p-values, the adjusted p-values based on a two-sided *Dunnett's test* can be obtained by using the SAS function PROBMC with the distribution "DUNNETT2", as shown below.

Let T_i represent the two-sample t-statistic (Chapter 5) for comparing the mean of Group i with the control Group mean.

$$T_i = \frac{\bar{X}_i - \bar{X}_0}{s \cdot \sqrt{\frac{1}{n_i} + \frac{1}{n_0}}}$$

where s is the square root of the ANOVA mean square error (MSE) and the subscript 0 refers to the control group. The *Dunnett* p-value can be found by using a SAS statement of the form

$$p_i = 1 - \text{probmc}(\text{"dunnett2"}, \text{abs}(t_i), ., df, K-1, L_1, L_2, \dots, L_{K-1})$$

where df is the number of ANOVA error degrees of freedom, K is the number of treatment groups (including the control group), and the L_i 's are distributional parameters used to adjust for unequal sample sizes, defined as

$$L_i = \sqrt{\frac{n_i}{n_i + n_0}}$$

For example, to compute *Dunnett's* adjusted p-value for comparing Group B (Middle-Dose) with Group E (Placebo) in Example E.1, find

$$T_B = (24.700 - 18.111) / 4.671((1/10) + (1/9))^{1/2} = 3.0701$$

and

$$L_1 = (8/(8+9))^{1/2} = 0.6860$$

$$L_2 = (10/(10+9))^{1/2} = 0.7255$$

$$L_3 = (9/(9+9))^{1/2} = 0.7071$$

$$L_4 = (8/(8+9))^{1/2} = 0.6860$$

then, evaluate

```
p_b=1-probmc("dunnett2",3.0701,.39,4,  
0.6860,0.7255,0.7071,0.6860);
```

to obtain the adjusted p-value, P_B , for which SAS returns a value of 0.0138.

These probabilities can be easily obtained for all comparisons by using PROC GLM. You must use the CONTROL option to identify Group E as the control group, and specify the ADJUST=DUNNETT option in the LSMEANS statement, as follows:

```
lsmeans trt / pdiff=control('E') adjust=dunnett;
```

The results from this statement are shown in Output E.3.

OUTPUT E.3 Dunnett's Results for Example E.1

EXAMPLE E.1: Multiple Comparisons Treatment Comparisons vs. Control Group (trt=E)		
The GLM Procedure Least Squares Means Adjustment for Multiple Comparisons: Dunnett		
trt	y LSMEAN	H0:LSMean= Control Pr > t
A	18.1250000	1.0000
B	24.7000000	0.0138
C	26.5555556	0.0017
D	21.1250000	0.4901
E	18.1111111	

p-Value Adjustment Methods

Both the *Tukey-Kramer* and *Dunnett* methods rely on numerical integration to evaluate complex probability distributions and require the assumption of normality. They are robust statistical methods that have good power and enjoy widespread usage in statistical programs, including SAS. You can also use a number of other methods that are easy to use and control the overall significance levels of the test. The *Bonferroni*, the *Sidak*, the step-down *Bonferroni* (Holm), and the step-up *Bonferroni* (Hochberg) methods, for example, are all useful, easily applied multiple comparison techniques. These methods attempt to control the overall significance level by adjusting p-values without requiring the evaluation of complex distributions. They can be used in making pairwise comparisons of means but also have a much wider application. However, while versatile and very easy to compute, these methods are generally conservative and might fail to detect important differences.

The adjusted p-values for the 10 pairwise comparisons of Example E.1 are shown in Table E.4 based on the methods listed in the preceding paragraph, in addition to the results previously obtained by using the *Tukey-Kramer* and *Dunnett* methods.

TABLE E.4 Adjusted p-Values for Example E.1

(i) Comparison	t-Test (‘Raw’)	Tukey- Kramer	Dunnett	Bonferroni	Sidak	Step-Down Bonferroni	Step-Up Bonferroni
	p _{RAW}	p _{TUK}	p _{DUN}	p _{BON}	p _{SID}	p _{STPDWN}	p _{STPUP}
(1) C v. E	0.0004*	0.0039*	0.0017*	0.004*	0.004*	0.004*	0.004*
(2) A v. C	0.0006*	0.0054*		0.006*	0.006*	0.005*	0.005*
(3) B v. E	0.0039*	0.0300*	0.0138*	0.039*	0.038*	0.031*	0.031*
(4) A v. B	0.0051*	0.0386*		0.051	0.050*	0.036*	0.036*
(5) C v. D	0.0216*	0.1389		0.216	0.196	0.130	0.130
(6) B v. D	0.1147	0.4980		1.000	0.704	0.574	0.574
(7) D v. E	0.1919	0.6759	0.4901	1.000	0.881	0.768	0.620
(8) A v. D	0.2066	0.7021		1.000	0.901	0.768	0.620
(9) B v. C	0.3926	0.9080		1.000	0.993	0.785	0.785
(10) A v. E	0.9951	1.0000	1.0000	1.000	1.000	0.995	0.995

The adjusted p-values using the *Bonferroni* and *Sidak* methods, are found from the raw p-values by using the formulas,

$$p_{BON} = m \cdot p_{RAW}$$

and

$$p_{SID} = 1 - (1 - p_{RAW})^m$$

where m is the number of comparisons and p_{RAW} is the raw (unadjusted) p-value. Values greater than 1.0 are truncated to 1.0. For example, adjusted p-values for Comparison 5 (C v. D) in Table E.4 are

$$p_{BON} = 10(0.0216) = 0.216 \text{ and } p_{SID} = 1 - (1 - 0.0216)^{10} = 0.1962$$

The *Bonferroni* step-down method adjusts the raw p-values in a stepwise fashion starting with the smallest. Comparison (1) is adjusted as $p_{STPDWN}(1) = m \cdot p_{RAW}(1)$ or $10(0.0004) = 0.004$. The next smallest raw p-value, Comparison (2), is adjusted as $p_{STPDWN}(2) = (m-1)p_{RAW}(2) = 9(0.0006) = 0.0054$. Comparison (3) is adjusted as $p_{STPDWN}(3) = (m-2)p_{RAW} = 8(0.0039) = 0.0312$, and so on.

Any adjusted p-value calculated to be greater than 1 is set to 1.0. Also, all comparisons that follow the first non-significant comparison must be non-significant, and an adjusted p-value is set equal to its predecessor if its calculated value is less. Therefore for Comparison (8), the adjusted p-value is calculated to be $3(0.2066) = 0.6198$, which is less than the preceding adjusted p-value (0.7676), so

the Comparison 8 p-value is set to 0.7676. The *Bonferroni* step-down method is sometimes referred to as Holm's method.

The *Bonferroni* step-up-method adjusts the raw p-values in a stepwise fashion, but it starts with the largest value. For Comparison (10) in Table E.4., you compute $p_{STPUP}(10)=1 \cdot p_{RAW}(10)=0.9951$. The next largest value, Comparison (9), is adjusted as $p_{STPUP}(9)=2 \cdot p_{RAW}(9)=2(0.3926)=0.7852$. Comparison (8) is adjusted as $p_{STPUP}(8)=3 \cdot p_{RAW}(8)=3(0.2066)=0.6198$, and so on. Each adjusted p-value must be no greater than its predecessor. Those values that are greater are set equal to the preceding adjusted p-value. The *Bonferroni* step-up method is also known as Hochberg's method.

Adjusted p-values using the *Bonferroni*, *Sidak*, and *Bonferroni* stepwise methods can be found by using PROC MULTTEST in SAS as shown below with the MC data set of Example E.1. The BONFERRONI, SIDAK, HOLM, and HOC options are specified in the PROC MULTTEST statement, and the results are written to the data set NEWMC ❸. The treatment group (TRT) is specified as the grouping variable in a CLASS statement ❹, and the MEAN option in the TEST statement ❺ identifies the response variable (Y) for which comparisons of means are to be performed. One CONTRAST statement is used for each pairwise comparison.

You see very similar results between the *Bonferroni* step-down and step-up methods for Example E.1 (stpbon_p and hoc_p, respectively, in Output E.4). However, the step-up method is actually more powerful and, in many cases, will find more significant comparisons, but it must be used with caution because the step-up method assumes that the raw p-values are mutually independent. Notice also that the *Bonferroni* step-down and step-up methods produce results that are very comparable to the *Tukey-Kramer* method for Example E.1.

