

Sample Size Determination from A Pilot Bioequivalence study for A Future Pivotal Bioequivalence Study: A SAS Procedure

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Abstract

The purpose of research study develops an automated computer statistical procedure to determine sample size from a pilot bioequivalence study for a future pivotal bioequivalence study. The sample size is critical in a pivotal bioavailability/ bioequivalence study, and was often estimated from a pilot study. A comprehensive review of current literature reveals that the software for sample size determination using data from a pilot study has not been reported (D. Inouye, Bull. Ecol. Soc. Am., 1997). A series computer code (SAS) which contains four macro-driven programs has been developed to perform the following steps: 1) The statistical analysis of a two-period crossover pilot bioequivalence study according to the FDA guidance 'Statistical procedures for bioequivalence studies using a standard two-treatment crossover design' and the paper by Sauter et al (Int. J. Clin. Pharmacol. Ther. Toxicol. 30:233-56(1992); 2) Consider $\alpha=0.05$ and the bioequivalence range of 90% confidence intervals (80%, 125%), the sample size was determined for a future pivotal BA/BE study according to the table generated by Diletti et al (int. J. Clin. Pharmacol. Ther. Toxicol. 29:1-8 (1991); 3) Using the estimated ratio of geometric means (μ_A/μ_B), MSS, MSE from step 1) and the sample size n from step 2), a new set of data were generated based on the error model described by Jones and Kenward (Design and Analysis of Cross-Over Trials, pp16-88, Chapman &Hall, New York, 1989); 4) The same statistical analysis procedure in step 1) was performed on the new data set to confirm whether so calculated sample size would warrant that the confidence intervals of ratio of geometric means falls within 80% - 125%. The results of this research study showed that the sample size was determined from the developed procedure readily. The results also showed that such a sample size warranted that the confidence intervals of ratio of geometric means falls within 80% - 125% in a simulated future pivotal bioequivalence trial. It is concluded that the applications of this computer procedure with real data from pilot bioequivalence studies were successfully demonstrated. The outcomes of this study has significant implications in clinical trials.

Introduction

Registration of a generic formulation requires a demonstration of bioequivalence with the accepted reference formulation of the same drug. As recommended by the FDA (1) and international harmonization of regulatory (2), a single dose, randomized, open label, two-way crossover design in a clinical phase I trial has been mostly used to demonstrate the bioequivalence. Three pharmacokinetic parameters, C_{max} , AUC_{0-t} , and AUC_{0-inf} , are used to determine the bioequivalence. Bioequivalence with respect to one of above mentioned three pharmacokinetic parameters is concluded if the 90% confidence intervals falls within the range of 80% to 125%. The 90% confidence intervals are obtained from the antilogs of the lower and upper bounds of the 90% confidence interval for the difference in the means of the log-transformed parameter.

To demonstrate the bioequivalence, the sample size, i.e, the number of subjects completed in the clinical trial, is critical. An inadequate small sample size would yield a low statistical power, and therefore, unreliable and wider confidence intervals. While an inadequate large sample size would be costly, and make the recruiting nearly impossible. An adequate sample size can be determined if intrasubject coefficient of variations, mean ratio, and the desired statistical power were known using the method proposed by Diletti et al (3).

Therefore, in common practice, two sequential clinical trials are often conducted for bioequivalence determination. The first one, called a pilot study, is used to determine the intrasubject coefficient of variations and mean ratio, therefore, to determine the sample size. The second one called a pivotal study, is used to demonstrate the bioequivalence using the appropriate sample size determined by the pilot study.

A comprehensive review of the current literature on software related to statistical power and sample size determination revealed that the software to determine the sample size using the data from a pilot study for log-transformed parameter has not been reported (4). The purpose of this research study is to develop an automated computer procedure using SAS, which can estimate the mean ratio, inter- and intra-subject variations from a pilot study, and can determine the sample size for a future pivotal study.

