Statistical analysis plan

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Prepared and approved for the BIOMArCS 2 glucose trial by
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Introduction
This Statistical Analysis Plan (SAP) is developed to guide the main analysis of the BIOMArCS 2 glucose trial data, i.e. the analysis of the relation between randomly allocated treatment (intensive glucose regulation or conventional glucose management) and infarct size as measured by cardiac Troponin T at 72 hours after admission. This document is based on the original study protocol and published trial design, and it was disclosed before trial data were released (Netherlands Trial Register, trial ID: NTR 1205). The SAP describes the patient characteristics and outcomes that will be analyzed, as well as the statistical methods that will be applied.
Role of the funding source

The BIOMArCS 2 glucose trial was sponsored by the Foreest Instituut, Alkmaar, The Netherlands and the Interuniversity Cardiology Institute of the Netherlands (ICIN), Utrecht, The Netherlands. The study was initiated by the study investigators (Boersma, Cornel, Umans), and was designed and conducted independently of the sponsors. This SAP was also prepared by the study investigators, entirely independent of the sponsors. Representatives of the funding sources did not have any influence on the planned analyses, nor will they have influence on the interpretation and reporting of the trial results.
Background of the study

Elevated admission plasma glucose (APG) is associated with increased mortality in patients who are admitted with an acute coronary syndrome (ACS). This is probably mediated by increased inflammation, apoptosis and coagulation and a disturbed endothelial function that can be found in hyperglycemic patients. Insulin has several characteristics that can (potentially) counteract these mechanisms.

Design

The BIOMArCS 2 glucose trial is a single centre prospective randomized open-label clinical trial. The primary objective is to evaluate the effectiveness and safety of intensive glucose regulation with intravenous insulin compared to conventional (expectative) glucose management in patients presenting with an ACS and an APG between 7.8 and 16 mmol/l. A total of 300 patients will be randomized in a 1:1 ratio to intensive or conventional glucose management. All patients will receive standard medical care.

The primary endpoint is myocardial infarct size, as expressed by the cardiac Troponin T level 72 hours after admission. We postulate that intensive glucose regulation may help to limit infarct size. To study the metabolic effects of insulin administration, we will investigate biomarker wash out patterns of various metabolic mechanisms. These will address inflammation, oxidative stress, hypercoagulability, endothelial activation and vasodilatation.


Follow up

The clinical follow up data that will be presented together with the results of the primary endpoint analysis will consist of data collected until the first outpatient visit, which is scheduled 6 weeks after randomization. We intent to collect endpoints on a yearly base, which will be analyzed in the future.
**Endpoint definitions** (based on the ARC Dublin criteria)

**Death**: All deaths are considered cardiac unless an unequivocal non-cardiac cause can be established.

**Cardiac death**: Any death due to proximate cardiac cause (e.g. MI, low-output failure, fatal arrhythmia), unwitnessed death and death of unknown cause, and all procedure-related deaths, including those related to concomitant treatment.

**Vascular death**: Death caused by non-coronary vascular causes, such as cerebrovascular disease, pulmonary embolism, ruptured aortic aneurysm, dissecting aneurysm, or other vascular diseases.

**Non-cardiovascular death**: Any death not covered by the above definitions, such as death caused by infection, malignancy, sepsis, pulmonary causes, accident, suicide or trauma.

**Re Myocardial Infarction**

Troponin I or CKMB as routinely measured in clinic > Upper normal limit (UNL) (i.e. >0.45 ug/l or >16 U/l respectively) or ECG showing new persistent or non-persistent ST-segment elevation >1.0 mm in two or more contiguous leads. Given that previous values have stabilized (= Stable or decreasing values on 2 samples and 20% increase 3 to 6 hours after second sample).

For **peri procedural infarction**:
- PCI > 3x UNL Troponin or CKMB, <48 hrs after PCI
- CABG > 5x UNL Troponin or CKMB, <7 days after CABG

**Stent thrombosis (definite)**

I.e. angiographic or pathologic confirmed stent thrombosis.

+ The presence of a thrombus that originates in the stent or in the segment 5 mm proximal or distal to the stent and (within 48 hours) typical anginal complaints, new suggestive ECG findings or positive MI biomarkers.

  - Early stent thrombosis; 0 - 30 days
  - Late stent thrombosis; >30 days

**Target Lesion Revascularization (TLR)**

The target lesion is defined as the treated segment from 5 mm proximal to the stent and to 5 mm distal to the stent.

Any repeat percutaneous intervention of the target lesion or bypass surgery of the target vessel performed for restenosis or other complication of the target lesion.
Elective Revascularization (Non Target Lesion)
Elective revascularization (PCI or CABG) for another lesion than index event related lesion.

Stroke
Ischemic or hemorrhagic stroke, Diagnosed by typical clinical presentation plus confirmed by CT scan of the brain and/or neurologist.

Endpoint Adjudication
Clinical events were recorded by the investigators and prior to data analysis all clinical events were adjudicated by an independent endpoint committee.
Statistical handling policy

Significance
All statistical tests will use a significance level of \( \alpha = 0.05 \), all tests will be two sided.
Confidence intervals (CI) will be presented with 95\% degree of confidence. All p values will be rounded to 3 decimal places; p values that round to 0.000 will be presented as <0.001.

Data summarization
Dichotome variables will be presented as numbers and percentages, continuous variables will be presented as medians with their 25\textsuperscript{th} and 75\textsuperscript{th} percentile, or means \( \pm \) one standard deviation (SD), as appropriate.

