Will the Number of Replicates In Bootstrapped Data Alter the Bioequivalence Results?

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Abstract: *The estimation of 90% parametric confidence intervals (CIs) of mean Cmax and AUC's ratios in bioequivalence (BE) tests are based upon the assumption that formulation effects of test and reference drug are comparable. Bootstrap nonparametric 90% CIs of formulation effects were estimated with the different replicates (500, 1000, 2000 and 5000) and then compared with the parametric 90% CIs of the original datasets. Histograms and density curves of formulation effects obtained from resampled datasets were similar to those of normal distribution. Currently, the 80 - 125% rule based upon the parametric 90% CIs is widely accepted under the assumption of normally distributed formulation effects in log-transformed data. However, number of replicates in the bootstrapping has significant impact on altering the study results.*

Keywords: Bootstrapping, CI estimation, replicated bootstrapping technique, bootstrapping in BE studies.

1. Introduction

Average bioequivalence is based on the PK parameters (e.g. AUC and Cmax) obtained from BE studies of crossover design or parallel design. Generally, log (AUC) and log (Cmax) values are statistically analyzed using the mixed effect or two-stage linear model.

Based on Shuirmann (1987) (FDA, 2001) two-one sided test (TOST), two formulations are claimed to be bioequivalent when the 90% confidence intervals (CIs) of mean log (AUC) differences and log (Cmax) differences fall within the regulatory acceptance limits (log (0.8) to log (1.25))[1]. The mean differences in log (AUC) or log (Cmax) between the test and the reference formulation represent the formulation effect, a key parameter in the Average Bio-Equivalence test (Patterson and Jones, 2002)[5].

AUC and Cmax are positive and right-skewed, they have been considered as log normally-distributed (Midha et al., 1993; Chow, 2003)[2]. Nonparametric methods may be indicated for data which do not follow a normal distribution even after some transformation. However, because of the poor sensitivity of nonparametric procedures for small data, other more reliable methods are needed (Pabst and Jaeger, 1990)[3].After the formulation effect estimate for each resampled dataset was obtained by BE tests using SAS, the distribution pattern of multiple replicates (500, 1000, 2000, 5000 and 10000) such estimates was analyzed instead of assuming it to be log-normal. The nonparametric CIs were then compared with the 90% CIs obtained from BE tests (parametric CIs) on the reported datasets.

2. Methods

Dummy Concentration data of 200 subjects of 400 sample (Individual small sample data) was created for the different time points of 30, 60, 90, 120, 150, 180, 300 and 480 minutes and used to estimate PK parameter using sparsh sampling techniques. In order to demonstrate bioequivalence, an adequate estimation of the rate (C_{max}) and extent (AUC) of dexamethasone absorption is needed.

The mean AUC for each product and time point t of measurement is calculated by using the mean concentrations (C_t) at each time point t to derive the mean profile for each product. On the basis of the trapezoid rule, mean AUCt is computed as the weighted linear combination of these mean concentrations at each time point through time t. The AUCt is the area under the concentration vs time curve from zero to the time t.

The ratio (Rt) of AUCt from the test product to the reference product is used to assess bioequivalence for each time t of interest. Estimation of the standard deviation(s) of R_t was done via the bootstrapping technique. The estimated mean concentration for different replicates as for Cmax and AUCs are as under

Table 1: The estimated mean concentration for Cmax

Replicates	Cmax		
	Mean	Median	Std Dev
500	1.1492	1.1502	0.1411
1000	1.1479	1.1462	0.1405
2000	1.1460	1.1427	0.1367
3000	1.1470	1.1422	0.1376
4000	1.1454	1.1408	0.1360
5000	1.1445	1.1400	0.1361

Table 2: The estimated mean concentration for AUCs

| Replicates	C_{max}		
	Mean	Median	Std
500	0.960	0.9581	0.067
1000	0.962	0.9605	0.068
2000	0.963	0.9615	0.066
3000	0.964	0.9615	0.067
4000	0.965	0.9617	0.067
5000	1.144	1.1400	0.136

Point estimate and 90% confidence interval for the two formulation with the different replications are as under:

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1371

Table 3: 90% confidence intervals of Cmax and AUCt

Replicates	rt_Cmax	rt_AUC0_t
500	91.71-138.14%	84.99-107.07%
1000	91.68-137.91%	85.01-107.55%
2000	92.11-137.09%	85.40-107.38%
3000	92.06-137.34%	85.37-107.58%
4000	92.17-136.91%	85.33-107.67%
5000	92.07-136.83%	85.38-107.71%

3. Discussion

The basic assumption of the parametric BE test is that log-transformed AUC and C_{max} are normally distributed. If this assumption is not true, BE may have to be tested non-parametrically. In this study, it was assumed that the log-normality of the formulation effect using bootstrap-resampling methods. There are several ways to determine whether samples originate from a normal distribution or not. A simple graphical way of testing normality is the normal probability (or Q-Q) plot method.
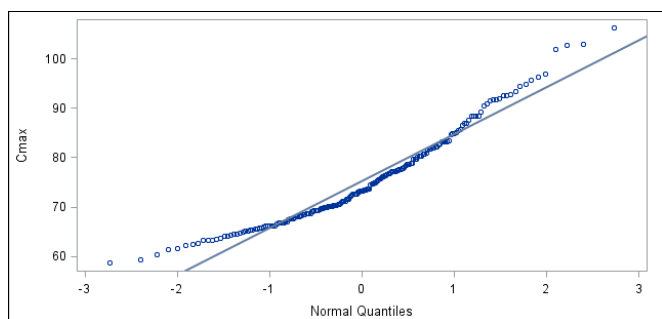
Figure 1: Q-Q plot for Cmax

Since bootstrapped data are large data, hence data was assumed to follows normality. In this context, we employed the bootstrap-resampling method for investigating the distribution of formulation effects in the replicates. Although bootstrapped results are usually approximate, they can sometimes be more reliable and more informative than a priori assumptions of the distribution

4. Conclusion

Nevertheless, this report exemplified the usefulness of nonparametric BE tests as an addition to the conventional BE test. The percent coverage of data is slightly varies in each interval but significant observation from the study is higher the replicates will bring the interval closer to the 20% defined interval. Hence, number of replicates in the bootstrapping has significant impact on altering the study results.

5. Acknowledgements

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Author Profile

Mudaliar Venkatesan received the M.Sc. degrees in Statistics from Gujarat University in 2006. Having 8 years of experience in clinical research industry from 2007-till date. Worked various phases of trials includes BA/BE, Phase I-III and QT/QTc studies with various therapeutic indications like Ophthalmology, Anti-viral (HIV), Alzheimer's, Respiratory and Cardiovascular for US-FDA, EU, EMA and MCC regulatory agencies. Currently working with Semler Research center Pvt. Ltd., Bangalore, India.