Multicenter double-blind placebo-controlled parallel-group randomized clinical trial of efficacy and safety of Subetta in the treatment of impaired glucose tolerance

Phase III

Sponsor	**OOO «NPF «MATERIA MEDICA HOLDING»**
Protocol number	**MMH-SU-006**
Version date:	**May 30, 2019**
ClinicalTrials.gov Id:	**NCT03725033**

Protocol Summary

This document represents the protocol summary for the study on human subjects. The study will be carried out in accordance with ICH GCP, National Standard of the Russian Federation GOST 52379-2005 "Good Clinical Practice", Helsinki Declaration of World Medical Association, relevant requirements of the regulatory authorities as well as the study procedures.

Title of Study

Multicenter double-blind placebo-controlled parallel-group randomized clinical trial of efficacy and safety of Subetta in the treatment of impaired glucose tolerance.

Phase: III

Sponsor: OOO "NPF "MATERIA MEDICA HOLDING", Moscow, Russia

Protocol No. MMH-SU-006

Objective of the study

- To assess efficiency and safety of Subetta in the treatment of impaired glucose tolerance[1] (IGT).

Endpoints

Primary endpoint

1. Changes in 2-hour fasting plasma glucose (OGTT[2]) at 12 weeks versus baseline.

Secondary endpoints

1. Percentage of subjects with 2-hour plasma glucose <7.8 mmol/L after 12-week therapy.
2. Changes in fasting plasma glucose at 12 weeks versus baseline.
3. Changes in HbA1c at 12 weeks versus baseline.

Safety assessment

- Presence and nature of adverse events during the therapy, their intensity (severity), relation to the product, outcome
- Percentage of patients with clinically relevant laboratory abnormalities after 12-week therapy
- Changes in vital signs.

[1] **Impaired glucose tolerance (IGT)** is a condition characterized by plasma glucose concentration of 7.8 to 11.0 mmol/L 120 min after oral glucose tolerance test (OGTT), with fasting plasma glucose <7.0 mmol/L.

[2] **Oral glucose tolerance test (OGTT)** should be carried out in the morning against regular diet and regular physical activity. The test should be preceded by an overnight fast (8-14 hours, water allowed). A carbohydrate-containing meal (30–50g) should be consumed on the evening before the test. Once fasting blood has been drawn, the patient should consume 75g anhydrous glucose (or 82.5g glucose monohydrate) in 250-300 ml water for 5 minutes. Smoking is prohibited during the test. Repeat blood samples are taken after 2 hours. Plasma glucose test will be made in central laboratory, therefore to avoid glycolysis and wrong results the blood should be collected into a tube containing preservative (sodium fluoride) provided by the sponsor.

Study design

Design: a multicenter, double-blind, placebo-controlled, parallel-group randomized trial.

The study will enroll outpatients adults aged 18-70 years with impaired glucose tolerance (IGT) who did not receive glucose-lowering agents previously. Persons with pre-diabetes[3], obesity (especially with visceral or abdominal obesity), dyslipidemia (with high triglycerides and/or low-density lipoproteins), hypertension, diabetes in first-degree relatives will be considered as potential candidates to participate in the study.

After the patient's information sheet and the form of informed consent for participation in the study are signed on the screening (visit 0; day from -7 to 0), complaints and history are collected, registration of concomitant conditions and diseases, objective examination, calculation of body mass index (BMI), oral glucose tolerance test (OGTT), fasting blood glucose. Furthermore, blood samples[4] (for testing HbA1c, chemistry and hematology, HLA genotyping) and urine sample will be collected; concomitant therapy will be recorded, diet and physical activity recommendations will be given. All women of childbearing potential will be administered pregnancy tests.

If eligibility criteria are met and there are no non-inclusion criteria on Visit 1 (Day 1), the patient is randomized to one of two groups: the Subetta group (patients will take 2 tablets twice a day for 12 weeks) or placebo group (patients will take a placebo according to the scheme of Subetta for 12 weeks).

In the course of the study, two more visits in 4 (Visit 2) and 12 (Visit 3) weeks are planned, during these weeks complaints are recorded, a patient's examination is recorded, therapy compliance and safety are assessed. On Visit 3 OGTT, fasting blood glucose, blood samples (for HbA1c, chemistry and hematology) and urinalysis will be performed.

The duration of observation period shall be up to 13 weeks.