Although the *Bonferroni* and *Sidak* methods are very easy to use, they are not recommended for the primary analysis in confirmatory or pivotal clinical trials because of their overly conservative nature.

SAS Code for Example E.1 (continued)

```
proc multtest bonferroni sidak holm hoc
  noprint out=newmc data=mc; ❸
  class trt; ❹
  test mean(y);
    contrast 'A v B' -1 1 0 0 0;
    contrast 'A v C' -1 0 1 0 0;
    contrast 'A v D' -1 0 0 1 0;
    contrast 'A v E' -1 0 0 0 1;
    contrast 'B v C' 0 -1 1 0 0;
    contrast 'B v D' 0 -1 0 1 0;
    contrast 'B v E' 0 -1 0 0 1;
    contrast 'C v D' 0 0 -1 1 0;
    contrast 'C v E' 0 0 -1 0 1;
    contrast 'D v E' 0 0 0 -1 1;
run;
```

```
proc print data=newmc;
  title1 'EXAMPLE E.1: Multiple Comparisons';
  title2 'p-Value Adjustment Procedures';
run;
```

OUTPUT E.4 p-Values for Pairwise Treatment Comparisons

The *Dunnett*'s p-values are not directly comparable to the other p-values in Table E.4 because they are testing different hypotheses. The *Dunnett* p-values are included to show how the values change when the alternative hypothesis is changed from "at least one pair of means differs" to "at least one treatment mean differs from the control mean". Adjusted p-values can be found for the latter hypothesis by using the *Bonferroni* and *Sidak* methods in the same way that is described for the all pairwise comparisons method. For example, when restricting attention only to the treatment versus control comparisons, the *Sidak* adjusted p-value for the B v. E comparison would be $1 - (1 - 0.0039)^4 = 0.0155$.

PROC GLIMMIX, available with SAS 9.2 and later, can also be used to obtain most of the adjusted p-values shown above, and others. A less conservative method available under PROC GLIMMIX is a stepdown procedure due to Shaffer, which uses logical constraints, found with the STEPDOWN(TYPE=LOGICAL) option in the LSMEANS statement. For Example E.1, use the following code:

```
proc glimmix data = mc;
   class trt;
   lsmeans trt / diff stepdown(type=logical);
run;
```

The STEPDOWN option in the LSMEANS statement without the TYPE option provides the raw p-values (p_{RAW}) along with the stepdown-adjusted p-values (p_{STPDWN}) shown in Table E.4.

'Closed' Testing

Multiple testing of individual null hypotheses that control for the overall significance level can be performed by using a principle called 'closure'. This concept is discussed in depth with good examples in Chi (1998) and Bauer (1991). Here, the concept is introduced for the case of $K=3$ (3 groups) and $m=3$ pairwise comparisons.

Under the principle of closure, each of the 3 pairwise comparisons can be conducted at the full α (0.05) level with no p-value adjustments, but only if the omnibus hypothesis (i.e., 'all groups equal') is first rejected at the α level. This method has great utility in the analysis of clinical trials data because many studies include 3 treatment groups. Pairwise comparisons of the mean responses among the 3 groups can be performed with *t-tests* for example, each at the 0.05 level of significance following a significant *F-test* when *analysis of variance* is used. If the Treatment effect is not significant based on the *ANOVA F-test*, each pairwise comparison must be declared non-significant under closed testing in order to maintain the experimentwise error rate at 0.05.

The principle of closed testing can become complex when the number of treatment levels gets large. You can find further details and examples of the closure principle in *Multiple Comparisons and Multiple Tests Using SAS* by Westfall, Tobias, Rom, Wolfinger, and Hochberg.

E.3 Multiple Comparisons of Binomial Proportions

Because the p-value adjustment methods discussed in the previous section are based only on the raw p-values and not the underlying distribution of response data, you can use these same methods for a wide range of applications, including those with non-normal data (e.g., categorical responses and time-to-event data). When responses are binomial, you can use methodology based on a set of raw p-values from a series of *chi-square tests*, *Fisher's exact tests*, *Cochran-Mantel-Haenszel tests*, and many other methods to adjust for multiple comparisons of group proportions, such as response rates among treatments. In fact, PROC MULTTEST in SAS can be executed from an input list of raw p-values, as shown next.

The comparison identification must be in the variable named TEST, and the raw (input) p-values must be values of the variable named RAW_P. The output shows the same adjusted p-values as previously obtained. Notice that in this program, no TEST or CLASS statements are used with PROC MULTTEST. Also, these adjusted p-values are not as accurate as those shown in Output E.4 because the raw p-values are entered using only 4-decimal place accuracy.

```

data adjst;
  input test raw_p @@;
  datalines;
1 0.0004  2 0.0006  3 0.0039  4 0.0051  5 0.0216
6 0.1147  7 0.1919  8 0.2066  9 0.3926 10 0.9951
;
proc multtest bonferroni sidak holm hoc pdata = adjst;
  title 'DATA SET ADJST';
run;

```

OUTPUT E.5 p-Value Adjustments Using PROC MULTTEST for Example E.1

DATA SET ADJST						
The Multtest Procedure						
Test	Raw	p-Values				
		Stepdown				
1	0.0004	0.0040	0.0040	0.0040	0.0040	0.0040
2	0.0006	0.0060	0.0054	0.0060	0.0054	0.0054
3	0.0039	0.0390	0.0312	0.0383	0.0312	0.0312
4	0.0051	0.0510	0.0357	0.0498	0.0357	0.0357
5	0.0216	0.2160	0.1296	0.1962	0.1296	0.1296
6	0.1147	1.0000	0.5735	0.7043	0.5735	0.5735
7	0.1919	1.0000	0.7676	0.8812	0.6198	0.6198
8	0.2066	1.0000	0.7676	0.9012	0.6198	0.6198
9	0.3926	1.0000	0.7852	0.9932	0.7852	0.7852
10	0.9951	1.0000	0.9951	1.0000	0.9951	0.9951

Beginning with SAS 9.2, you can use the LSMEANS statement of PROC GLIMMIX to perform multiple comparisons on proportions directly without having to first compute the p-values for use with PROC MULTTEST (see PROC GLIMMIX in the *SAS/STAT User's Guide*).

Resampling Methods

'Resampling' is another method that can be used for performing multiple comparisons with binomial data. This method uses a randomization principle developed by Fisher and requires the use of a computer program. In SAS, you can use PROC MULTTEST to perform 'permutation' resampling, as shown in Example E.2. In essence, the algorithm randomly selects, with replacement, a sample of all possible permutations of outcomes assuming the null hypothesis is true. The adjusted p-value is the proportion of those samples that have outcomes more extreme than the observed outcome.

◆ Example E.2

Suppose a binomial response ('response' or 'non-response') is measured on a new sample of 168 patients randomized to the 5-group parallel study given in Example E.1, with summary results as follows.

TABLE E.5 Response Rates for Example E.2

	A	B	C	D	E
	Lo-Dose	Mid-Dose	Hi-Dose	Reference	Placebo
N	33	31	34	35	35
Responders	9	14	23	10	5
Non-Responders	24	17	11	25	30
Response Rate	27.3%	45.2%	67.6%	28.6%	14.3%

Use PROC MULTTEST to perform permutation resampling and compare the results with other p-value adjustment methods.

