

Standard Operating Procedure Statistical Analysis of Clinical Trial Data

Identification No : **DAT02**

Version No: **1**

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TABLE OF CONTENTS

1. ABBREVIATIONS.....	3
2. SCOPE.....	3
3. FIELD OF APPLICATION	3
4. RESPONSIBILITIES.....	3
5. PROCEDURE	3
5.1 Creation of trial analysis files	3
5.2 Analysis programs	4
5.3 Analysis Sets.....	4
5.3.1 Full Analysis Set.....	4
5.3.2 Per Protocol Set	4
5.4 Analysis items.....	4
5.5 Level of significance and confidence coefficient	5
5.6 Analysis technique	5
5.6.1 Analysis of continuous outcome - Parallel groups.....	5
5.6.2 Analysis of continuous outcome - Paired groups (cross-over trial).....	5
5.6.3 Analysis of categorical outcome - Parallel groups	6
5.6.4 Analysis of categorical outcome - Paired groups (cross-over trial)	7
5.6.5 Analysis of survival outcome.....	7
6. REFERENCES.....	8
7. TEMPLATES	8
8. VERSION HISTORY	8

1. ABBREVIATIONS

ASST-PG23:	Azienda Socio-Sanitaria Territoriale - Papa Giovanni XXIII
BS:	Biostatistician
CI:	Confidence Interval
DB:	Database
FROM:	Fondazione per la Ricerca Ospedale Di Bergamo
KM:	Kaplan-Meier
PI:	Principal Investigator
SAP:	Statistical Analysis Plan
SOP:	Standard Operating Procedure

2. SCOPE

This SOP describes the procedure for carrying out statistical analysis of clinical trials.

3. FIELD OF APPLICATION

This SOP is applicable to trials sponsored or supported by FROM, or sponsored by ASST-PG23.

4. RESPONSIBILITIES

Biostatistician

- Writes statistical analysis plan (where appropriate) according to the protocol
- Programs any planned analysis in collaboration with the PI
- Performs data listings, analysis and reporting
- Discusses any primary or secondary results with the PI.

5. PROCEDURE

5.1 Creation of trial analysis files

At the time of analysis (i.e. when all trial data are cleaned) the trial DB is locked. The BS downloads all the data from the DB website into files in Comma-separated Values format. The download system produces a zip archive with many CVS files as there are e-CRF visits of the trial. Along with this archive, a data management document containing the transcoding table of each individual element of the database is produced.

All original files are kept in a trial specific directory with limited access (single sign-on access) in a validated cloud storage service, with several back-up (i.e. the master copy on the hard drive is always synchronized with cloud account, which is then subjected again to backup for added security).

Subsequent versions of data files (i.e. merge of two or more files) are tracked with number and/or date of variation.

DAT02/2

Statistical Analysis of CT Data

The BS will create annotated programs to prepare the data for analysis (including recording, labeling and scoring of data) according to the protocol and the **Statistical Analysis Plan (T.DAT02.01/02)**, where appropriate.

5.2 Analysis programs

The BS is responsible for conducting the main analyses according to the protocol and the **Statistical Analysis Plan (T.DAT02.01/02)**, where appropriate. BS is responsible for writing appropriate statistical programs to carry out these analyses with adequate explanatory annotation. Any changes made to the statistical analysis planned in the protocol should be documented and justified. All analyses will be carried out using well established statistical software.

5.3 Analysis Sets

The set of subjects whose data are to be included in the main analyses should be defined in the statistical section of the protocol. In addition, documentation for all subjects for whom trial procedures (e.g. run-in period to evaluate that all inclusion and exclusion criteria are verified) were initiated may be useful.

5.3.1 Full Analysis Set

The intention-to-treat principle implies that the primary analysis should include all randomized subjects according to the group they were originally assigned, regardless of what treatment (if any) they received. Preservation of the initial randomization in analysis is important in preventing bias and in providing a secure foundation for statistical tests.

5.3.2 Per Protocol Set

The 'per protocol' set of subjects, sometimes described as the 'valid cases', the 'efficacy' sample or the 'evaluable subjects' sample, defines a subset of the subjects in the full analysis set who are more compliant with the protocol and is characterized by criteria such as the following:

- the completion of a certain pre-specified minimal exposure to the treatment regimen;
- the availability of measurements of the primary variable(s);
- the absence of any major protocol violations including the violation of entry criteria.

