Statistical Analysis Plan

Protocol Number: ARG-E08

SAICoDis - Safety of Argatroban Infusion in Conduction Disturbances.
A prospective, open, multicenter safety study to investigate conduction disturbances in patients receiving argatroban therapy.

Version: 1.0
Date: 30 July 2021
NCT number: NCT05740371

[redacted]

[redacted]

Statistical Analysis Plan

Sponsor: Mitsubishi Tanabe Pharma GmbH

Protocol Number: ARG-E08

EudraCT number: 2016-003521-42

SAICoDis - Safety of Argatroban Infusion in Conduction Disturbances.

A prospective, open, monocentric safety study to investigate conduction disturbances in patients receiving argatroban therapy.

Version 1.0

(final, 30.07.2021)

Signatures

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Approvals		
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1 Table of contents

2 Abbreviations and definitions

Abbreviation	Explanation
ACT	Activated clotting time
AE	Adverse event
ATC	Anatomic therapeutic classification
BMI	Body Mass Index
CABG	Coronary Artery Bypass Graft
CRF	Case Report Form
DMP	Data management plan
dTT	diluted Thrombin Time
ECG	Electrocardiogram
ECG-1	12-lead baseline electrocardiogram performed at screening visit
ECG-2	12-lead electrocardiogram performed immediately after cardiac intervention in steady state anticoagulation with argatroban.
ECG-3	12-lead electrocardiogram performed > 8 but ≤ 28 hours after termination of prolonged argatroban infusion.
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ITT	Intention to treat population
MedDRA	Medical Dictionary for Regulatory Activities
ms	Milliseconds
PCI	Percutaneous coronary intervention
PP	Per protocol population
PT	Preferred Term (MedDRA)

QTc	Corrected QT interval
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SOC	System Organ Class (MedDRA)
SOP	Standard Operating Procedure

3 Introduction

The objective of this statistical analysis plan (SAP) is to define the planned statistical analyses in more detail than what is outlined in the clinical study protocol. It follows the principles of the guidelines International Conference on Harmonization (ICH) E3 and ICH E9 and the relevant [redacted] standard operation procedure (SOP). This SAP is the guideline for all statistical programming and creation of tables planned to be performed for the analysis of the SAICoDis study.

This SAP has to be finalized and signed prior to database lock and before start of statistical analyses.

4 Study documents

This SAP is based on the study documents in Table 1.

Document	Version, date
Study Protocol	Version 2.0 dated 08-May-2020
CRF	Version 1.0, dated 23-Nov-2016
Data Management Plan	Version 2.0, dated 15-Apr-2021

Table 1: Study documents

5 Standard operating procedure

Statistical analysis follows [redacted] Studien 4.0, effective from 09.01.2019, unless specified differently in this SAP.

6 Responsibilities

Responsible for the determination of statistical analyses	[redacted]
Responsible for the programming	[redacted]
Responsible for internal quality control	[redacted]

Table 2: Responsibilities

7 Timelines

This SAP is to complete and to sign prior to the database being locked and final analysis begin.

8 Study objectives

The primary objective of this study is to determine change of corrected QT interval (QTc) during intravenous argatroban infusion in patients undergoing percutaneous coronary intervention (PCI). To observe whether argatroban has a pharmacological effect on cardiac repolarization, it will be investigated, if a mean QTc prolongation of more than 10 ms will occur between ECG-2 (electrocardiogram immediately after cardiac intervention when patient is fully anticoagulated with argatroban) and ECG-1 (baseline ECG).

The primary endpoint of this study is the mean difference in QTc interval between ECG-2 and ECG-1.

9 Study preparations

Investigational product:

- Argatroban-monohydrate (Arginine-derived direct thrombin inhibitor).

Therapy used as a comparator:

- None, this is not a comparative study.

10 Study design

10.1 Overview

The present study is an interventional Phase IV prospective, multicenter, single-group, open-label, ECG-reader (cardiac specialist) blinded safety study (PASS) to investigate conduction disturbances in patients receiving argatroban therapy during an elective percutaneous coronary intervention (PCI).

10.2 Sample Size

A one-sided one sample t-test with a significance level of 5% will be used to test the primary hypothesis of this study that QTc will not increase more than 10 ms under argatroban. Given a sample size of 45 patients, up to a population mean of Δ QTc = 6 the power of the test is adequate, i.e. > 0.8. To account for potential dropouts at least 50 patients are to be enrolled into the study, assuming a dropout rate of about 10%.