SAS Code for Example E.2

```
data rr;
    input trt $ resp count @@;
    /* resp=0 is "non-response", resp=1 is "response" */
    datalines;
A 0 24    A 1  9    B 0 17    B 1 14
C 0 11    C 1 23    D 0 25    D 1 10
E 0 30    E 1  5
;

ods select
    Multtest.pValues;
proc multtest order = data permutation
    nsample=20000 seed=28375 data = rr;          ⑦
    /* (prespecify the seed value so results can be duplicated) */
    class trt;
    test fisher(resp);                         ⑥
    freq count;
        contrast 'A vs B' -1  1  0  0  0;
        contrast 'A vs C' -1  0  1  0  0;
        contrast 'A vs D' -1  0  0  1  0;
        contrast 'A vs E' -1  0  0  0  1;
        contrast 'B vs C'  0  -1  1  0  0;
        contrast 'B vs D'  0  -1  0  1  0;
        contrast 'B vs E'  0  -1  0  0  1;
        contrast 'C vs D'  0  0  -1  1  0;
        contrast 'C vs E'  0  0  -1  0  1;
        contrast 'D vs E'  0  0  0  -1  1;
title1 'EXAMPLE E.2: Multiple Comparisons of Proportions';
title2 'Permutation Resampling Method';
run;
```

The ‘raw’ p-values are the values that result from unadjusted pairwise comparisons using two-tailed *Fisher’s exact tests* (Chapter 17). The values are found directly by using PROC MULTTEST with the FISHER option in the TEST statement ⑥, which eliminates the need to run PROC FREQ. A resampling size of 20,000 is requested, and a random seeding value is provided ⑦. (Note that with SAS 9.2, the random number generator has been changed to the “Mersenne Twister”. To get the results that you would get with versions prior to SAS 9.2, specify the RANUNI option in PROC MULTTEST.) If no value is specified for SEED, SAS randomly selects a value, which results in slightly different adjusted p-values each time the program is run.

OUTPUT E.6 Resampling Method Using PROC MULTTEST for Example E.2

EXAMPLE E.2: Multiple Comparisons of Proportions
Permutation Resampling Method

The Multtest Procedure

p-Values

Variable	Contrast	Raw	Permutation
resp	A vs B	0.1932	0.5430
resp	A vs C	0.0014	0.0078
resp	A vs D	1.0000	1.0000
resp	A vs E	0.2365	0.7024
resp	B vs C	0.0831	0.3625
resp	B vs D	0.2038	0.5914
resp	B vs E	0.0072	0.0404
resp	C vs D	0.0017	0.0088
resp	C vs E	<.0001	<.0001
resp	D vs E	0.2436	0.7024

For comparison, compute adjusted p-values by using the *Bonferroni*, *Sidak*, step-down *Bonferroni*, and step-up *Bonferroni* methods from the raw p-values based on *Fisher’s exact test* (Output E.6).

```
data binp;
    input test raw_p @@;
    datalines;
1 0.1932  2 0.0014  3 1.0000  4 0.2365  5 0.0831
6 0.2038  7 0.0072  8 0.0017  9 0.0001 10 0.2436
;

proc multtest bonferroni sidak holm hoc pdata = binp;
run;
```

PROC MULTTEST finds the raw p-values with the pdata option. SAS will look for the ‘raw_p’ variable in the data set named by pdata (in this case binp) for the p-values. The p-values from the SAS output are reproduced in Table E.6, which compares the adjusted p-values for the permutation resampling method with the methods previously described. Notice that permutation resampling results in 4 significant comparisons ($p < 0.05$), while each of the other methods find only 3 that are significant. Resampling techniques are preferred, when possible, because they generally have greater power than the other methods previously discussed.

In the past, application of resampling methods has been limited by the huge computing resources required, but they are gaining in use with the increasing power of desktop computing. One disadvantage of resampling is that the adjusted p-values will differ slightly with each implementation. When using PROC MULTTEST, results can be duplicated by specifying the same value for SEED each time you run the program.

TABLE E.6 Adjusted p-Values for Example E.2

Comparison	Fisher's ('Raw')	Permutation Resampling	Bonferroni	Sidak	Stepdown Bonferroni	Stepup Bonferroni
C v. E	0.0001*	0.0001*	0.001*	0.001*	0.001*	0.001*
A v. C	0.0014*	0.0080*	0.014*	0.014*	0.013*	0.013*
C v. D	0.0017*	0.0093*	0.017*	0.0170*	0.014*	0.014*
B v. E	0.0072*	0.0397*	0.072	0.070	0.050	0.050
B v. C	0.0831	0.3666	0.831	0.580	0.499	0.487
A v. B	0.1932	0.5530	1.000	0.883	0.966	0.487
B v. D	0.2038	0.6039	1.000	0.898	0.966	0.487
A v. E	0.2365	0.7095	1.000	0.933	0.966	0.487
D v. E	0.2436	0.7095	1.000	0.939	0.966	0.487
A v. D	1.0000	1.0000	1.000	1.000	1.000	1.000

The adjusted p-values found by PROC MULTTEST are based on the entire set of CONTRAST statements used. To compare each treatment group with the control, use the same SAS statements as shown in the SAS Code for Example E.2, and replace the ten CONTRAST statements with the following set of four statements:

```
contrast 'A vs E' -1 0 0 0 1;
contrast 'B vs E' 0 -1 0 0 1;
contrast 'C vs E' 0 0 -1 0 1;
contrast 'D vs E' 0 0 0 -1 1;
```

E.4 Summary

This introduction to multiple comparisons illustrates just some of the many methods available for conducting multiple comparisons for hypothesis testing. Many of the same methods can be used when addressing the multiplicity problem caused by simultaneous analysis of multiple response variables, such as that which occurs with multiple primary endpoints or study objectives. Adjustments based on methods discussed here can also be implemented for simultaneous confidence intervals.

The *Tukey-Kramer* and *Dunnett*'s methods presented in this section using *one-way ANOVA* (Chapter 6) are widely used when response data have a normal distribution. These methods can also be used with more complex models, such as *two-way ANOVA* (Chapter 7), *repeated measures ANOVA* (Chapter 8), and *analysis of covariance* (Chapter 11), although these methods can be somewhat more conservative with these models.

You have also seen how to adjust the raw pairwise p-values to maintain a pre-specified, overall α -level without requiring *ANOVA* assumptions. The p-value adjustment methods discussed here can be used for pairwise treatment comparisons when response data do not follow the normal distribution. This approach can be applied to most of the methods discussed in this book that involve comparisons of more than two treatment groups.

In addition, resampling techniques and simulation methods are gaining greater attention with the increasing power of low-cost computing.

The field of multiple comparisons is a broad and still evolving field. Entire books have been written and professional conferences are held, about this one subject. Standard tests of the past, such as *Duncan*'s test, have been supplanted by more powerful methods. 'Stepwise' methods can be used with the *Tukey*, *Dunnett*, and *Sidak* methods, re-sampling methods, and tests under the closure principle. Improvement in power often results by implementing stepwise versions of the methods discussed in this chapter.

Note: For a more detailed discussion of multiple comparisons with SAS examples, refer to the excellent book, *Multiple Comparisons and Multiple Tests Using SAS*, by Westfall, Tobias, Rom, and Wolfinger, which was written under the SAS Press program. The multiple testing issue in clinical trials is also discussed thoroughly in *Analysis of Clinical Trials Using SAS: A Practical Guide*, by Dmitrienko, Molenberghs, Chuang-Stein, and Offen, also a SAS Press book.

APPENDIX F

Data Transformations

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F.1 Introduction

Many statistical tests discussed in this book, including the *two-sample t-test* (Chapter 5) and *ANOVA* (Chapters 6, 7, and 8) are performed under the assumption of normally distributed data with variance homogeneity among groups. Although such tests are known to be robust against mild deviations to these assumptions, especially with larger sample sizes, clinical data are often encountered that appear to have skewed distributions or larger variability within certain groups. The underlying distribution in such tests might actually be non-normal, or there might be a small proportion of the data called ‘outliers’ that occur outside the range of usual expectation under the normality assumption, for known or unknown reasons.

Data transformations can often be used effectively in normalizing the data and/or stabilizing the variability. The logarithmic, square root, arcsine, and rank transformations are among the most frequently used data transformations in applied statistics. The square root and arcsine transformations are infrequently used in clinical statistics, however. Converting the observations to their ranks before analysis is one of the most common methods of transforming the data (see Chapters 12, 13, and 14). The logarithmic ('log') transformation is also frequently used, and its application is discussed in the next section.

Log transformations are commonly used in analyzing many types of clinical research data. One example is in vaccine and immunogenicity studies in which antibody titer values are measured. These values (x_i 's) are usually modeled with the log-normal distribution, and results are summarized in terms of the geometric mean titer and the geometric mean ratio (or ‘n-fold increase’). Another common application of the log-transformation is in the analysis of area-under-the-curve (AUC) data based on blood levels from bioavailability and pharmacokinetic studies.