The precise reasons for excluding subjects from the per protocol set should be fully defined and documented.

Special classification of incomplete cases is required, in particular the incomplete cases should be roughly divided into two categories: one being ineligible, related to a pre-drug problem, and the other being discontinuance, dropout, and deviation (violation), related to treatment after trial initiation.

5.4 Analysis items

In general, the main statistical analysis items are individual evaluations of efficacy and safety. Other evaluation items (i.e. specific observation/measurement parameters and/or laboratory examinations/physical tests) tailored to the primary objective of the clinical trial may also be selected beforehand to be included in the main statistical items. With respect to handling these data, however, it is necessary to make a thorough examination, as secondary statistical analyses. These statistical analyses may

DAT02/2

Statistical Analysis of CT Data

not only support the main statistical analyses, but also suggest a new clinical hypothesis for future investigations.

5.5 Level of significance and confidence coefficient

The level of significance, confidence coefficient, and two-tailed or one-tailed test during analysis should be specified. Usually, 5% and 1% are used as the levels of significance and 95% and 99% as confidence coefficient. In testing, it is recommendable to describe not only whether or not the difference is significant, but also the significant probability (p value).

5.6 Analysis technique

The analysis technique must be selected in accordance with the study design and evaluation scale.

5.6.1 Analysis of continuous outcome - Parallel groups

The following guidelines are designed to assist in the analysis of a randomized controlled trial with two parallel groups and a numerical (continuous) outcome. The outcome is denoted by y, the binary treatment variable x (taking values 0 and 1), and baseline values of the outcome are denoted by z.

Exploratory analysis

1. Produce the following descriptive statistics for the outcome for each group separately and combined: mean, standard deviation, median, minimum, maximum, interquartile range, and count;
2. Investigate the distribution of the data using graphical methods. Transform the outcome if clinically appropriate;
3. Investigate the equality of the variance;
4. Investigate the relationship between the baseline and outcome measures using graphical methods and summary statistics.

Comparing means (or medians) between two groups

Determine an appropriate analysis based on the size of the groups, the distribution of the outcome, the variances and the presence or absence of baseline measures. In all cases an estimate of the treatment effect will be presented with a 95% confidence interval (CI). Use the first appropriate analysis in the list below:

1. If we have baseline measures and a relationship between y and z, we use a regression/ANCOVA based analysis;
2. Where assumptions of Normality are appropriate (e.g. if we have approximately normal data -with or without transformation- or a large sample size) we use a two sample t-test;
3. If assumptions of Normality cannot be made (e.g. if we have a small sample size ($n < 30$) and/or non-normal data), we use the Mann Whitney test;
4. If we have a large sample we might use the Normal z-test.

5.6.2 Analysis of continuous outcome - Paired groups (cross-over trial)

The following guidelines are designed to assist in the analysis of a cross-over randomized controlled trial with a numerical (continuous) outcome. The outcome is denoted by y, the binary treatment variable x ($A=1$,

DAT02/2

Statistical Analysis of CT Data

B=0), and cross-over period is denoted by period (taking values 1 and 2). Also, sequence denotes the randomization group (AB=1, BA=2) and id the patient number.

Exploratory analysis

1. Produce summary statistics for each treatment/period combination;
2. Produce subject profile plots by plotting each subject response in the two periods sort id period;
3. Plot the mean value (\pm Standard Deviation) or median value (with interquartile range) of each treatment for each period.

5.6.3 Analysis of categorical outcome - Parallel groups

The following guidelines are designed to assist in the analysis of a randomized trial with two parallel groups and a categorical outcome. The categorical outcome is denoted by y , the binary treatment variable x , and baseline factors for adjustment by z . In this section we will assume that the outcome variable y is either a binary or ordinal outcome.

The appropriate analysis will depend on the form of the categorical outcome. For all analyses an estimate of the treatment effect will be presented with a 95% CI and P-value from an appropriate test.

Exploratory analysis

1. Tabulate the outcome categories by treatment group. Check if there is a need to collapse categories of y into fewer categories;
2. Check the expected frequency in each cell of y by groups.

Comparing binary outcome between two groups

Unadjusted analysis

A) Large samples

Estimates and CIs for risk difference, risk ratio and odds ratio and the relative Chi squared test with its associated p-value.

The CIs given are calculated using a normal distribution to approximate binomial. This is acceptable if samples are large i.e. $n \cdot p \geq 5$ and $n(1-p) \geq 5$.