10.3 Randomization

Not applicable.

11 Study Schedule

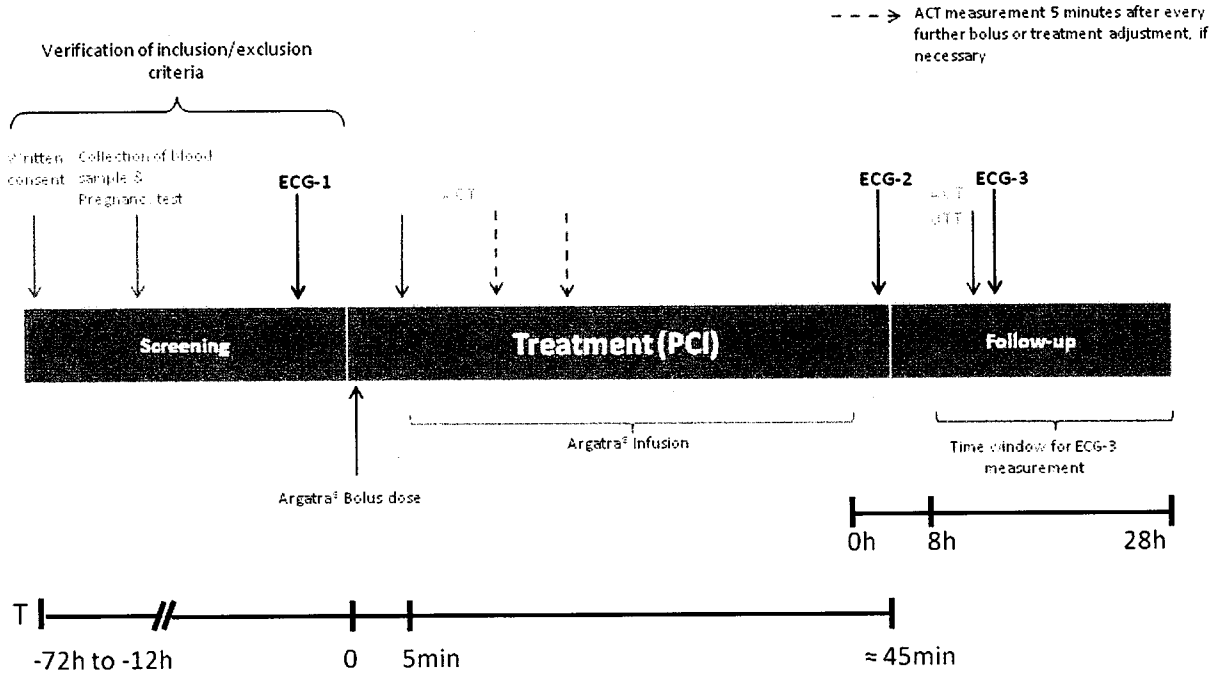


Figure 1: Timing of study procedures

Day/ visit type ► procedures ▼	Screening (hour -72 to -12)	Treatment (hour 0)	Follow-Up (ends > 8 but ≤ 28 hours after PCI)
Informed consent	✓		
Inclusion/exclusion	✓		
Demographics	✓		
Medical history	✓		
Past & concomitant treatment	✓	✓	✓
Vital signs and bodyweight	✓		
Pregnancy test (urine)	✓		
ECG	✓	✓	✓
Haematology	✓		
Haematocrit	✓	(✓)	✓
Hs-Troponin-T	✓		
Liver function profile	✓		
Coagulation profile	✓	✓	✓
Kidney function profile	✓		
Electrolytes	✓		
Study drug infusion		✓	
AE evaluation		✓	✓

Table 3: Schedule of assessment

12 Criteria for evaluation

12.1 Primary objective

The primary objective is to determine the change of QTc interval during argatroban infusion in patients undergoing PCI. To observe whether argatroban has a pharmacological effect on cardiac repolarization it will be investigated, if a mean QTc prolongation of more than 10 ms occurs between ECG-2, which needs to be performed immediately after cardiac intervention in a status of full anticoagulation with argatroban, and ECG-1, the baseline ECG.

The primary endpoint of this study is the mean difference in QTc interval between ECG-2 and ECG-1.