F.2 The Log Transformation

■ The Log-Normal Distribution

If the logarithm of a response, Y , denoted as $\log(Y)$, has a normal distribution, the random variable Y is said to come from a ‘log-normal’ distribution. The log-normal distribution looks like a normal distribution (see Chapter 1 or Figure B.1 in Appendix B) with its left tail compressed and its right tail stretched out due to a small number of very large values. (This is also called a ‘skewed’ distribution). It is appropriate to perform the log transformation on clinical data prior to analysis if the data come from a log-normal distribution. More generally, the log transformation is often used to ‘improve’ the normality of data sets that have any type of skewed distribution as described above.

■ What Is a Logarithm?

A logarithm has an associated base, b . The base b logarithm of X is expressed as $\log_b X$. A logarithm is the inverse function of exponentiation. That is, if $Y = \log_b X$, then $X = b^Y$. When not specified, b is usually 10. Most often, b is selected to be e (“Euler’s constant”). In fact, this selection is so common, the base e logarithm has a special name, the ‘natural logarithm’, which is abbreviated as ‘ \ln ’. Thus, $\ln(X) = \log_e(X)$, which implies $X = e^Y$ (also denoted, $X = \exp(Y)$). Only the natural logs are referred to in the discussion that follows.

■ Analyzing Data Using the Log Transformation

Following transformation, analyses are carried out using an ANOVA on the transformed data, and summary statistics are computed in the usual way. These statistics can be expressed in terms of the original data by using the relationships discussed next.

Observe the response measures x_1, x_2, \dots, x_n . Apply the log transformation as $y_i = \ln(x_i)$, and conduct analyses as usual on the y_i ’s. Let \bar{Y} and s_y denote the mean and standard deviation of the y_i ’s, and let L_Y and U_Y represent the lower and upper limits of the $100(1-\alpha)\%$ confidence interval, found by

$$\bar{Y} \pm t_{\alpha/2} \cdot s_{\bar{Y}}$$

that is,

$$L_Y = \bar{Y} - t_{\alpha/2} \cdot s_{\bar{Y}} \quad \text{and} \quad U_Y = \bar{Y} + t_{\alpha/2} \cdot s_{\bar{Y}}$$

A $100(1-\alpha)\%$ confidence interval based on the original data (x_i ’s) is found by exponentiating L_Y and U_Y .

$$(e^{L_Y} - e^{U_Y})$$

Because

$$\bar{Y} = \frac{\sum y_i}{n} = \sum \frac{\ln(x_i)}{n} = \ln\left(\prod x_i\right)^{\frac{1}{n}}$$

you see that $\exp(\bar{Y})$ is simply the geometric mean of the x_i 's (denoted by \bar{X}^g). This represents the ‘geometric mean titer’ or GMT used in immunogenicity studies. You can make inferences on this GMT by noting that the standard error of $\exp(\bar{Y})$ may be approximated with a second-order Taylor series expansion as

$$SE(e^{\bar{Y}}) = \frac{e^{\bar{Y}} \cdot s_y}{\sqrt{n}}$$

Finally, let Δ represent the difference between two treatment means, say, A and B,

$$\Delta = \bar{Y}_A - \bar{Y}_B$$

This difference is easily converted to the “geometric mean ratio” by exponentiating

$$e^\Delta = e^{\bar{Y}_A - \bar{Y}_B} = \frac{\bar{X}_A^g}{\bar{X}_B^g}$$

Notice that the transformation does not substantively change the hypothesis that is being tested. The hypothesis of no treatment difference is equivalent to the hypothesis that the ratio of treatment means is 1.

Similarly, changes from a baseline value, expressed as $\Delta = \bar{Y} - \bar{Y}_0$, can be converted to a percent change in geometric means as $100 \cdot (e^\Delta - 1)\%$.

APPENDIX G

SAS Code for Exercises in Chapter 23

SAS Code for Exercises (see Chapter 23)

```
libname examp 'c:\bookfiles\examples\sas';
options nodate nonumber ls=85 ps=55;

*=====
|   STATISTICAL ANALYSES for Response Variable = "score"|
|       (continuous numeric response variable)           |
*=====;

/* ANALYSIS #1: Two-Sample t-Test */
proc ttest data = examp.trial;
  class trt;
  var score;
  title 'ANALYSIS #1: Two-Sample t-Test';
run;

/* ANALYSIS #2: Wilcoxon Rank-Sum Test */
proc nparlway wilcoxon data = examp.trial;
  class trt;
  var score;
  title 'ANALYSIS #2: Wilcoxon Rank-Sum Test';
run;

/* ANALYSIS #3: One-Way ANOVA */
proc glm data = examp.trial;
  class trt;
  model score = trt;
  means trt;
  title 'ANALYSIS #3: One-Way ANOVA';
run;
```

```

/* ANALYSIS #4: Two-Way ANOVA with Interaction */
proc glm data = examp.trial;
  class trt center;
  model score = trt center trt*center;
  lsmeans trt / stderr pdiff;
  title 'ANALYSIS #4: Two-Way ANOVA With Interaction';
run;
quit;

/* ANALYSIS #5: Two-Way ANOVA without Interaction */
proc glm data = examp.trial;
  class trt center;
  model score = trt center;
  title 'ANALYSIS #5: Two-Way ANOVA Omitting Interaction';
run;
quit;

/* ANALYSIS #6: Three-Way ANOVA, with All Interactions */
proc glm data = examp.trial;
  class trt center sex;
  model score = trt | center | sex;
  lsmeans trt / stderr pdiff;
  title 'ANALYSIS #6: Three-Way ANOVA - all Interactions';
run;
quit;

/* ANALYSIS #7: Three-Way Main Effects ANOVA */
proc glm data = examp.trial;
  class trt center sex;
  model score = trt center sex;
  title 'ANALYSIS #7: Three-Way Main Effects ANOVA';
run;
quit;

/* ANALYSIS #8: Regression of Score on Age */
ods graphics on;
/* score by age plot found in "fitplot" ods graph*/
proc glm data = examp.trial;
  model score = age / solution;
  title 'Linear Regression of Score on Age';
run;
quit;
ods graphics off;

/* ANALYSIS #9: Analysis of Covariance - Test for Equal Slopes */
proc glm data = examp.trial;
  class trt;
  model score = trt age trt*age;
  /* use interaction to check for equal slopes */
  title1 'ANALYSIS #9: ANCOVA Using Age as Covariate,';
  title2 'Test for Equal Slopes';
run;
quit;

```

```

/* ANALYSIS #10: Analysis of Covariance - Assuming Equal Slopes */
ods graphics on;
/* plot of score by age for each treatment
   found in "ancovaplot" ODS graph*/
proc glm data = examp.trial;
  class trt;
  model score = trt age / solution;
  lsmeans trt / stderr pdiff;
  title 'ANALYSIS #10: ANCOVA Using Age as Covariate';
run;
quit;
ods graphics off;

/* ANALYSIS #11: Stratified ANCOVA */
proc glm data = examp.trial;
  class trt center;
  model score = trt center age / solution;
  title1 'ANALYSIS #11: ANCOVA -- using Age as covariate,';
  title2 'Stratified by Center';
run;
quit;

*=====
|   STATISTICAL ANALYSES for Response Variable = "resp"  |
|          (dichotomous response variable)                  |
*=====; ;

/* format used in ANALYSES 12-14 */
proc format;
  value rspfmt 0 = 'NO' 1 = 'YES';
run;

/* ANALYSES #12 and #13: Chi-Square and Fishers Exact Test
   -- Dichotomous Response */
proc freq data = examp.trial;
  tables trt*resp / chisq nocol nopct;
  format resp rspfmt. ;
  title1 'ANALYSIS #12: Chi-Square Test';
  title2 'ANALYSIS #13: Fishers Exact Test';
run;

/* ANALYSIS #14: Stratified CMH Test for Response Rate Analysis */
proc freq data = examp.trial;
  tables center*trt*resp / cmh nocol nopct;
  format resp rspfmt. ;
  title 'ANALYSIS #14: CMH Test Controlling for Center';
run;