Statistical Methods

1.1. Analysis of a pilot study

The statistical analysis of a two-period crossover pilot bioequivalence study is performed according to the FDA guidance 'Statistical procedures for bioequivalence studies using a standard two-treatment crossover design' (2) and the paper by Sauter et al (5). In brief, a parametric (normal-theory) general linear model (GLM procedure) or Proc Mixed procedure was applied to the logarithmic transformation of C_{max} , AUC_{0-t} , and AUC_{0-inf} . However, using Proc Mixed procedure has certain advantages over GLM model in that it handles the data with missing values or unbalanced data better. The analysis of variance (ANOVA) model included the following factors: Subjects, sequence, subjects within sequence, period, and model can be expressed in matrix notation

$$Y = XB + e$$

Where y is an $n \times 1$ vector of dependent variables, X is an $n \times k$ design matrix, B is a $k \times 1$ vector of parameters to be estimated, and e is an $n \times 1$ vector of unknown/random errors.

The two one-sided hypotheses were tested at the 5% level by constructing 90% confidence intervals for the parameter values. The estimate for ratio of geometric means was as (6)

$$(\mu_A/\mu_B) = \exp (LS \text{ Mean } A - LS \text{ Mean } B) \quad [1]$$

The estimate for confidence intervals was calculated as (6)

$$\text{Lower limit} = \exp (LS \text{ Mean} - LS \text{ Mean} B - (T_{0.05} MSE)).....[2]$$

$$\text{Upper limit} = \exp (LS \text{ Mean} - LS \text{ Mean} B + (T_{0.05} MSE)).....[3]$$

Where for equations [1-3], LS Mean = least square mean for log-transformed parameters; A = Test formulation and B = Reference formulation; $T_{0.05}$ = t value at 0.05% level; MSE = Mean Square Error.

LS Mean A, LS Mean B, and MSE can be calculated from ANOVA analysis, and $T_{0.05}$ can be found in the student t-test table.

The intrasubject and intersubject variations were estimated using the formula proposed by Hauschke et al (7)

Intrasubject coefficient of variation is defined as

$$CV\varepsilon = \sqrt{\exp(\sigma^2_{\varepsilon}) - 1}.....[4]$$

and estimated by

$$CV\varepsilon = \sqrt{\exp(MSE) - 1}.....[5]$$

Intrasubject coefficient of variation is defined as

$$CV_s = \sqrt{\exp(\sigma^2_s) - 1}.....[6]$$

And estimated by

$$CV_s = \sqrt{\exp[(MSS - MSE)/2] - 1}.....[7]$$

Where MSS = Mean square error for subject within sequence.

Consider $\alpha=0.05$, the bioequivalence range (0.80, 1.25), and the desired statistical power (70%, 80%, or 90%), then the sample size was determined for a future pivotal BA/BE study according to the table generated by Diletti et al (3).

Three log-transformed parameters ($C_{\max}$, AUC_{0-t} , and $AUC_{0-inf.}$) were analyzed, resulting in three values of sample size. The maximum of these three sample sizes was chosen, so that the 90% confidence intervals for all these three parameters would fall into 80% to 125%.

Using the estimated ratio of geometric means ($\mu A/\mu B$), MSS , MSE and the sample size n , a new set of data can be generated.

1.2. Simulation of a pivotal study

Based on the error model described by Jones and Kenward (8), the procedure to generate a new set of data for a future pivotal study was described as follows.

For Treatment B:

$$Y_{ab} = \sqrt{(MSS - MSE)/2} * R_{annor}....[8]$$

$$Y_{B, K} = Y_{B, mean} + Y_{ab} + \sqrt{MSE} * R_{annor}.....[9]$$

For Treatment A:

$$Y_{A, K} = Y_{B, mean} + Y_{ab} + \ln(\mu_A/\mu_B) + \sqrt{MSE} * R_{annor}.....[10]$$

Where $Y_{B, mean}$ represents the mean value of $\ln(C_{max})$, $\ln(AUC_{0-t})$, or $\ln(AUC_{0-inf})$ of the Kth subject for Treatment A (test formulation); K is from 1 to n.

Two groups with equal sample size (n/2) were selected randomly, with one group using treatment sequence AB, and the other using treatment BA.

For simplicity, the sequence and period effects in this error model were assumed not significant, and there was no carry-over effect.