12.2 Secondary objectives

The secondary objectives are:

- Determination of the QTc interval after sufficient wash-out period by ECG-3 which needs to be performed > 8 but ≤ 28 hours after termination of prolonged argatroban infusion.
- Investigation of dependence of QTc interval on gender and applied doses.
- Determination of coagulation status during argatroban therapy.
- Assessment of safety-related events within the scope of anticoagulation with argatroban, for example bleeding events or thromboembolic events.

The secondary endpoints in this study are:

- Mean difference in QTc interval between ECG-3 and ECG-1.
- Proportion of patients with a prolongation of QTc interval to > 500 ms at ECG-2.
- Proportion of patients with a prolongation of QTc interval to > 500 ms at ECG-3.
- Proportion of patients with a prolongation of QTc interval of > 60 ms at ECG-2 compared to ECG-1.
- Proportion of patients with a prolongation of QTc interval of > 60 ms at ECG-3 compared to ECG-1.

12.3 Safety endpoints

The safety endpoints of this study are:

- Incidence of haemorrhagic events according to the definition of CABG-related bleeding.
- Incidence of adverse events.

13 Analysis sets

13.1 Definition of analysis sets

Each patient will be assigned to appropriate analysis populations for statistical analysis. The different analysis populations are:

Enrolment set (all enrolled population): The enrolment set includes all patients who were included in the study (eligibility screening conducted), even if no treatment was performed. The enrolment set is the starting point for the description of the patients' disposition.

Intention-to-treat set (ITT population): This set contains all the patients of the enrolment set in whom argatroban was administered. The primary analysis set for the description of the patients, for analyses of efficacy and safety is the ITT population.

Per-protocol set (PP population): The PP population is a subset of the ITT population and includes patients where all inclusion criteria are met, where none of the exclusion criteria applies and where the study was carried out according to the study protocol.

The procedures in case of protocol violations and the assignment of the individual patients to the analysis collectives are determined in consultation with the principal investigator prior to the statistical analysis.

The primary outcome criterion of this study, the mean difference in QTc interval between ECG-2 and ECG-1, is analyzed for the ITT and additionally for the PP sample. Differences in the results between the ITT and PP samples are discussed.

13.2 Protocol deviations

The classification of protocol deviations into not significant and significant protocol deviations, meaning protocol deviations leading to the exclusion of patients from the PP-set, will be done after data review in co-operation between the sponsor, the principal investigator and [redacted]. The decision about the assignment of patients to the analysis sets will be made before data lock. A data review protocol will be prepared.

14 Statistical methodology

14.1 Data handling

14.1.1 Definition of time points

See *Timing of study procedures* (Figure 1, Page 9).

14.1.2 Handling of missing values

All available data will be included in the analyses. There will be no substitution of missing data, i.e., missing data will not be replaced by any method of imputation (observed case analysis). Frequencies of missing values will be presented.

14.1.3 Inconsistent data

During study, data is continuously checked for inconsistencies. In case any inconsistencies are recognized, the investigator is asked to resolve these. There should be no inconsistencies in data at time of data lock.

Should there be inconsistencies that cannot be solved, handling of these inconsistencies will be specified after data review in co-operation between the sponsor, the principal investigator and [redacted] before data lock. The decisions will be written down in the data review protocol.

14.1.4 Handling of outliers

Outliers are described in tables, individual patient data listings and figures (Box-Plots).

14.1.5 Data calculations

Distances between dates will be calculated as (date 2 - date 1). Periods of time are calculated as (date 2 - date 1) + 1 (e.g. duration of adverse event).

14.1.6 Data transformations and computed variables

No data transformations (e.g. square root, logarithm) to confirm statistical assumptions will be performed. All variables will be used in the analysis as reported.

The Body Mass Index (BMI) is computed as follows:

BMI=Weight (kg) / Height $(m)^2$.

14.1.7 Classification of variables

BMI will be classified according to the WHO:

	BMI (kg/m²)
Underweight	< 18.50
Normal weight	18.50 < 25.00
Pre-obesity	25.00 < 30.00
Obesity class I	30.00 < 35.00
Obesity class II	35,00 < 40.00
Obesity class III	≥ 40.00

Classification of blood pressure (mm Hg)[1]

Category	Systolic blood pressure		Diastolic blood pressure
Optimal	<120	and	<80
Normal	120–129	and/or	80–84
High normal	130–139	and/or	85–89
Grade 1 hypertension	140–159	and/or	90–99
Grade 2 hypertension	160–179	and/or	100–109
Grade 3 hypertension	≥180	and/or	≥110
Isolated systolic hypertension	≥140	and	<90

1) The blood pressure (BP) category is defined by the highest level of blood pressure, whether systolic or diastolic.