During the study the treatment for underlying conditions will be allowed with the exception of the drugs indicated in the section “Prohibited concomitant therapy”.

Inclusion and exclusion criteria

Inclusion criteria

1. Outpatients aged 18 to 70 years.
2. Impaired glucose tolerance (plasma glucose from 7.8 to 11.0 mmol / L 2 hours after a 75 g oral glucose consumption during an oral glucose tolerance test, while fasting plasma glucose <7.0 mmol / L).

[3] **Prediabetes**: Fasting plasma glucose = 6.1–6.9 mmol/L orand 2) 2-hour plasma glucose post-OGTT = 7.8–11.0 mmol/lL or/and 3) HbA1c 5.7–6.4%.
[4] All investigations are carried out in a central laboratory.

3. HbA1c is 5.7–6.4%.
4. The body mass index is 25.0-39.9 kg / m^2.
5. Consent to use reliable contraceptive methods during the study (for men and women with reproductive potential).
6. The presence of the signed informed consent form to participate in the clinical trial.

Exclusion criteria

1. Type I or II diabetes mellitus.
2. Use of any medications indicated in the section "Prohibited concomitant medications".
3. Acute or exacerbation/decompensation of chronic disease of any etiology at the time of examination or during the previous week.
4. Uncontrolled arterial hypertension with blood pressure: systolic blood pressure > 160 mm Hg and/or diastolic blood pressure > 100 mm Hg.
5. Acute coronary syndrome, myocardial infarction, acute impairment of cerebral circulation during the previous 6 months prior to enrollment.
6. Unstable or life-threatening arrhythmia during the previous 3 months prior to enrollment.
7. Acute and chronic heart failure with functional class III or IV (according to the classification of the New York Heart Association, 1964).
8. Respiratory failure.
9. Chronic kidney disease (classes C3-5 A3).
10. Hepatic insufficiency (class C according to Child-Pugh).
11. Presence or suspicion of oncology disease.
12. The presence of an allergy / hypersensitivity to any component of the medication administered during the treatment.
13. Alcohol consumption > 2 alcohol units[5] for males and > 1 alcohol unit for females per day.
14. Mental illness or drug abuse in anamnesis.
15. Bariatric surgery in anamnesis, any surgery for 3 months before enrollment.
16. Pregnancy, breast-feeding; childbirth less than 3 months before enrollment.
17. Participation in other clinical trials for 3 months before enrollment in this study.
18. Patients who are related to any of the on-site research personnel directly involved in the conduct of the trial or are an immediate relative of the study investigator. 'Immediate relative' means husband, wife, parent, son, daughter, brother, or sister (regardless of whether they are natural or adopted).

[5] 1 alcohol unit − 15 g of ethanol or approximately 40 g of hard liquors or 140 g of wine or 300 g of beer.

19. Patients who work for MATERIA MEDICA HOLDING (i.e. the company's employees, temporary contract workers, appointed officials responsible for carrying out the research or immediate relatives of the aforementioned).

Criteria for Withdrawal or Termination

1. Screening failure.
2. Acute or exacerbation/decompensation of chronic disease of any etiology, with clinically relevant symptoms persisting for more than 5 days.
3. Inability or refusal of patient to comply with the protocol requirements.
4. Necessity in medical products prohibited within the study.
5. Development of adverse event requiring cancellation of investigational product.
6. Desire of patient to complete the study ahead of schedule due to inefficacy of therapy or any other reason.
7. Pregnancy.
8. Cases not specified by the protocol when, according to the investigator's opinion, further participation in the study harms the patient.
9. Incorrect inclusion of ineligible patient.
10. Participation in another clinical study.
11. Unblinding.

Number of subjects

It is planned to include 842 patients, which is expected to allow at least 286 patients (143 patients in both groups - Subetta and Placebo) to complete all protocol procedures.

Interim analysis

The study provides 1 intermediate "non-blind" analysis. At the request of the sponsor, a blind interim analysis can be performed to clarify population characteristics and possible correction of the sample size (only in the direction of increase).

Treatment

Group 1

Name of the medicinal product: Subetta

Active ingredient: affinity purified antibody to C-terminal fragment of insulin receptor beta-subunit – 0.006 g*, affinity purified antibodies to endothelial NO synthase - 0.006 g*

** Mixture of water-ethanol dilutions 100^{12}, 100^{30}, 100^{200} of active substance used for saturation of isomalt.*

Excipients: isomalt, crospovidone, magnesium stearate.