```

```

/* ANALYSIS #15: Logistic Regression - Dichotomous Response */
proc logistic data = examp.trial;
  class trt / param = ref;
  model resp = trt;
  title1 'ANALYSIS #15: Logistic Regression Analysis for';
  title2 'Treatment Group Differences Using Dichotomized
Response';
run;

/* ANALYSIS #16: Logistic Regression - Dichotomous Response,
   Adjusted for Age */
proc logistic data = examp.trial;
  class trt / param = ref;
  model resp = trt age;
  title1 'ANALYSIS #16: Logistic Regression Analysis for';
  title2 'Treatment Group Differences Using Dichotomized
Response';
  title3 'Adjusted for Age';
run;

/* ANALYSIS #17: Logistic Regression - Dichotomous Response,
   Adjusted for Age, Sex, Center */
proc logistic data = examp.trial;
  class trt sex center / param = ref;
  model resp = trt age sex center trt*center;
  title1 'ANALYSIS #17: Logistic Regression Analysis for';
  title2 'Treatment Group Differences Using Dichotomized
Response';
  title3 'Adjusted for Age, Sex, Center & Interactions';
run;

*=====
|   STATISTICAL ANALYSES for Response Variable = "sev"   |
|           (ordinal categorical response variable)      |
*===== ; 

/* Obtain Severity Distributions based on the Response = "sev" */

/* formats used for ANALYSES 18, 19, 20, 21, 23, and 24 */
proc format;
  value sev1fmt  0 = '0 = None'        1 = '1 = Mild'
                2 = '2 = Mod '       3 = '3 = Sev '       ;
  
  value sev2fmt  0 = '0 = None'      ' 1 = '1.036 = Mild'
                2 = '2.185 = Mod '  ' 3 = '3 = Sev      ' ;
  
  value sev3fmt  0 = 'None'          1 = 'Mild'
                2 = 'Mod.'          3 = 'Sev.'         ;
run;

```

```

/* ANALYSES #18 and #19: Chi-Square / Fishers Exact Test --
   Multinomial Response */
proc freq data = examp.trial;
   tables trt*sev / chisq exact nocol nopct;
   format sev sev1fmt.;
   title1 'ANALYSIS #18: Chi-Square Test';
   title2 'ANALYSIS #19: Generalized Fishers Exact Test';
run;

/* ANALYSIS #20: CMH Test for Comparing Severity Distributions -
   Using Table Scores */
proc freq data = examp.trial;
   tables trt*sev / cmh nocol nopct;
   format sev sev1fmt.;
   title1 'ANALYSIS #20: Mantel-Haenszel Test on Severity';
   title2 '(Using Table Scores)';
run;

/* ANALYSIS #21: CMH Test for Comparing Severity Distributions
   - Using Modified Ridit Scores */
proc freq data = examp.trial;
   tables trt*sev / cmh scores = modridit nocol nopct;
   format sev sev2fmt.;
   title1 'ANALYSIS #21: Mantel-Haenszel Test on Severity';
   title2 '(Using Modified Ridit Scores)';
run;

/* ANALYSES #22: Wilcoxon Rank-Sum Test on Response = "SEV" */
proc nparlway wilcoxon data = examp.trial;
   class trt;
   var sev;
   title 'ANALYSIS #22: Wilcoxon Rank-Sum Test on "SEV"
Response';
run;

/* ANALYSIS #23: Stratified CMH Test for Comparing Severity
   Distributions - Using Table Scores */
proc freq data = examp.trial;
   tables center*trt*sev / cmh nocol nopct;
   format sev sev3fmt.;
   title1 'ANALYSIS #23: CMH Test on Severity Distributions';
   title2 '(Using Table Scores), Controlling for Center';
run;

```

```

/* ANALYSIS #24: Stratified CMH Test for Comparing Severity
   Distributions - Using Modified Ridit Scores */
proc freq data = examp.trial;
   tables center*trt*sev / cmh scores = modridit nocol nopct;
   format sev sev3fmt.;
   title1 'ANALYSIS #24: CMH Test on Severity Distributions';
   title2 '(Using Modified Ridit Scores), Controlling for
Center';
run;

/* ANALYSIS #25: Logistic Regression: Proportional Odds Model */
proc logistic data = examp.trial;
   class trt / param = ref;
   model sev = trt;
   title1 'ANALYSIS #25: Proportional Odds Model for Treatment';
   title2 'Differences Using Ordinal Response Categories';
run;

/* ANALYSIS #26: Logistic Regression: Proportional Odds Model,
   Adjusted for Age */
proc logistic data = examp.trial;
   class trt / param = ref;
   model sev = trt age;
   title1 'ANALYSIS #26: Proportional Odds Model for Treatment';
   title2 'Differences Using Ordinal Response Categories';
   title3 'Adjusted for Age';
run;

/* ANALYSIS #27: Logistic Regression: Proportional Odds Model,
   Adjusted for Age, Sex, Center */
proc logistic data = examp.trial;
   class trt sex center;
   model sev = trt age sex center;
   title1 'ANALYSIS #27: Proportional Odds Model for Treatment';
   title2 'Group Differences Using Ordinal Response Categories';
   title3 'Adjusted for Age, Sex & Center';
run;

```

APPENDIX H

Finding Adjusted Alphas for Interim Analyses—SAS Examples

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H.5	Lan-DeMets α-Spending Function	506

H.1 Introduction

Beginning with SAS 9.2, the procedures SEQDESIGN and SEQTEST can be used to help design and analyze interim clinical investigations. In the examples discussed in Section 2.6, you may use PROC SEQDESIGN to obtain the adjusted alphas by using the appropriate options and parameters. You may also obtain the stopping boundaries using the same procedure. The SEQTEST procedure is used in conjunction with SEQDESIGN to conduct the testing at the interim stages. Normally, the statistician uses PROC SEQDESIGN to establish the stopping boundaries, and then apply PROC SEQTEST to conduct the test.

Chapter 2 presents an introduction to interim analyses under the discussion of the multiple testing problem and shows how conducting interim evaluations can affect the overall alpha levels. In that context, adjusted alphas are presented rather than use of stopping boundaries and other approaches to sequential analyses. This appendix shows how to obtain the adjusted alpha values given in Chapter 2, Tables 2.4 through 2.7 using SAS. For details of the SAS code and obtaining stopping boundaries using PROC SEQDESIGN and PROC SEQTEST, please refer to the *SAS/STAT User's Guide*.

H.2 Pocock's Approach

The following SAS code uses PROC SEQDESIGN to replicate the adjusted significance levels, α_P , found in Table 2.4 of Chapter 2. This example only produces the reduced alpha level when there are five analyses. You can obtain the results shown in Table 2.4 for two through four analyses by changing the value of NSTAGES in the SAS code ❶. The adjusted alpha (0.0158) corresponding to Table 2.4 for five analyses is found under “Pocock Reduced Alpha” in Output H.1 ❷.

SAS Code for Example H.I

```
title "Pocock's Approach with Five Analyses";
proc seqdesign boundaryscale=pvalue;
    TwoSidedPocock: design method=poc nstages=5 ①
        alt=twosided stop=reject;
    ods output boundary=bv;
run;

data bv;
    set bv;
    reduced_alpha=2*bound_la;
run;

proc print data=bv label;
var bound_la bound_ua reduced_alpha;
label bound_la      = "Boundary Lower Alpha"
      bound_ua      = "Boundary Upper Alpha"
      reduced_alpha = "Pocock Reduced Alpha";
run;
```

OUTPUT H.I SAS Output of Pocock's Approach for Example H.I

```
Pocock's Approach with Five Analyses

The SEQDESIGN Procedure
Design: TwoSidedPocock

Design Information

Statistic Distribution          Normal
Boundary Scale                 P-Value
Alternative Hypothesis        Two-Sided
Early Stop                     Reject Null
Method                          Pocock
Boundary Key                   Both
Number of Stages                5
Alpha                           0.05
Beta                            0.1
Power                           0.9
Max Information (Percent of Fixed Sample) 120.6603
Null Ref ASN (Percent of Fixed Sample)   117.6742
Alt. Ref ASN (Percent of Fixed Sample)   68.49131
```

OUTPUT H.1 SAS Output of Pocock's Approach for Example H.1
(continued)