Laboratory reference range values are defined in DMP.

14.1.8 Multi-center data

This study is conducted in two centers. A subgroup analysis per center will be performed.

14.1.9 Data review

A data review protocol will be prepared before data lock. At the minimum, the protocol will contain the decisions on:

- the affiliation of patients to the samples ITT and PP,
- identification of patients without PCI,
- handling of outliers, if any,
- MedDRA version used,
- ATC version used,
- Version of [redacted] Coding SOP.

14.2 Descriptive statistics

Absolute and relative frequencies (percentages) will be determined for nominal and ordinal characteristics.

For quantitative analysis variables, mean, standard deviation, minimum, 1st quartile, median, 3rd quartile and maximum will be determined, and these statistics will be presented in tables for each treatment and/or point in time. If appropriate, continuous variables may be categorized and additionally treated as ordinal variables.

Adverse events and concomitant diseases will be coded according to the MedDRA system and described according to their preferred term and the system organ class. Concomitant medication will be coded according to the WHO-ATC system. The coding is carried out according to [redacted] SOP CO.

Graphical presentation of data will be given by means of histograms, bar charts, pie charts, and box plots, as appropriate.

14.3 Confirmatory statistics

Following measurements of QTc will be made for each patient:

- Prior to first bolus dose of argatroban: QTc_1
- Immediately after cardiac intervention in a status of full anticoagulation with argatroban: QTc_2
- > 8 but ≤ 28 hours after termination of prolonged argatroban infusion: QTc_3

The statistical hypothesis of this study refers to the difference QTc_2 - QTc_1 = Δ QTc:

H_0: Δ QTc > 10 ms

H_A: Δ QTc ≤ 10 ms.

The null hypothesis H_0 states that the mean difference QTc_2 minus QTc_1 is greater 10 ms. The alternative hypothesis H_1 asserts that the mean difference QTc_2 minus QTc_1 is lower or equal 10 ms.

H_0 will be tested by a one-sided one sample t-test with a significance level of alpha=5%. A one-sample t-test can be used to compare a sample mean to a given value. The variable analysed is assumed to be normally distributed.

Data from all patients with measurements QTc_1 and QTc_2 will be included in the analysis of Δ QTc.

Additionally, Δ QTc as well as QTc_1 and QTc_2 will be described by descriptive statistics (mean, standard deviation, median, minimum, maximum, and 95%-confidence

interval). An individual patient listing will be provided with QTc_1, QTc_2, Δ QTc, QTc_3 and the difference QTc_3 minus QTc_1.

The analysis of the primary variable will be based on the ITT sample. It will be repeated for the PP sample. The study report will contain a statement about difference in study results between the ITT and PP sample.

The SAS-statement used to test H_0 is:

```
PROC TTEST DATA=data H0=10 SIDE=L;
  VAR deltaQTc;
RUN;
```

H0=10 requests tests against a null value of 10 ms,

SIDE=L specifies a lower one-sided test, in which the alternative hypothesis indicates a mean less than the null value, and lower one-sided confidence intervals between minus infinity and the upper confidence limit.

14.4 Exploratory analyses

If p-values are calculated for secondary endpoints they need to be interpreted descriptively and non-confirmatory only.

14.5 Interim analyses

No interim analyses are planned.

14.6 Analysis of subgroups

Study data will be analyzed split by sex of patients and by center.

15 Statistical analyses

15.1 Study patients

15.1.1 Disposition of patients

The flow chart of patient disposition in the clinical study report will provide the respective frequency counts regarding the following patient subsets:

- Patients who were enrolled
- Patients who were enrolled but not treated
- All patients with premature discontinuation, if any
- All patients who completed the study as planned.

The flow chart will depict the patient IDs of the patients who did not complete the study as planned.

15.1.2 Reasons for withdrawal

The number and percentage of patients who discontinued the study prematurely will be tabulated as well as the reasons of premature end of study. An individual patient data listing of patients with premature end of study completes the information.

15.1.3 Protocol deviations

The number and percentage of patients with protocol deviations will be given in the clinical study report classified as significant and not significant deviation as defined in the data review protocol. In addition, the investigators' assessment of inclusion and exclusion criteria at screening will be presented.

