Statistical Analysis Plan

PRAISE

PRediction of Acute coronary syndrome in acute Ischemic StrokE

BMBF Projekt FKZ: 81X1710109

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Abbreviations

SAP	Statistical Analysis Plan
IEC	Independent Ethics Committee
IRB	Institutional Review Board
mITD	Modified Intention-to-diagnose
PP	Per Protocol population
EFS	Evalued for Safety Set
DSMB	Data and safety monitoring board
EAC	Endpoint adjudication committee
ACS	Acute coronary syndrome
hs-cTn	High-sensitivity cardiac Troponin
mRS	Modified Rankin Scale NIHSS
MoCA	Montreal Cognitive Assessment
PSS	Perceived Stress Scale
IADL	Lawton Instrumental Activities of Daily Life Scale
NIHSS	National Institutes of Health Stroke Scale
TICS	Telephone Interview with Cognitive Status
mRS	Modified Rankin Scale NIHSS
TIA	Transient ischemic attack
MI	Myocardial infarction
ROC	Receiver-Operating-Characteristic
AUC	Area under the curve

1 Introduction

This Statistical Analysis Plan (SAP) is based on the study protocol version 1.1 of June 19, 2018 (also published in (Nolte, et al. 2020)) and follows the guideline for statistical analysis plans (Gamble, et al. 2017)

Some points of the statistical methods and of the study design are already described in the study protocol. This Statistical Analysis Plan (SAP) aims to further specify the procedures and statistical methods applied during the final analysis of the study data.

1.1 Background and rationale

Elevation of cardiac troponin can be found in about 30% of patients with acute ischemic stroke (depending on the assay and cut-off used). Elevated troponin indicates increased mortality in stroke patients. There is currently little evidence regarding the ideal care of these patients. The investigators know from previous studies that approximately 25% of acute stroke patients with elevated levels of cardiac troponin have culprit lesions on coronary angiogram.

The primary goal of the PRAISE study is to develop a diagnostic algorithm that allows the prediction of an acute coronary syndrome (ACS) in stroke patients. To achieve this, clinical symptoms, troponin levels as well as findings on ECG, echocardiography and coronary angiography will be systematically evaluated. The PRAISE study is a multicenter study with more than 25 sites in Germany. Acute ischemic stroke patients with elevated troponin level are eligible for participation in the study. The primary endpoint is the diagnosis of acute coronary syndrome as established by an independent endpoint committee.

1.2 Objectives

The primary goal of the multicenter PRAISE study is to develop a diagnostic algorithm to better identify ACS as origin of troponin elevation in patients with an acute ischemic stroke. The primary hypothesis will test whether dynamic troponin levels (as compared to stable ones) indicate presence of ACS. The following hypotheses will be tested:

Primary hypothesis: A dynamic troponin elevation (>50% change at measurement after 3 hours) indicates an ACS in patients with an acute ischemic stroke. The change in troponin level (dynamic vs. stable after 3 hours) within patients with an acute ischemic stroke can be used to differentiate whether an ACS is present.

Endpoint: diagnosis of "ACS", determined by an independent endpoint adjudication committee.

1. Secondary hypothesis: Stroke patients with dynamic troponin change have a worse functional outcome three or twelve months after the event in comparison to those with stable troponin.

Endpoints: Modified Rankin-Scale (mRS) after three and twelve months.

2. Secondary hypothesis: Stroke patients with dynamic troponin change have an increased risk for recurrence of cardiovascular events and a higher mortality rate in comparison to those with stable troponin.

Endpoints: Brain infarcts, transient ischemic attack (TIA), myocardial infarction (MI) and death as well as the combined endpoint thereof (MACE) during the acute inpatient stay, after three and twelve months.

In addition, in an exploratory approach we want to establish a combined rule-in/rule-out diagnostic algorithm to identify the subgroup of troponin-positive stroke patients in whom the diagnosis of ACS (i.e. type I vs. type II MI) vs. not is made by means of coronary angiography including stroke-specific cut-off values for troponin and other biomarkers, the value of ECG, echocardiography, clinical parameters, and combinations thereof.