Pocock's Approach with Five Analyses							
The SEQDESIGN Procedure							
Design: TwoSidedPocock							
Method Information							
-----Unified Family-----							
Boundary	Method	Alpha	Beta	Rho	Tau	C	Drift
Upper Alpha	Pocock	0.02500	0.10000	0	0	2.41318	3.560656
Lower Alpha	Pocock	0.02500	0.10000	0	0	2.41318	-3.56066
Boundary Information (P-Value Scale)							
Null Reference = 0							
-Information Level-		-----Alternative-----		----Boundary Values----			
Stage		-----Reference-----		---Lower--		---Upper--	
1	0.2000	Lower Upper		Alpha Alpha		Alpha Alpha	
2	0.4000	-1.59237 1.59237		0.00791 0.99209		0.00791 0.99209	
3	0.6000	-2.25196 2.25196		0.00791 0.99209		0.00791 0.99209	
4	0.8000	-2.75807 2.75807		0.00791 0.99209		0.00791 0.99209	
5	1.0000	-3.18475 3.18475		0.00791 0.99209		0.00791 0.99209	
Boundary Lower Boundary Upper Pocock Reduced							
Obs		Boundary Lower Boundary Alpha		Pocock Reduced Alpha		②	
1		0.00791 0.99209		0.015814 0.015814			
2		0.00791 0.99209		0.015814 0.015814			
3		0.00791 0.99209		0.015814 0.015814			
4		0.00791 0.99209		0.015814 0.015814			
5		0.00791 0.99209		0.015814 0.015814			

H.3 O'Brien-Fleming Approach

The following SAS code uses PROC SEQDESIGN to replicate the progressively increasing alpha levels, α_{OF} , found in Table 2.5 of Chapter 2. Here again, NSTAGES is set to 5 corresponding to 5 analyses ③. You can obtain the entries in Table 2.5 for two through four analyses by changing the value of NSTAGES in the SAS code. Output H.2 shows the adjusted alphas under “O’Brien-Fleming Reduced Alpha” ④.

SAS Code for Example H.2

```
title "O'Brien-Fleming Approach with Five Analyses";
proc seqdesign boundaryscale=pvalue;
    TwoSidedOBrienFleming: design method=obf nstages=5 ③
        alt=twosided stop=reject;
    ods output boundary=bv;
run;

data bv;
    set bv;
    reduced_alpha=2*bound_la;
run;

proc print data=bv label;
var bound_la bound_ua reduced_alpha;
label bound_la      = "Boundary Lower Alpha"
      bound_ua      = "Boundary Upper Alpha"
      reduced_alpha = "O'Brien-Fleming Reduced Alpha";
run;
```

OUTPUT H.2 SAS Output of O'Brien-Flemming Approach for Example H.2

O'Brien-Fleming Approach with Five Analyses							
The SEQDESIGN Procedure							
Design: TwoSidedOBrienFleming							
Design Information							
Statistic Distribution							
Boundary Scale							Normal
Alternative Hypothesis							P-Value
Early Stop							Two-Sided
Method							Reject Null
Boundary Key							O'Brien-Fleming
Number of Stages							Both
Alpha							5
Beta							0.05
Power							0.1
Max Information (Percent of Fixed Sample)							0.9
Null Ref ASN (Percent of Fixed Sample)							102.6486
Alt. Ref ASN (Percent of Fixed Sample)							101.9146
							75.0254
Method Information							
-----Unified Family-----							
Boundary	Method	Alpha	Beta	Rho	Tau	C	Drift
Upper Alpha	O'Brien-Fleming	0.02500	0.10000	0.5	0 2.	04009	3.284189
Lower Alpha	O'Brien-Fleming	0.02500	0.10000	0.5	0 2.	04009	-3.2841

OUTPUT H.2. SAS Output of O'Brien-Flemming Approach for Example H.2 (continued)

O'Brien-Fleming Approach with Five Analyses

The SEQDESIGN Procedure

Design: TwoSidedOBrienFleming

Boundary Information (P-Value Scale)

Null Reference = 0

Stage	Information Level	Proportion	--Alternative----		----Boundary Values--	
			Reference	Lower	Upper	Alpha
1		0.2000	-1.46873	1.46873	2.53612E-6	1.00000
2		0.4000	-2.07710	2.07710	0.0006284	0.99937
3		0.6000	-2.54392	2.54392	0.00422	0.99578
4		0.8000	-2.93747	2.93747	0.01128	0.98872
5		1.0000	-3.28419	3.28419	0.02067	0.97933

Obs	O'Brien-Fleming Reduced			
	Boundary		Boundary	Fleming
	Lower	Upper	Reduced	
Alpha	Alpha	Alpha	Alpha	④
1	2.53612E-6	1.00000	0.000005	
2	0.0006284	0.99937	0.001257	
3	0.00422	0.99578	0.008445	
4	0.01128	0.98872	0.022555	
5	0.02067	0.97933	0.041341	

H.4 Lan-DeMets Cumulative α -Spending Function

The Lan-DeMets cumulative alpha-spending values shown in Table 2.6 can be obtained from SAS using the SEQDESIGN procedure, as shown in the following code. Notice that the information fraction may be entered manually as an option in PROC SEQDESIGN ⑤. The results shown in Table 2.6 can be found under “Cumulative Alpha” ⑥ in Output H.3.

SAS Code for Example H.3

```

title "Cumulative Lan-DeMets Alpha Spending Function for the";
title2 "O'Brien-Fleming Spending Function, Two-Tailed Test
       at Alpha=0.05";
proc seqdesign errspend boundaryscale=pvalue;
  TwoSidedErrorSpending: design method=errfuncobf
    nstages=14 alpha=0.05
    alt=twosided stop=reject
    info=cum(0.1 0.2 0.25 0.3 0.333
              0.4 0.5 0.6 0.667
              0.7 0.75 0.8 0.9 1.0); 5
  ods output errspend=es;
run;

```

```

data es;
  set es;
  cumulative_alpha = alpha_l + alpha_u;
run;

proc print data=es label;
var alpha_l alpha_u cumulative_alpha;
label alpha_l = "Cumulative Alpha Lower"
      alpha_u = "Cumulative Alpha Upper"
      cumulative_alpha = "Cumulative Alpha";
run;

```

OUTPUT H.3 Lan-DeMets Cumulative α -Spending for Example H.3

Cumulative Lan-DeMets Alpha Spending Function for the
O'Brien-Fleming Spending Function, Two-Tailed Test at Alpha=0.05

The SEQDESIGN Procedure
Design: TwoSidedErrorSpending

Design Information

Statistic Distribution	Normal
Boundary Scale	P-Value
Alternative Hypothesis	Two-Sided
Early Stop	Reject Null
Method	Error Spending
Boundary Key	Both
Number of Stages	14
Alpha	0.05
Beta	0.1
Power	0.9
Max Information (Percent of Fixed Sample)	103.6638
Null Ref ASN (Percent of Fixed Sample)	102.7219
Alt. Ref ASN (Percent of Fixed Sample)	71.48972

Method Information

----Error Spending----						
Boundary	Method	Alpha	Beta	Function	Drift	
Upper Alpha	Error Spending	0.02500	0.10000	Approx. O'Brien-Fleming	3.300377	
Lower Alpha	Error Spending	0.02500	0.10000	Approx. O'Brien-Fleming	-3.30038	

OUTPUT H.3 Lan-DeMets Cumulative α -Spending for Example H.3 (continued)

Cumulative Lan-DeMets Alpha Spending Function for the
O'Brien-Fleming Spending Function, Two-Tailed Test at Alpha=0.05

The SEQDESIGN Procedure
Design: TwoSidedErrorSpending

Boundary Information (P-Value Scale)
Null Reference = 0

Stage	Information Level Proportion	Alternative		Boundary Values	
		Reference Lower	Upper	Lower Alpha	Upper Alpha
1	0.1000	-1.04367	1.04367	1.3612E-12	1.00000
2	0.2000	-1.47597	1.47597	5.38871E-7	1.00000
3	0.2500	-1.65019	1.65019	7.09249E-6	0.99999
4	0.3000	-1.80769	1.80769	0.0000396	0.99996
5	0.3330	-1.90452	1.90452	0.0000899	0.99991
6	0.4000	-2.08734	2.08734	0.0003593	0.99964
7	0.5000	-2.33372	2.33372	0.00139	0.99861
8	0.6000	-2.55646	2.55646	0.00331	0.99669
9	0.6670	-2.69542	2.69542	0.00482	0.99518
10	0.7000	-2.76129	2.76129	0.00531	0.99469
11	0.7500	-2.85821	2.85821	0.00702	0.99298
12	0.8000	-2.95195	2.95195	0.00874	0.99126
13	0.9000	-3.13101	3.13101	0.01379	0.98621
14	1.0000	-3.30038	3.30038	0.01861	0.98139