15.1.4 Analysis sets

The number and percentage of patients included in the analysis sets, together with a breakdown of the reasons for the assignment to the analysis sets, will be provided in the clinical study report. A tabular listing of all patients excluded from the ITT and from the PP sample will be provided.

15.2 Demographic and other baseline characteristics

Summary tables will be provided for the ITT population by means of descriptive statistics and frequency tables and may be repeated for other populations as appropriate.

The following demographic data and baseline characteristics will be presented:

Demographics:

- Gender
- Ethnicity
- Age
- Height, weight, BMI
- Pregnancy test (female patients only)

Vital signs:

- Blood pressure
- Pulse rate

Current status of coronary heart disease:

- Duration of coronary heart disease
- Angina prectoris (stable/unstable, CCS-classification)
- Indication for PCI (single-vessel disease, multiple-vessel disease, in-stent-stenosis, revision of venous bypass stenosis, no PCI – including details)
- Stenosis severity pre-PCI

Laboratory results at screening:

- Coagulation status
- Hemogram
- Liver function profile
- Kidney function profile
- Electrolytes
- Hs-Troponin-T

For reference ranges see DMP. A listing of each abnormal laboratory value will be provided.

15.3 Previous and concomitant treatment and medications

Summary tables will be provided for the ITT population for:

Relevant medical history and current medical conditions, including indication to concomitant medication (MedDRA-coded: preferred term and organ system class).

Previous and concomitant medications (ATC-coded: anatomical main group, therapeutic subgroup, chemical substance) – separately for screening and follow-up.

Previous treatment (non-drug therapy) related to cardiac disease (surgical interventions ...) with be listed and grouped ad-hoc if applicable. Same applies for new or changed non-drug therapies recorded in follow-up.

15.4 Study treatment

Summary tables will be provided for the ITT population for:

- Duration of PCI (end minus start time)
  – all patients, separately for patients with/without PCI
- ACT measurement during PCI
  - Number of measurements
  - below / within / above target range, i.e. < 300, 300 - 450, > 450 s
    – separately for ACT measurement prior to argatroban treatment, first ACT measurement after first bolus, ...
- Extent of argatroban exposure
  - Duration of argatroban treatment
  - Number of bolus administered
  - Dosing interuptions and adaptations
  - Argatroban dose
    – separately for each bolus dose, infusion dose
  - Argatroban cumulative dose per kg (in mg/kg)
    $$\left(\sum_b bolus_b(\mu g/kg) + \sum_i infusion_i\,(\mu g/kg/min) * duration_i\,(min)\right)/1000$$
  - Argatroban cumulative dose (in mg)

In addition, a listing will be provided depicting the individual PCI duration, ACT measurements and argatroban dosing scheme per patient.

- Measured argatroban plasma concentration at follow-up
- ACT measurement at argatroban-free status (follow-up)
  - Summary statistics per center

- below / within / above normal range,
  i.e. < 80, 80 – 160, > 160 s (center1), < 97, 97 – 133, > 133 s (center 2)
- listing of ACT values outside the normal range

- Diluted thrombin time (dTT) at follow-up

In addition, a listing will present the argatroban concentration and coagulation status (dTT and ACT) at follow-up together with the QT interval at ECG-3.

Additional drugs administered will be presented (ATC-coded: therapeutic subgroup, chemical substance)

15.5 Efficacy evaluation

The primary data set for the efficacy analysis will be the ITT analysis set. Additional analyses will be performed for the PP analysis set. Note that whereas trials to show superiority always use the ITT population as primary analysis, results for both ITT and PP population are of interest in non-inferiority trials. It may actually be easier to show non-inferiority in the bigger sample of the ITT population, especially in case of many protocol deviations. This underpins the importance of the PP analysis in non-inferiority trials.

Subgroup analyses will be performed as described in Section14.6.

Summary tables will be provided by means of descriptive statistics and frequency tables, as appropriate.