Method of administration: Tablet for oral use. 2 tablets twice daily. The tablets should be held in mouth until completely dissolved 15 min prior to meal.

Dosage form: Tablets.

Description: White to off-white, flat, cylinder-shaped, beveled edge tablets scored on one side, with integral edges.

Storage conditions: Store at a temperature not exceeding 25°C.

Group 2

Name of the medicinal product: Placebo

Active ingredient: NA

Excipients: isomalt, crospovidone, magnesium stearate.

Method of administration: Tablet for oral use. 2 tablets twice daily. The tablets should be held in mouth until completely dissolved 15 min prior to meal.

Dosage form: Tablets.

Description: White to off-white, flat, cylinder-shaped, beveled edge tablets scored on one side, with integral edges.

Storage conditions: Store at a temperature not exceeding 25°C.

Treatment duration

Subetta/Placebo treatment duration is 12 weeks.

Observation period

The duration of observation period shall be up to 13 weeks (screening up to 1 week, study therapy 12 weeks).

Symptomatic (Standard) treatment

Throughout the study, patients should adhere to the recommendations for lifestyle modifications (good nutrition and regular physical activity).

Patients may receive treatment for co-morbid conditions with medicines that are not listed in the "Prohibited concomitant treatment" section.

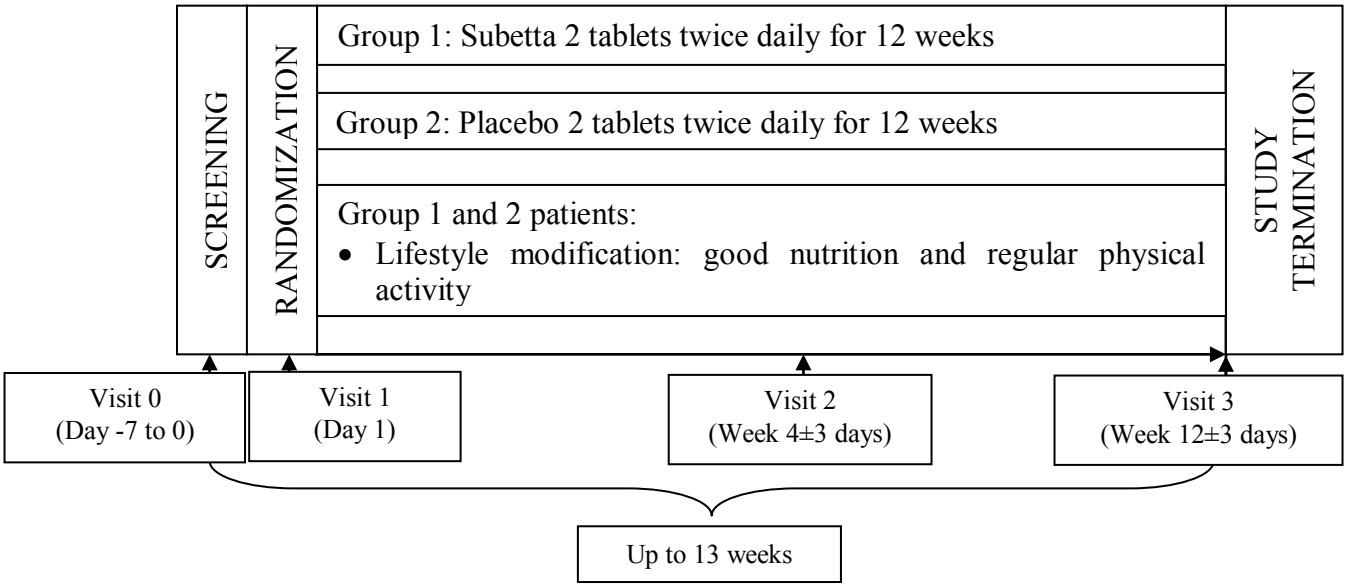
Prohibited concomitant therapy

The medicines specified in cl. 1 and 2 belong to prohibited ones at baseline (during any period of time) and during the study.

The medicines specified in cl. 3-17 belong to prohibited ones within 3 months prior to enrollment and during the study.