Error Spending Information

Stage	Information Level Proportion	Cumulative Error Spending			
		Lower Alpha	Beta	Beta	Upper Alpha
1	0.1000	0.00000	0.00000	0.00000	0.00000
2	0.2000	0.00000	0.00000	0.00000	0.00000
3	0.2500	0.00001	0.00000	0.00000	0.00001
4	0.3000	0.00004	0.00000	0.00000	0.00004
5	0.3330	0.00010	0.00000	0.00000	0.00010
6	0.4000	0.00039	0.00000	0.00000	0.00039
7	0.5000	0.00153	0.00000	0.00000	0.00153
8	0.6000	0.00381	0.00000	0.00000	0.00381
9	0.6670	0.00606	0.00000	0.00000	0.00606
10	0.7000	0.00738	0.00000	0.00000	0.00738
11	0.7500	0.00965	0.00000	0.00000	0.00965
12	0.8000	0.01221	0.00000	0.00000	0.01221
13	0.9000	0.01814	0.00000	0.00000	0.01814
14	1.0000	0.02500	0.10000	0.10000	0.02500

OUTPUT H.3 Lan-DeMets Cumulative α -Spending for Example H.3 (continued)

Cumulative Lan-DeMets Alpha Spending Function for the O'Brien-Fleming Spending Function, Two-Tailed Test at Alpha=0.05			
The SEQDESIGN Procedure			
Design: TwoSidedErrorSpending			
Obs	Cumulative Alpha Lower	Cumulative Alpha Upper	Cumulative Alpha 6
1	0.00000	0.00000	0.000000
2	0.00000	0.00000	0.000000
3	0.00001	0.00001	0.000015
4	0.00004	0.00004	0.000084
5	0.00010	0.00010	0.000206
6	0.00039	0.00039	0.000789
7	0.00153	0.00153	0.003053
8	0.00381	0.00381	0.007615
9	0.00606	0.00606	0.012122
10	0.00738	0.00738	0.014769
11	0.00965	0.00965	0.019299
12	0.01221	0.01221	0.024424
13	0.01814	0.01814	0.036289
14	0.02500	0.02500	0.050000

H.5 Lan-DeMets α -Spending Function

The following SAS code uses PROC SEQDESIGN to show how you can use SAS to replicate the Lan-DeMets alpha levels, α_{LD} , using the O'Brien-Fleming spending function, as summarized in Table 2.7 of Chapter 2. Note that this example produces only the alpha level when there are five analyses **7**, as determined by the NSTAGES option. You can obtain results shown in Table 2.7 for two through four analyses by changing the value of NSTAGES in the SAS code. SAS can produce alpha levels for several other error spending methods by changing the METHOD option (see SAS documentation for details). Look under “Lan-DeMets Reduced Alpha” **8** in Output H.4 for the reduced alpha levels.

SAS Code for Example H.4

```
title "Lan-DeMets Alpha Using O'Brien-Fleming Spending
Function";
proc seqdesign boundaryscale=pvalue;
  TwoSidedErrorSpending: design
    method=errfuncobf
    nstages=5 7
    alt=twosided
    stop=reject
    alpha=0.05
  ;
  ods output boundary=bv;
run;
```

```

data bv; set bv;
    reduced_alpha=2*bound_la;
run;
proc print data=bv label;
var bound_la bound_ua reduced_alpha;
label bound_la      = "Boundary Lower Alpha"
      bound_ua     = "Boundary Upper Alpha"
      reduced_alpha = "Lan-DeMets Reduced Alpha";
run;

```

OUTPUT H.4 Lan-DeMets α -Spending for Example H.4

Lan-DeMets Alpha Using O'Brien-Fleming Spending Function

The SEQDESIGN Procedure
Design: TwoSidedErrorSpending

Design Information

Statistic Distribution		Normal
Boundary Scale		P-Value
Alternative Hypothesis		Two-Sided
Early Stop		Reject Null
Method		Error Spending
Boundary Key		Both
Number of Stages		5
Alpha		0.05
Beta		0.1
Power		0.9
Max Information (Percent of Fixed Sample)		102.3077
Null Ref ASN (Percent of Fixed Sample)		101.636
Alt. Ref ASN (Percent of Fixed Sample)		75.86658

Method Information

Boundary	Method	----Error Spending----				Drift
		Alpha	Beta	Function		
Upper Alpha	Error Spending	0.02500	0.10000	Approx. O'Brien-Fleming	3.278713	
Lower Alpha	Error Spending	0.02500	0.10000	Approx. O'Brien-Fleming	-3.27871	

Boundary Information (P-Value Scale)

Null Reference = 0

Stage	-Information Level-	-----Alternative-----		----Boundary Values----		
		-----Reference-----		---Lower--	---Upper--	
		Proportion	Lower	Upper	Alpha	Alpha
1		0.2000	-1.46629	1.46629	5.38871E-7	1.00000
2		0.4000	-2.07364	2.07364	0.0003939	0.99961
3		0.6000	-2.53968	2.53968	0.00368	0.99632
4		0.8000	-2.93257	2.93257	0.01102	0.98898
5		1.0000	-3.27871	3.27871	0.02113	0.97887

Obs	Boundary	Boundary	Lan-DeMets
	Lower	Upper	Reduced
1	5.38871E-7	1.00000	0.000001
2	0.0003939	0.99961	0.000788
3	0.00368	0.99632	0.007357
4	0.01102	0.98898	0.022031
5	0.02113	0.97887	0.042252

APPENDIX I

Commonly Used Parameterizations in Logistic Regression Models

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I.1 Introduction

There are several ways in which a statistical model may be written. In a logistic regression model (Chapter 20), you might include numeric covariates (either continuous or ordinal) and nominal categorical factors. Generally, you will have one parameter (β_i) associated with each numeric covariate (X_i). For a nominal covariate (or any effect used in a CLASS statement in SAS), the number of parameters depends on your model specification or ‘parameterization’ as well as the number of levels of the effect. Here, we look at two of the most commonly used parameterizations, the ‘reference’ and the ‘effect’ parameterizations for nominal main effects models, and how to interpret the SAS output in each case.

I.2 The ‘Reference’ Parameterization

Examples 20.1–20.3 in Chapter 20 all use a ‘reference’ parameterization, specified by the PARAM=REF option in the CLASS statement of PROC LOGISTIC. This means that model estimates for each level of the nominal factor are incremental to that of a specified reference level. If there are m levels of a main effect X , there would be $m-1$ parameters associated with the incremental effects over the reference.

In Output 20.1 for Example 20.1, a portion of which is replicated in Output I.1, the Intercept estimate of 2.6135 refers to the reference value of the Group effect, namely PBO (recall that PBO was designated as the reference level). Since

Group has only two levels ($m=2$), there is one parameter ($m-1$) for the group factor, with estimate of -1.1191. This is the incremental effect for the active group (ACT) relative to the placebo group (PBO), so that the estimate for the active group would be $2.6135 - 1.1191 = 1.4944$.

SAS sets up a design matrix consisting of x values for the numeric covariates and dummy values for the classification factors. For the reference parameterization, the dummy codes of 0 and 1 are used in the design matrix, as seen under “Class Level Information” in the output below. The effect associated with a level of the class variable is the intercept plus the parameter estimate for that level times the corresponding design variable.

OUTPUT I.I Selected SAS Output for Example 20.I – Reference Parameterization

Class Level Information					
Class	Value	Design Variables			
group	ACT			1	
	PBO			0	
Analysis of Maximum Likelihood Estimates					
Parameter	DF	Estimate	Standard Error	Chi-Square	Pr > ChiSq
Intercept	1	2.6135	0.7149	13.3662	0.0003
group ACT	1	-1.1191	0.4669	5.7446	0.0165
x	1	-0.1998	0.0560	12.7187	0.0004
Odds Ratio Estimates					
Effect		Point Estimate	95% Wald Confidence Limits		
Group ACT vs PBO		0.327	0.131	0.815	
x		0.819	0.734	0.914	

Based on the parameter estimates, you can write the estimated logit function as

$$\text{logit} = (2.6135 - 1.1191 \cdot \text{group}_{(\text{ACT})} - 0.1998 \cdot x)$$

Substituting the design variables of 1 and 0 in for group, you get

$$\begin{aligned}\text{logit}_{(\text{ACT})} &= (2.6135 - 1.1191 \cdot (1) - 0.1998 \cdot x) \\ &= 1.4944 - 0.1998 \cdot x\end{aligned}$$

and

$$\begin{aligned}\text{logit}_{(\text{PBO})} &= (2.6135 - 1.1191 \cdot (0) - 0.1998 \cdot x) \\ &= 2.6135 - 0.1998 \cdot x\end{aligned}$$

The odds ratio for ACT vs. PBO is found by exponentiating the incremental effect of ACT over PBO, namely -1.1191, so that the odds ratio is $e^{-1.1191} = 0.327$.