1.2.1 Primary Outcome

The primary outcome is defined as presence of ACS within seven days of admission to hospital ("V2") defined by an independent endpoint adjudication committee on individual patient basis (i.e. ST-elevation myocardial infarction STEMI, Non-STEMI, and unstable angina vs. other cardiac and non-cardiac).

1.2.2 Main Secondary Outcomes

The secondary outcome parameters are defined as:

- Mortality (recorded at 1 week, 3- and 12-months follow-up)
- Cardiovascular events (new stroke, TIA, MI; at 1 week, 3- and 12-months follow-up)
- Combined vascular endpoint (MACE, i.e. mortality plus cardiovascular endpoints as above)
- Functional outcome (mRS; at baseline, 1 week, 3- and 12-months follow-up)
- Imaging (Stroke location and size, degree of cerebral white matter damage)
- New hospital admission

During the course of the study it turned out that the imaging procedure and count of hospital admission were not realisable. Therefore, these endpoints cannot be analysed.

1.2.3 Safety Outcome Parameters

Documentation of SAEs and assessment by a critical event committee (CEC), regular review by a data and safety monitoring board (DSMB).

Procedure-related complications of coronary angiography: clinically manifest stroke, renal failure, hematoma at site of puncture (groin); assessment by independent data and safety monitoring board. For definition of serious adverse events of special interest see study protocol.

2 Study Methods

2.1 Trial design

Observational, prospective, phase III ((Pepe 2003), (Zhou, Obuchowski und McClish 2011)), multicenter diagnostic trial with blinded endpoint assessment of pre-defined endpoints.
All patients receive troponin measurement at hospital admission (defined as 0 hrs) and again at 3 hrs.
For details of target condition, index text and reference standard see study protocol.

2.2 Randomization

For this type of study, a randomization was not required (within-subject design). Troponin progression measurement and coronary angiography are performed in all patients.

2.3 Sample size

To be assessed for eligibility (n = 8,750 patients with ischemic stroke, thereof n = 1,250 with troponin elevation according to the inclusion criteria)
To be allocated to trial (n = 251)
To be analyzed (n = 203)
Full details of sample size calculation can be found in the study protocol.

2.4 Framework

Prospective, single-arm, multicenter, phase 3 diagnostic accuracy study.

2.5 Interim analyses

Interim analyses in terms of efficacy will not be performed.

2.6 Timing of final analysis

All outcomes will be analysed collectively after the last follow-up and data cleaning process are finished. The final analysis of the primary endpoint will take place after the database has been reviewed for completeness and accuracy and is determined by the Endpoint Adjudication Committee to be complete. The results will be reported according to the STARD Statements ((Bossuyt, et al. 2003))

2.7 Timing of outcome assessments

A detailed schedule of the planned outcome collection is given in table 1 of the study protocol (Nolte, et al. 2020).
The primary outcome, presence of ACS, is assessed within 7 days of hospital admission ("V2"). Follow-up measurements will be carried out after 3 ("V3") and 12 months ("V4").

3 Statistical Principles

3.1 Confidence intervals and *P* values

All applicable statistical tests will be two-sided and will be performed using a 5% significance level. Analyses of secondary outcomes will be performed without adjustment for multiplicity. All confidence intervals presented will be 95% and two-sided.

3.2 Adherence and protocol deviations

Adherence is not relevant in this study, since there is no therapeutic intervention for study purposes to be followed.

All protocol violations will be documented and classified as major or minor in a blinded manner by an independent staff member who is not involved in the study process.

A major protocol deviation occurs if contrary to the study protocol a patient could not receive a coronary angiography. The following reasons are listed:

- Data from coronary angiography are not provided for technical reasons.
- Patient refuses coronary angiography.
- Clinician declines coronary angiography for medical reasons.
- Coronary angiography is not performed for capacity reasons.
- A transfer of images from the ultrasound scanner is not possible for technical reasons.