1. Oral and other antidibetic drugs:
    - biguanides (metformin, phenformin, etc.)
    - sulphonylurea derivatives (glibenclamide, tolbutamide, etc.)
    - thiazolidinediones (rosiglitazone, pioglitazone, etc.)
    - alpha-glucosidase inhibitors (acarbose, miglitol)
    - dipeptidyl peptidase 4 inhibitors (DPP-4): sitagliptine, vildagligptine, saxagliptine, linagliptine, alogliptine, gosogliptine
    - glucagon-like peptide receptor analogues (GLP-1): exenatide, liraglutide, lixisenatide, dulaglutide, etc.
    - sodium-dependent glucose cotransporter type 2 inhibitors (SGLT2): dapagliflozin, empagliflozin, canagliflozin
2. Insulins and their analogs
3. Glucocorticosteroids for systemic use and topical glucocorticosteroids
4. Thyroid hormones
5. Oral contraceptives
6. Danazol
7. Chlorpromazine
8. Beta-2-sympathomimetics for injections
9. Appetite-stimulating drugs
10. Drugs for treatment of obesity
11. Nitric oxide release modulators, including nebivolol
12. Nitric oxide donators (long-acting nitrates and nitrate-like agents, except for on-demand nitrates used in angina episodes)
13. Diuretics
14. Biologically active food supplements
15. Phytodrugs
16. Products manufactured by Materia Medica Holding (Divaza, Impaza, Afalaza)
17. Drugs known to have caused allergic reactions

Study design scheme



Schedule of study procedures

Procedure \ Visit	Visit 0 (Day -7 to 0) Screening	Visit 1 (Day 1) Screening, randomization	Visit 2 (Week 4±3 days)	Visit 3 (Week 12±3 days)
Informed consent	+			
Study subject registration in IVRS and assignment of a personal code	+			
Collection of complaints	+	+	+	+
Medical history	+			
Registration of co-morbid conditions and diseases	+	+		
Physical examination	+	+	+	+
Registration of vital signs (heart rate, respiratory rate, blood pressure)	+	+	+	+
BMI calculation	+			
Concomitant therapy	+	+	+	+
Eligibility assessment	+	+		
Randomization of the study subject		+		
Study drug supply		+	+	
Study drug accountability and return, compliance assessment			+	+
Recommendations (good nutrition and regular physical activity)	+	+	+	+
Evaluation of treatment safety		+	+	+
End of visit	+	+	+	+

Completion of the patient's participation in the study				+
Investigations:				
OGTT*	+			+
Blood sampling for HbA1c	+			+
Blood sampling for HLA genotyping	+			
Blood and urine samples for safety analysis	+			+
Pregnancy test**	+			
** Fasting blood glucose will be determined at OGTT (prior to glucose loading)* *** If applicable*				

Statistical Analyses.

SAS 9.4 will be used for data processing and statistical calculations[6].

Samples

Total set: all patients included in the study who have signed Informed Consent Form for participation in the study. All AEs including those occurred prior to the study therapy will be considered throughout the study for this sample.

Safety population: all patients who received at least one dose of the study drug. This sample will be used for the study treatment ***safety and tolerability analyze***, since all the AEs identified after the study drug administration will be recorded.

Full Analysis Set. This sample includes all enrolled and randomized patients, except for those who met at least one of the following criteria:

1) non-compliance with inclusion / exclusion criteria;
2) the patient has not taken as minimum a single dose of the study drug;
3) lack of any data of the patient after prescription of the study drug.

This sample is the most consistent with the "Intention-to-treat" principle**,** will be used for the ***Intention-to-treat analysis (ITT analysis) of the the study therapy efficacy***.

Per protocol set. This sample includes all patients who completed the therapy as per the study protocol without any missing scheduled visits. This sample will be used for the ***Per Protocol analysis (PP- analysis) of the the study therapy efficacy***.

Per Protocol set will not include the patients whose data are completely or partially invalid for analysis due to a protocol deviation.