15.5.1 12-lead ECG

The following will be presented at screening, during treatment and at follow-up:

- Overall interpretation (investigator and blinded ECG-reader assessment); if abnormal, clinically significant
- Measured QT interval, corrected QT interval
- Interpretation of QT interval: prolonged; if prolonged, clinically significant

Primary endpoint (see section 12.1):

- Difference in QTc interval between ECG-2 and ECG-1 (Δ QTc= QTc2 - QTc1)
- Test of statistical hypothesis (see Section 14.3)

Secondary endpoints (see section 12.2):

- Difference in QTc interval between ECG-3 and ECG-1 (QTc_3 - QTc_1)

- Proportion of patients with QTc_2 > 500 ms
- Proportion of patients with QTc_3 > 500 ms
- Proportion of patients with QTc_2 - QTc_1 > 60 ms
- Proportion of patients with QTc_3 – QTc_1 > 60 ms

In addition, a listing will tabulate the individual response data, i.e. QTc_1, QTc_2, QTc_3 as well as gender and cumulative Argatroban dose.

The dependence of QTc interval on gender and cumulative Argatroban dose will furthermore be investigated in plots. If applicable, linear correlation of QTc interval and cumulative Argatroban dose will be assessed.

Heart rate and Measured RR interval will be presented at screening, during treatment and at follow-up.

15.5.2 Laboratory results

Hematocrit at screening, during treatment and at follow-up; classification of hematocrit values to reference range values (see DMP).

A listing of each abnormal laboratory value will be provided.

15.6 Analysis of safety

Evaluation of safety will be performed for the ITT analysis set. If present, adverse events in patients who are not included in the ITT population will be described separately.

15.6.1 Adverse events

Adverse events (AE) data will be processed in the statistical analyses after coding according to the MedDRA dictionary. In an individual patient data listing verbatim adverse events term will be depicted along with the assigned MedDRA preferred term. Statistical analysis will not take in account MedDRA low level terms but will start at preferred term level.

In addition to an analysis of all adverse events, adverse events with a causal relation to the treatment, that is adverse reactions, will be analyzed separately.

The incidence of haemorrhagic events according to the definition of CABG-related bleeding will be presented.

15.6.2 Individual patient data listing of adverse events

All adverse events, treatment emergent or not, will be listed in an individual patient data listing. Verbatim adverse event terms will be depicted along with the assigned

MedDRA preferred term, treatment, intensity, seriousness, relation, measures regarding study treatment and patient, start of AE, end of AE, and visit dates.

A separate listing will be provided for deaths, other serious adverse events, and other significant adverse events.

16 Statistical software used for analysis

Statistical analysis will be conducted using the program system SAS (Version 9.4 or later) under the Microsoft Windows 10 Professional operating system (or later) at the computer facilities of [redacted].

17 Reporting of analyses

Percentage values will be printed with two digits to the right of the decimal point. All p-values will be given by four digits to the right of the decimal point.

Verbatim terms documented on the CRF will be presented as entered.

The statistical analysis output will be prepared in English. The separate individual patient data listings will be in original CRF language. The separate individual patient data listing will include patients excluded from the ITT analysis set, if any.

18 Changes to planned analyses

This statistical analysis plan does currently not include any relevant changes to the planned analyses that are described in the study protocol.

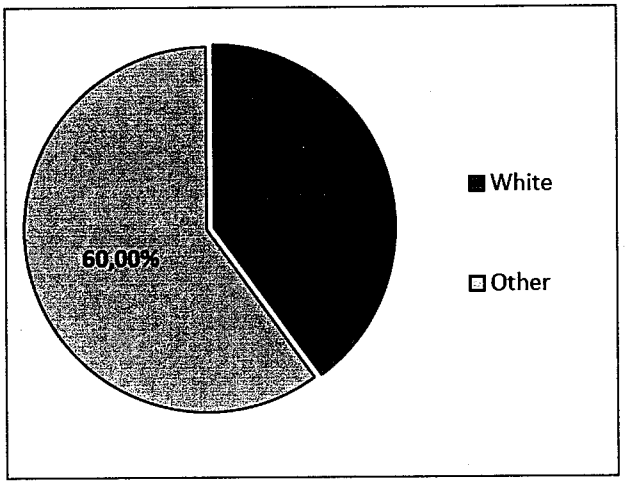
Any changes made to the SAP after sign-off must be signed-off as a SAP Amendment, detailing the old text, revised text and reason for change.