Further, a major protocol deviation occurs if a troponin measurement is not available.

These patients will not be taken into regard into the primary analysis.

A minor protocol deviation occurs if a patient could not receive echocardiography. The following reasons are listed:

- Based on clinical indication, a transesophageal echocardiography is performed, which is not relevant for the objective of this trial.
- Echocardiography is not performed for capacity reasons.

Patients with minor protocol deviations are included in the primary analysis.

3.3 Analysis populations

3.3.1 Modified Intention to diagnose Population (mITD)

The primary analysis population is the mITD (intention to diagnose) population. The mITD population consists of all patients in whom troponin measurements at 0 and 3 hours were available.

3.3.2 Per Protocol population (PP)

The Per Protocol population includes all patients who have no major protocol violation. In particular coronary angiography must have been performed.

3.3.3 Evaluated for Safety Set (EFS)

All patients who received a coronary angiography and thus provided at least one troponin measurement will be included into the Evaluated for Safety (EFS) set.

4 Trial Population

4.1 Screening data

The following summaries will be presented for all screened patients:

- The reported number of stroke patients treated at the respective center
- the number and percentage of patients included

This summary will be provided overall and by study center.

4.2 Eligibility

The number of ineligible patients will not be reported.

4.3 Recruitment

All available recruitment information will be included and presented in a flow diagram according to the STARD Statements (Bossuyt, et al. 2003) (see Appendix).

4.4 Withdrawal/follow-up

In case of withdrawal from follow-up this is documented in different levels:

1. Patient says explicitly, that he/she does not want to take part on further phone interview regarding specific score assessments (timing: measurement time point of statement, documentation of reasons for the decision)
2. Patients are not able to attend follow-up examinations due to health issues.
3. Patient says explicitly, that he/she does not want to be contacted in further follow-ups.
4. Patients immediately withdraw consent for study participation after study inclusion.
5. Patients who are subsequently identified as not meeting the inclusion and exclusion criteria will be treated as patients who have withdrawn from the study.

The number by level of withdrawal will be tabulated.

4.5 Baseline patient characteristics

Baseline is defined as the measurement recorded at hospital admission (< 72 h after admission) ("V1"). All relevant characteristics to be measured at baseline are listed in table 1 of the study protocol (Nolte, et al. 2020)). Patients will be described with respect to

- Age
- Gender
- Medical history/comorbidities
    - Diabetes mellitus
    - Hypertension
    - Hyperlipidemia
    - History of coronary artery disease
    - Heart failure
    - Atrial fibrillation
    - Previous history of stroke
    - history of cancer
    - smoking status
- acute therapy of ischemic stroke
    - i.v. thrombolysis
    - thrombectomy
- Physical examination
    - Heart rate on admission
    - systolic blood pressure on admission
    - diastolic blood pressure on admission
- Current Medication
    - Antiplatelet therapy
    - dual antiplatelet therapy
    - oral anticoagulation
    - statin
    - beta-blockers
- NIHSS
- mRS
- GRACE Score
- MoCA
- Creatinine
- Haemoglobin
- Peak troponin T

both overall and separately for presence/absence of dynamic troponin elevation and additionally for the presence/absence of ACS as well as for the diagnostic categories no ACS, MI Type 1 and MI type 2. Categorical data will be summarised by numbers and percentages. Continuous data will be summarised by mean, SD, median, IQR and range. Number of available observations and number of missing observations will be presented for the diagnostic groups (dynamic vs. stable) separately and overall for both groups. Differences in baseline variables between the two diagnostic groups will be analysed by appropriate tests, e.g. Chi-squared test, Mann-Whitney-U test, or analyses of variance.

5 Analysis

5.1 Analysis methods

5.1.1 Primary endpoint

The primary outcome analysis will be performed in the mITD population using the adjudicated endpoint data of the endpoint adjudication committee.