Data distribution
Chi-square tests will be used to analyze differences in categorical data between independent groups. If expected cell frequencies are less than 5, Fisher’s exact tests will be used instead. The distribution of continuous data will be tested with the Kolmogorov-Smirnov test. We assume a normal distribution in case of a non significant test (p >0.05). Student’s t-tests will be used to analyze differences in continuous data with normal distribution between independent groups. Mann-Whitney tests will be used in case of non normal distributions.
Statistical analyses

Intention to treat population
All analyses will be performed according to the intention-to-treat (ITT) principle. The ITT population consists of all subjects who were correctly assigned a randomization number. We will exclude patients from the ITT population who were evidently randomized erroneously, i.e. patients without ACS who were identified as such within 1 hour after randomization, and who had no further study procedures except randomization. The ITT population is considered the main analysis population of the BIOMArCS 2 glucose trial.

Per protocol population
We will also perform a per protocol (PP) analysis. The PP population is defined as a subset of the ITT population, and consists of patients who are actually treated as randomized.

Primary efficacy analysis
The primary efficacy endpoint of the BIOMArCS 2 glucose trial is the myocardial infarct size, defined as the cardiac Troponin T level at 72 hours after admission. Troponin T at 72 hours will be measured in a core laboratory (Erasmus MC) by using the Cobas 8000 analysis system. During admission, blood samples are collected at regular time points until 96 hours. The 72 hours sample is defined as the sample that is collected a) within 48 to 96 hours after admission, and b) that has smallest absolute time distance to the 72 hour time point.

We will determine the mean (median) Troponin T value at 72 hours in both treatment arms. A Student’s t test (or Mann-Whitney test, as appropriate) will be applied to evaluate the (statistical significance of the) difference between the mean (median) values.
**Further analysis of cardiac Troponin - I**
The relation between allocated treatment and Troponin T at 72 hours will be further studied with help of linear regression, adjusting for the Troponin T value at admission (model A), and adjusting for age, gender and the Troponin T value at admission (model B).

**Further analysis of Troponin - II**
Patients will be classified as having had a ‘small’ or ‘large’ myocardial infarction, as follows. Patients with a Troponin T value at 72 hours < than the mean value of all patients (irrespective the allocated treatment) will be classified as having had a ‘small’, whereas those with a value ≥ the mean will be classified as having had a ‘large’ infarction. If Troponin data are non normally distributed, the median (in stead of the mean) value will be used as threshold. The relation between allocated treatment and dichotomized infarct size will then be studied by logistic regression analysis. We will run a univariable model with allocated treatment as single determinant (model A), as well as a multivariable model with adjustment for Troponin T values at admission (model B), and a model with adjustment for age, gender and admission Troponin T (model C). Results will be reported as odds ratios and 95% CIs.

**Secondary endpoint analysis**
We will also estimate infarct size with two other techniques.

First, the area under the CKMB curve will be calculated by the linear-trapezoidal method that is described by Vollmer et al *Am.J.Clin.Pathol*. 1993;100:293-8. CKMB values will be obtained at admission and 6, 12, 24, 36 and 72 h after the onset of symptoms. Missing baseline and 72 hour values will be substituted with the value 0. The log normal function will be used to estimate missing values at intermediate time points.

Second, a rest gated myocardial perfusion scintigraphy (MPS) will be performed 6 (±1) weeks after the index event, using 99mTc-myoview single photon emission computed tomography (SPECT). Based on this technique, infarct size, left ventricular ejection fraction (LVEF), and summed rest score (SRS) will be determined.

**Missing data**
When using modeling techniques, missing data will be imputed when ≥1% of the input of a variable is missing. Data will be imputed using multiple imputation.
Subgroups

Treatment effects will be studied in relation to the following characteristics:

- Sex
- Age; <75 vs. ≥75 yrs
- Killip class; class 1 vs. class 2 or higher
- Anterior vs. non anterior infarction
- Non-insulin treated diabetes vs. previous unknown diabetes
- ST segment elevation- vs. non-ST-segment elevation ACS
- Time from onset of symptoms to randomization
- Admission plasma glucose level < 10 mmol/l vs. ≥ 10 mmol/l and <10; 10 – 12; ≥ 12 mmol/l
- HbA1c level; < 5.7%; 5.7 – 6.4% and ≥ 6.5%

Furthermore we will compare the treatment effect of intensive versus conventional glucose regulation in the following subgroups:

1) For ST segment elevation ACS patients, those with admission plasma glucose level < 10 mmol/l vs. ≥ 10 mmol/l.
2) For Killip class 2 or higher patients, those with admission plasma glucose level < 10 mmol/l vs. ≥ 10 mmol/l.
3) For ST segment elevation ACS patients, those admitted < 2 hours after onset of symptoms vs. ≥ 2 – 6 and ≥ 6 hours.

Further analyses on patients who received intensive glucose regulation

We will separately investigate patients who received intensive glucose regulation. In this subset, we will study:

1) The relation between the duration of IV insulin administration (<24 versus ≥24 hours) and Troponin T at 72 hours after admission.
2) The relation between the glucose level (<7.8 versus ≥7.8 mmol/l) at the time the insulin perfusor was actually started and Troponin T at 72 hours after admission.
3) As 2, but now using the value of 10 mmol/l as threshold.
4) The relation between the timing (≤2 versus >2 hours after admission) of the start of the insulin perfusor and Troponin T at 72 hours after admission.