19 Graphics, analysis tables and listings

19.1 Graphics

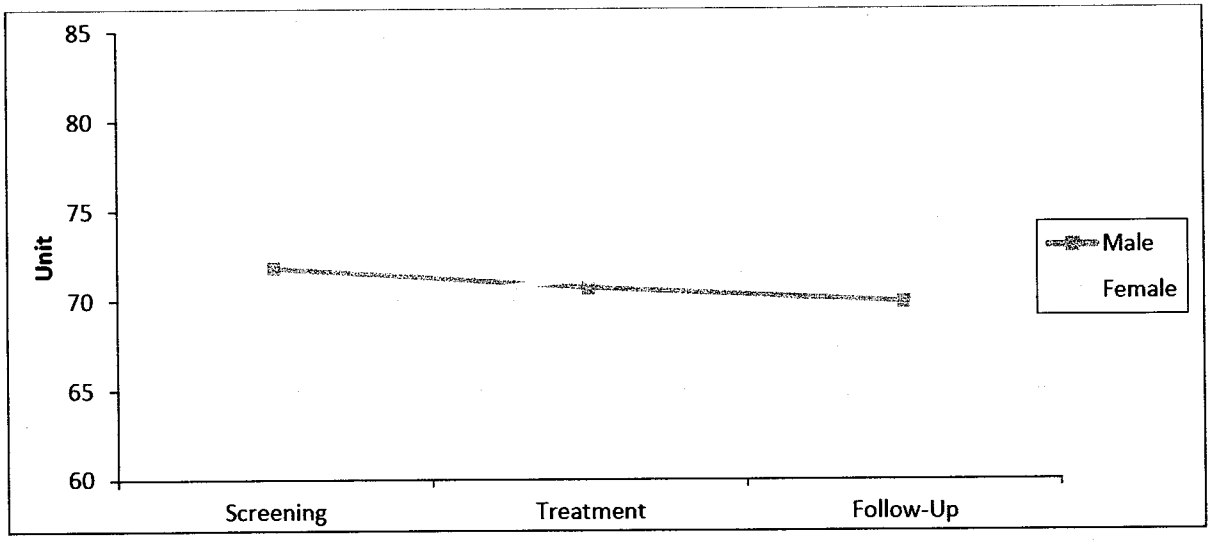
19.1.1 Pie charts

Pie charts will be used to illustrate numerical proportion.

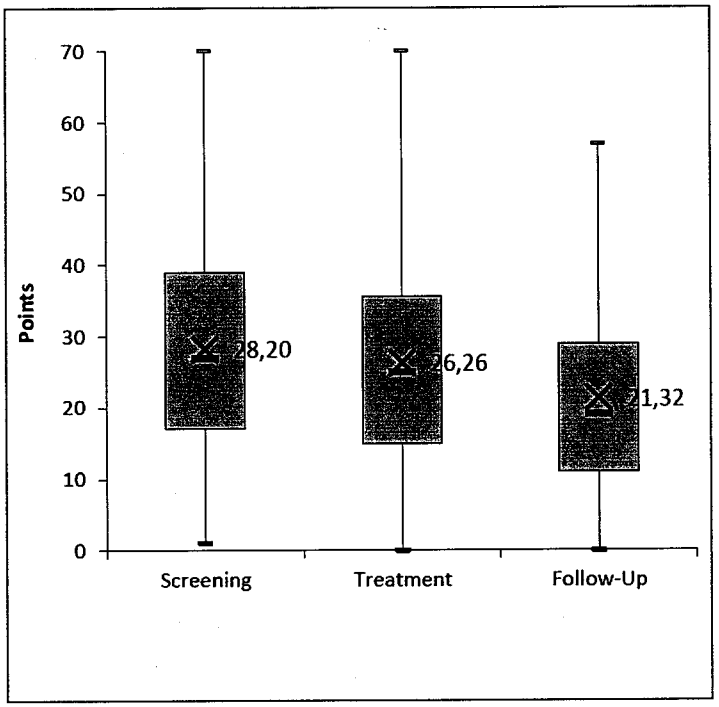


19.1.2 Plots of means and box plots

Mean plots may be used to see if means varies between different groups of patients or how means are changing over time.