Use of the Chi squared test to test for an association between y and x is acceptable only if 80% of the cells have expected frequencies ≥ 5 and all the expected values are > 1 .

B) Small samples

For small samples where the conditions specified above are not met, exact CIs and tests should be reported. Fishers exact test is provided.

Adjusted for baseline factors

The treatment effect can be adjusted for baseline factors using logistic regression assuming that none of the cells is too small or empty. To adjust a risk difference estimate Binomial regression may be used.

Comparing ordinal outcome between groups

Unadjusted analysis

Test for differences between treatment groups using Wilcoxon Rank Sum test.

Adjusted for baseline factors

Test for differences between treatment groups using ordinal logistic regression. Check proportional odds assumption.

5.6.4 Analysis of categorical outcome - Paired groups (cross-over trial)

The following is designed to assist in the analysis of 2 by 2 cross-over trials with a binary outcome. The outcome is denoted by y , the binary treatment variable by x .

Exploratory analysis

A tabulation of the paired responses in each group describes the data to be analyzed.

Analysis of categorical outcome - Paired

Estimates and CIs for the difference and ratio of proportions and the odds ratio are provided along with a McNemars test (unadjusted for period effect).

Estimates and CIs for the difference and ratio of proportions and the odds ratio, adjusted for period effect can be obtained using a random effect model.

5.6.5 Analysis of survival outcome

Let t represent the elapsed time between entry to the study and observation of an outcome of interest. We assume t is continuous. A clear definition of: start time, origin e.g. date of diagnosis, end time e.g. date of death or censoring, unit of time e.g. months, status at end time e.g. dead. In all analyses the following will be presented: estimation of effect size, precision (CI or SE), number of events and number of patients at risk. Let Y be the censoring variable, where $y=1$ outcome occurred and $y=0$ censoring has occurred. We assume that censoring is uninformative and that we have no competing events. Let $z_1, z_2, z_3 \dots, z_i$ be prognostic factors that may affect survival time. Let x represent the k treatment groups (0 to k) e.g. placebo ($x=0$) and control ($x=1$).

Exploratory analysis

Define survival parameters: survival time and censoring variables;

Obtain summary descriptive statistics:

- Produce summary of survival data, e.g. mean median, min, max;
- Produce summary the following summary statistics: time at risk, incidence rate, number of subjects, and the 25th, 50th, and 75th percentiles of survival time;
- Compute median survival time by treatment groups;
- Tabulate rate of outcome of interest by treatment group.

Produce life table: displays and graphs life tables for individual-level or aggregate data. For large datasets, specify the intervals into which the data are to be aggregated for tabular presentation.

Check proportionality

Visually examine the raw survival plots for subgroups to check they do not cross: graph Kaplan-Meier (KM) curves for each treatment group (A);

Visually examine the raw hazard plots for subgroups to check for non-parallel trends: graph a smoothed curve of baseline hazard (B);

Use the graphs produced in (A) and (B) to assess proportionality as follows:

- If the KM curves cross the assumption is almost certainly violated;
- If the KM curves do not cross, the assumption is probably acceptable but will need to be checked formally.

Comparison of survival curves - non-parametric methods

Use log-rank test to compare survival curves, which test for equality of survival functions.

Comparison of survival curves - semi-parametric methods

Use the Cox proportional hazard model below from a to b to estimate the effect of a risk factor(s) on the hazard:

- (A) Unadjusted (single variable analysis): this is equivalent to the logrank test, but allows formal assessment of the PH assumption, using Schoenfeld residuals and gives estimates of the treatment effect;
- (B) Adjusted for other covariates (multivariate analysis).

Test the PH assumption formally: global test and by each variable.

Produce diagnostic plots after Cox proportional hazard model (at least one):

- Plot a $-\ln(-\ln(st))$ plot by level of variable;
- Plot of predicted survival from Cox model with observed survival by KM;
- Plot estimates of the hazard or survival function at mean values of covariates;
- Plot survival scaled Schoenfeld residuals versus time and look for flatness of the smooth.

6. REFERENCES

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7. TEMPLATES

T.DAT02.01/02 Statistical Analysis Plan

8. VERSION HISTORY

Version	Date	Reason for revision
1	30 Sept 2016	Starting document.
2	15 Dec 2019	Minor wording correction.