Using the same statistical analysis procedure, the ratio of geometric means (untransformed), 90% confidence intervals, cvs, cve, MSS, and MSE were computed to confirm whether so calculated sample size would warrant that the ratio of geometric means falls within 80% - 125%. The result from a simulation pivotal study is shown in Table 2. The results showed that such a sample size (N=40) warranted that the confidence intervals of ratio of geometric means falls within 80% - 125% in a simulated future pivotal bioequivalence trial with both of CIs (90% or 95%). Table 1 shows the result from a pilot study where the confidence intervals of ratio of geometric means falls out of 80% - 125%.

1.3. Flow Charts of Computer Procedures

There were four macro driven subroutines for this procedures named as ANOVA1, SIZE1, SIZE2, ANOVA2. (Program details are not demonstrated here. Interested readers should contact authors for the detailed information of SAS procedures.)

To perform the computing procedure, the three pharmacokinetic parameters $C_{\max}$, AUC_{0-t} and $AUC_{0-\infty}$ obtained from a pilot bioequivalence study were input into the first procedure ANOVA1, and the parameters were transformed to natural log-based parameters. The ratio of geometric means(un-transformed), 90% confidence intervals, cvs, cve, MSS, and MSE were calculated from the log-transformed pharmacokinetic parameters in ANOVA1, and a data file containing those parameter values was generated, and ready to be used by the next subroutine SIZE1. At the same time, a similar table was generated for reporting.

The next procedure, SIZE1 subroutine is to find an adequate sample size using the data file generated by ANOVA1. At the beginning, a data files containing the table generated by Diletti et al (3) was loaded into the program to determine the sample size based on the defined power level and other generated parameter values. In step 3, the new data set containing the determined sample size from the calculation of step 2 was generated and loaded into SIZE2 for determining the sample size. After this step, all the generated values were put into the final procedure ANOVA2 to determine the correct sample size to be used for a future pivotal trial.

Conclusion

Four automated computer procedures have been developed based on the theory of a statistical error model to determine the sample size from a pilot bioequivalence study for a future pivotal bioequivalence study. The simulation tests using data from different bioequivalence studies have been conducted to determine the validity of the computer procedures developed. The results showed that the test procedures developed are valid and the procedures are ready for applications in clinical trials. The results also showed that such a sample size warranted that the confidence intervals of ratio of geometric means falls within 80% - 125% in a simulated future pivotal bioequivalence trial. Thus, it is concluded that the applications of this computer procedure with real data from pilot bioequivalence studies were successfully demonstrated. The outcomes of this research study will have significant implications in clinical trials.

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Appendix:

Table 1

Statistical Comparisons for Treatments A Versus B, Pilot Study			
	Lcmax	Lauc	Laucinf
Mean A	2.794	5.237	5.263
Mean B	2.695	5.218	5.242
CI (90%)	94.3-129.3	89.5-115.9	90.1-115.8
CI (95%)	91.0-134.0	86.9-119.4	87.5-119.2
P Value	0.281	0.803	0.764
Mean Ratio	1.104	1.018	1.022
Power	62.94	80.36	82.76
N	12	12	12
MSE	0.0441	0.0298	0.0280
CV	0.212	0.174	0.169
MSS	0.122	0.332	0.334
NU	40	16	16

Note: N = Number of subject used in pilot study

NU = Number of subject required in future pivotal study

Table 2

Statistical Comparisons for Treatments A Versus B, Pivotal Study			
	Lcmax	Lauc	Laucinf
Mean A	2.864	5.381	5.409
Mean B	2.721	5.327	5.352
CI (90%)	106.3-125.2	98.7-112.9	99.1-112.9
CI (95%)	104.6-127.3	97.4-114.5	97.8-114.4
P Value	0.0055	0.182	0.154
Mean Ratio	1.154	1.056	1.058
Power	98.03	99.64	99.73
N	40	40	40
MSE	0.0472	0.0319	0.0300
CV	0.220	0.180	0.175
MSS	0.135	0.380	0.383

Note: N = Number of subject used in pivotal study

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