Protocol deviations that lead to complete or partial data invalidity:

1. Violation of visit schedule.
2. Inappropriate distribution/supply of the study drug.

[6] Licensee: OOO "NPF "Materia Medica Holding", No. 70100045

3. Administration of prohibited therapy.
4. Increase or decrease ≥ 25% in the amount of the study therapy administered.
5. Inability to assess the patient's compliance using the formula (e.g. loss of pack with the product).
6. Major discrepancies between source documents and CRF detected during monitoring or another authorized check.
7. Violation of the procedure for obtaining Informed consent.
8. Non-compliance with the clinical study protocol procedures.
9. Inability to collect all patient's data used for evaluation of the study endpoints (e.g. lack of entries in source documents required for verification of inclusion/exclusion criteria, safety and efficacy criteria).
10. Other protocol deviations resulting in full or partial data invalidity.

Evaluation of sample size

The sample size has been assessed on the basis of the following rules and assumptions:

1. Statistical provisions.
   1.1 the power of the statistical tests "P = (1 - β)" is assumed to be 80% (the probability of correct rejection of the null hypothesis is 0.8);
   1.2 the probability of a type I error "α" is allowed to be less than 5% (the probability of the erroneous acceptance of an alternative hypothesis is less than 0.05);
   1.3 the statistical tests are 2-sided unless otherwise specified;
   1.4 the calculation of the sample size is based on the assumptions about the expected effects, declared in the primary efficacy endpoint of the Protocol;
   1.5 the ratio between the sample sizes of Subetta and Placebo groups is 1:1 (1 Subetta patient - 1 Placebo patient);
   1.6 statistical hypotheses - null and alternative hypotheses on superiority of the study product over placebo under the dosing regimen used
   1.7 one unblinded interim analysis on 50% sample was expected allowing to terminate the study both due to the effect confirmation (according to O'Brien-Fleming) and due to acceptance of null hypothesis (Pocock).
      a) primary endpoint:

$$\mathbf{H_0: \Delta\mu_1 - \Delta\mu_2 = 0}$$

$$\mathbf{H_a: \Delta\mu_1 - \Delta\mu_2 \neq 0}$$

where $\mathbf{\Delta\mu_1}$ - mean reduction (initial – final score) in Subetta group,

$\mathbf{\Delta\mu_2}$ – mean reduction (initial – final score) in Placebo group;

1.8 calculation of sample size for statistical criteria will be made using the following formula:

$$\mathbf{n_1 = kn_2}$$

$$\mathbf{n_2} = \boldsymbol{R}\frac{\left(\boldsymbol{z_{\alpha/2}} + \boldsymbol{z_{\beta}}\right)^2 * (\mathbf{1} + \mathbf{1}/\boldsymbol{k}) * \boldsymbol{\sigma}^2}{\boldsymbol{\varepsilon}^2}$$

where $\mathbf{n_1}$, $\mathbf{n_2}$ is sample size in IP and placebo groups, respectively

$\boldsymbol{\varepsilon} = \boldsymbol{\Delta\mu_1}\text{-}\boldsymbol{\Delta\mu_2}$ is expected difference between mean reductions of value between Subetta and Placebo groups

k – coefficient of Subetta/Placebo sample ratio (1 : 1)

σ – standard deviation of reduction

$z_{\alpha/2}$ – tabular value of two-sided z-test for **α**

z_{β} – tabular value of one-sided z-test for β, R-coefficient taking into account interim analysis depending on the accepted functions of error and analysis position (in this design equal to 1.1334).

1.9 terminal sample size was determined using the formula:

$$\mathbf{N_T = N_{PP}/(1\text{-}R_w)}$$

where $\mathbf{N_T}$ – terminal sample size

$\mathbf{N_{PP}}$ – result of calculation in cl. 1.8, i.e. scheduled number of subjects completing the study per protocol

$\mathbf{R_w}$ – withdrawal rate.

2. Assumptions on expected clinical study effects: difference in reduction of 2-hour plasma glucose (at OGTT) between Product and Placebo groups will be no less than **ε** = 1.1 at standard deviation of **σ** = 3.11.

A priori estimates for tested parameter variables in the groups:

Primary endpoint:

Changes in 2-hour fasting plasma glucose (OGTT) at 12 weeks vs. baseline.

Placebo group:

characteristics of 2-hour plasma glucose level (at OGTT) at the start of the study.

SD=0.94 [39]

After 3 months

SD=2.3 [1.87; 2.987] [40]

Change (3 m) –0.2 [–1; 0,6] [40]

Standard deviation of change in 3 months (in assumption of independence of scoring for DM)

SD_{Δ}~311.