I.3 The ‘Effect’ Parameterization

Another popular parameterization is the ‘deviation from the mean’ or ‘effect’ parameterization, specified with the PARAM=EFFECT option in SAS (this is the default in PROC LOGISTIC). The estimates for this parameterization for each level of group are incremental to the overall mean effect. The data for Example 20.1 is rerun using the effect parameterization with the following SAS code:

```
proc logistic data = aml;
  class group(ref='PBO') / param = effect;
  model relapse(event='YES') = group x;
run;
```

The relevant output is shown in Output I.2 below.

OUTPUT I.2 Selected SAS Output for Example 20.1 – Effect Parameterization

Class Level Information					
Class	Value	Design Variables			
group	ACT	1			
	PBO	-1			
Analysis of Maximum Likelihood Estimates					
Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	2.0539	0.5967	11.8477	0.0006
group ACT	1	-0.5595	0.2335	5.7446	0.0165
x	1	-0.1998	0.0560	12.7187	0.0004

In the case of two levels for the class variable, as in this example, the design structure assigns dummy variables of +1 and -1 associated with the levels (ACT and PBO).

For this parameterization, you can write the estimated logit function as

$$\text{logit}_{(\text{ACT})} = (2.0539 - 0.5595 \cdot \text{group}_{(\text{ACT})} - 0.1998 \cdot x)$$

Substituting the design variables in for group, you get

$$\begin{aligned}\text{logit}_{(\text{ACT})} &= (2.0539 - 0.5595 \cdot (+1) - 0.1998 \cdot x) \\ &= 1.4944 - 0.1998 \cdot x\end{aligned}$$

and

$$\begin{aligned}\text{logit}_{(\text{PBO})} &= (2.0539 - 0.5595 \cdot (-1) - 0.1998 \cdot x) \\ &= 2.6134 - 0.1998 \cdot x\end{aligned}$$

While both parameterizations yield the same estimated logistic regression equations, the calculations using the parameter estimates are slightly different. The odds ratio for ACT vs. PBO is found by exponentiating the incremental effect of ACT over PBO, namely $1.4944 - 2.6134 = -1.1191$, again yielding the odds ratio for Group of 0.327.

I.4 More Than Two Nominal Levels

If there are more than two nominal levels, you compare each level with the reference level when using the reference parameterization. To illustrate this, we alter the data in Example 20.1 so that patients with a Patient Number of 70 or higher are assigned to an agent already being marketed, so there are now three treatment groups: active, marketed, and placebo, or more briefly, three levels of the nominal group factor: ACT, MKT, and PBO. You get Output I.3 by running PROC LOGISTIC, again using PBO as the reference group, with the reference parameterization.

The estimated logit function is

$$\text{logit} = (2.7086 - 1.6081 \cdot \text{group}_{(\text{ACT})} - 1.2924 \cdot \text{group}_{(\text{MKT})} - 0.1656 \cdot x)$$

Notice there are now two dummy variables for each level of group under “Design Variables”. Substituting these values for group you get

$$\begin{aligned}\text{logit}_{(\text{ACT})} &= (2.7086 - 1.6081 \cdot (1) - 1.2924 \cdot (0) - 0.1656 \cdot x) \\ &= 1.1005 - 0.1656 \cdot x\end{aligned}$$

$$\begin{aligned}\text{logit}_{(\text{MKT})} &= (2.7086 - 1.6081 \cdot (0) - 1.2924 \cdot (1) - 0.1656 \cdot x) \\ &= 1.4162 - 0.1656 \cdot x\end{aligned}$$

and

$$\begin{aligned}\text{logit}_{(\text{PBO})} &= (2.7086 - 1.6081 \cdot (0) - 1.2924 \cdot (0) - 0.1656 \cdot x) \\ &= 2.7086 - 0.1656 \cdot x\end{aligned}$$

OUTPUT I.3 Selected SAS Output for Example 20.I Altered to Have 3 Group Levels – Reference Parameterization

Class Level Information			
Parameter	DF	Estimate	Standard Error
Intercept	1	2.7086	0.7023
group ACT	1	-1.6081	0.5613
group MKT	1	-1.2924	0.5666
x	1	-0.1656	0.0557

Analysis of Maximum Likelihood Estimates			
Effect	Point Estimate	95% Wald Confidence Limits	Wald Chi-Square
group ACT vs PBO	0.200	0.067 0.602	14.8742
group MKT vs PBO	0.275	0.090 0.834	8.2069
x	0.847	0.760 0.945	5.2034

The odds ratio for ACT vs. PBO is found by exponentiating the incremental effect of ACT over PBO, namely $1.1005 - 2.7086 = -1.6081$, yielding the odds ratio of $e^{-1.6081} = 0.200$. The odds ratio for MKT vs. PBO is found similarly as $e^{(1.4162-2.7086)} = e^{-1.2924} = 0.275$. Note that when using the reference parameterization, the odds ratio can easily be confirmed by exponentiating the parameter estimate printed by SAS.

Now we repeat this same analysis using effect parameterization, which produces the results indicated in Output I.4.

OUTPUT I.4 Selected SAS Output for Example 20.I Altered to Have 3 Group Levels – Effect Parameterization

Class Level Information			
Class	Value	Design Variables	
group	ACT	1	0
	MKT	0	1
	PBO	-1	-1

Analysis of Maximum Likelihood Estimates					
Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	1.7418	0.5880	8.7760	0.0031
group ACT	1	-0.6413	0.3145	4.1570	0.0415
group MKT	1	-0.3256	0.3176	1.0506	0.3054
x	1	-0.1656	0.0557	8.8444	0.0029

Odds Ratio Estimates			
Effect	Point Estimate	95% Wald Confidence Limits	
group ACT vs PBO	0.200	0.067	0.602
group MKT vs PBO	0.275	0.090	0.834
x	0.847	0.760	0.945

The estimated logit function is

$$\text{logit} = (1.7418 - 0.6413 \cdot \text{group}_{(\text{ACT})} - 0.3256 \cdot \text{group}_{(\text{MKT})} - 0.1656 \cdot x)$$

The dummy variables are now +1, 0, and -1, as shown under “Class Level Information”. Substituting these values for group you get

$$\begin{aligned}\text{logit}_{(\text{ACT})} &= (1.7418 - 0.6413 \cdot (1) - 0.3256 \cdot (0) - 0.1656 \cdot x) \\ &= 1.1005 - 0.1656 \cdot x\end{aligned}$$

$$\begin{aligned}\text{logit}_{(\text{MKT})} &= (1.7418 - 0.6413 \cdot (0) - 0.3256 \cdot (1) - 0.1656 \cdot x) \\ &= 1.4162 - 0.1656 \cdot x\end{aligned}$$

and

$$\begin{aligned}\text{logit}_{(\text{PBO})} &= (1.7418 - 0.6413 \cdot (-1) - 0.3256 \cdot (-1) - 0.1656 \cdot x) \\ &= 2.7086 - 0.1656 \cdot x\end{aligned}$$

These estimated logistic regression equations under the effect parameterization are the same as those obtained using the reference parameterization, as are the odds ratios.

I.5 Other Parameterizations

When interactions are included in the model for the nominal factors, the analysis is a bit more complex, but the process of how to use the parameter estimates given in the SAS output is the same. While the reference and effect parameterizations are the most commonly used, many other types of parameterizations are also available in SAS ('ordinal', 'GLM', 'polynomial', and variations of these based on orthogonal coding). Please refer to the *SAS/STAT User's Guide* for details. Note that parameterization issues introduced here are not unique to logistic regression but also apply to other modeling procedures such as ANOVA, ANCOVA, and Cox Regression.

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