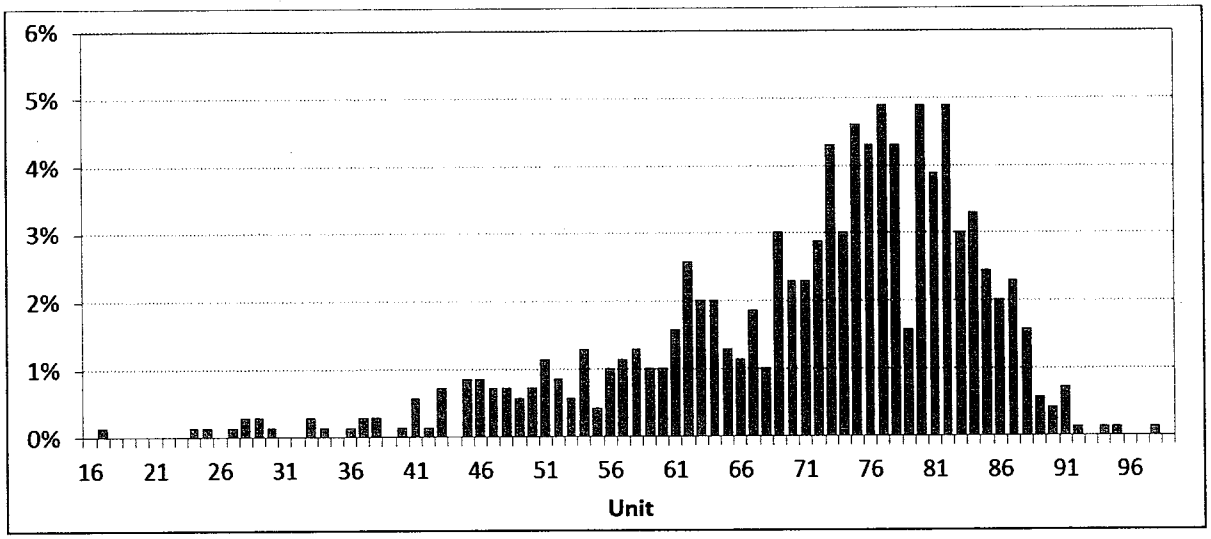


Box plots may be used to detect and illustrate location (mean, median) and variation changes between different groups of patients or between different points in time.

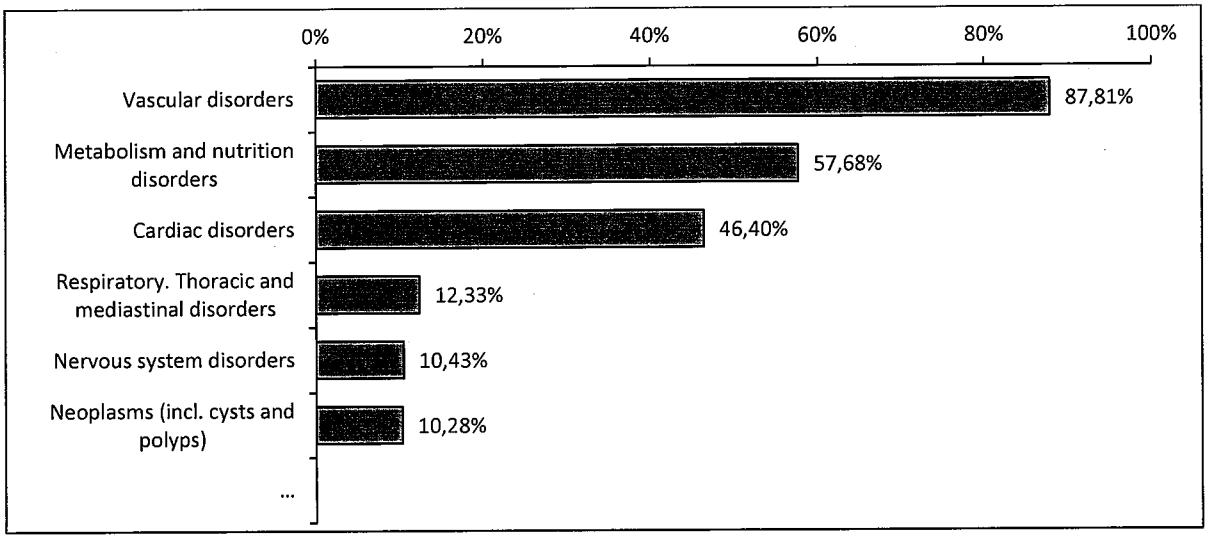


19.1.3 Histograms

To graphically summarize the distribution of univariate data histograms will be used where appropriate.



Histograms will also be used to show relative frequencies in case of multiple response variables like concomitant diseases.



Histograms may also be used to compare numerical proportions.