Study drug group:

Based on interim study, change in glucose level in experimental group in 3 months was:

Change (3 m) = 1.96 [1,7; 2,22]

Let us take standard deviation of change in experimental group similar to control group.

Software code for evaluation of sample size:

```
Proc seqdesign errspend;
Design nstages=2
Info=cum(0.5,1)
Method(alpha)=UNI(rho=0.5 tau=0)
Method(beta)=UNI(rho=0 tau=0)
Stop=both
Alpha=0.05
Beta=0.2;
Samplesize model=twosamplemeans(meandiff=1.1 stddev=3.11);
Run;
```

Therefore, the value of expected effect is rated as **ε_{Δ}=1.1** mmol/L (the most conservative scoring) – the worst case for Subetta and the best for Placebo) at standard deviation **σ_{Δ}=3.11** mmol/L. Thus, sample size will be 143 subjects per study group. Taking into account the claimed withdrawal coefficient, this will require enrollment of 842 subjects.

Therefore, overall size of the required sample will be 842 subjects ensuring the study completion per protocol by at least 286 subjects.

Therefore, the sample size has been found to be (143) patients in each of the groups (Product and Placebo) to estimate superiority of the study drug over placebo.

Taking into account potential withdrawal of at least 66% subjects[7] (Cw=0.66) during the study for various reasons, at least 842 patients should sign PIS and ICF.

Statistical criteria

All statistical calculations will be performed using two groups of statistical criteria:

- parametric – to obtain effective estimates for random parameters in case the relevant conditions of method/model applicability are not violated (e.g. sphericity, normality, risk proportionality, etc.);

[7] Based on blinded interim analysis.

- non-parametric – in all other cases.

Parametric criteria

The application of parametric criteria will be accompanied by a check of models for applicability (e.g. Kolmogorov-Smirnov test, Shapiro-Wilk test).

The following parametric methods and approaches are to be used:

1. To assess the differences of continuous variables obtained in one group at two different visits – Student t-test for paired samples.
2. To assess the temporal dynamics of the compared values – analysis of variance (ANOVA) or covariance (ANCOVA) in the modification with repeated measures.
3. In case of multiple comparisons between the groups will apply a variety of corrections for multiplicity Dunnett, Tukey, Scheffe, Holm adaptive test, etc.
4. In case of abnormal data distribution, approaches with the Generalized Linear Models and /or Mixed Linear Models will be used.
5. Selection of the type of distribution, clarification of the factor and covariance structures of the model is carried out with fit statistics such as AIC (Akaike information criterion).

To perform the above-mentioned statistical tests and techniques, it is assumed that the following SAS procedures are used:

- UNIVARIATE – check for normality of the compared distributions;
- CORR, MEANS – calculation of descriptive statistics;
- TTEST – Student t-test with all the modifications;
- GLM – analysis of Generalized Linear Models for studying temporal dynamics (ANOVA, ANCOVA);
- GENMOD – analysis of Generalized Linear Models.
- MIXED – analysis of Generalized Linear Models.

Non-parametric criteria

Below, there are the main types of possible comparisons with the respective criteria:

1. To assess the dynamics of the compared indicators – Friedman test, non-parametric analogue of analysis of variance with repeated measures.
2. For the frequency analysis of 2×2 cross tables – χ^2-test (if the compared frequencies are greater than 5) or Fisher exact test (if one of the compared frequencies is less than 5).
3. For the frequency analysis of cross tables with independent strata – Cochran–Mantel–Haenszel test (modification of the χ2-test for multiple comparisons).

4. For the frequency analysis of data on the presence / absence of an event or outcome during repeated measures (cross tables with dependent strata) – survival analysis.

To perform the above-mentioned non-parametric statistical analysis options, it is assumed that the following SAS procedures are used:

- FREQ – Friedman test, χ^2-test and / or Fisher exact test; Cochran–Mantel–Haenszel test
- LIFETEST, PHREG – survival analysis
- NPAR1WAY – Mann-Whitney U-test.

Safety parameters

Adverse events recorded during the study will be grouped into frequency tables by severity, seriousness and relationship with the study drug.

Data presentation

Descriptive statistics will be provided for each study continuous / interval variable. Statistic data will be presented by mean, standard deviation, min and max values. Comparisons suggesting statistical conclusion will have the relevant confidence intervals. Outliers will be analyzed individually. The data will be grouped by visits. The categorical variables will be presented as frequency tables by visits.