The presence/absence of ACS in relation to presence/absence of dynamic troponin elevation (defined as rise/falling pattern of >50% (Δ-change at 0 vs. 3 hrs)) will be analysed by calculation of the diagnostic odds ratio together with the 95%-confidence interval. Corresponding p-value will result from the likelihood ratio chi-square test.

5.1.2 Secondary endpoints

Secondary endpoint analysed are performed in an explorative manner without adjustment for multiplicity.

The secondary endpoints

- Mortality
- cardiovascular events (stroke, TIA, Myocardial infarction)
- MACE (combined endpoint of death, recurrent stroke, TIA, myocardial infarction)

are time-to event data. Event rates at 1 week, 3- and 12-months follow-up are reported based on Kaplan-Meier estimators. Cox proportional hazards will be used to estimate hazard ratios (95%-CI) comparing the diagnostic groups (stable vs. dynamic) for mortality and cause-specific hazard ratios for cardiovascular events taking death as competing event into account.

The secondary endpoint

- mRS (measured at baseline, 1 week, 3- and 12-months follow-up)

is an ordinal scaled score which ranges from 0 (no symptoms) to 6 (death). This endpoint will be analysed by fitting a proportional-odds logistic regression model to calculate the common odds ratio (95%-CI) as a measure of the likelihood that individuals in the stable group would lead to lower scores on the mRS scale than would patients in the dynamic group.

During the course of the study it turned out that the imaging procedure and count of hospital admission were not realisable. Therefore, these endpoints cannot be analysed.
Table 1 gives an overview of measurement time points of all endpoints.

Endpoints	Time point			
	V1 < 72 h after admission	V2 < day 7 days	V3 3-months FU	V4 12-months FU
Primary endpoint				
Acute coronary syndrome (ACS)		X		
Secondary endpoints				
Cardiovascular events (stroke, TIA, death, MI)		X	X	X
MACE (combined endpoint)		X	X	X
Mortality		X	X	X
mRS	X	X	X	X
MI Type 1 / MI Type 2		X		

Table 1: Frequency and scope of study visits

5.2 Missing data

In case at least 5 % of the sample is missing, as a sensitivity analysis a multiple imputation is carried out in the mITD population to investigate the effects of missing values on the results of the primary and secondary analyses. To set up a proper imputation model, we will follow the recommendations of White, Royston and Wood (White IR 2011). The imputation model will include all outcome parameter variables secondary endpoints) at baseline and all further available time points as well as further variables for model refinement (as defined in 4.5). The number of imputations will be defined by the percentage of missing values in at least one of those defined variables at all time points.
Further sensitivity analyses for the primary endpoint will be conducted using other mechanisms for replacement of missing values (e.g. best/worst case).

5.3 Additional analyses

The primary outcome analysis is repeated in the PP population. Additional analyses of the presence/absence of ACS in relation to presence/absence of dynamic troponin elevation include the calculation of further diagnostic accuracy measures like sensitivity and specificity together with the 95%-confidence interval.

A mediation analysis will be conducted to identify direct and indirect effects of the diagnostic group (dynamic vs. stable) and additionally peak troponin T in patients with available troponin T values (exposure variables) and the presence of ACS (yes vs. no) as well as presence of MI type 1 (yes vs. no) and in patients with ACS MI (Type 1 vs. Type 2) (mediator variables) on mortality and MACE (target outcome) using accelerated failure time models. This will be performed after unblinding of the diagnostic groups and analysed in an explorative manner.

5.3.1 Subgroup analyses

Subgroup analyses with respect to the primary endpoint will be performed by the appropriate interaction tests. Each test will be performed at a significance level of alpha=5%.

Predefined subgroups will include:

- Gender (male/female)
- Age (dichotomized at 75 years)
- Renal failure (dichotomized at eGFR 60 ml/min)

5.3.2 Adjusted analyses

For the primary endpoint univariate and multiple logistic regression models will be fitted to the data to calculate unadjusted and adjusted odds ratios, including a series of baseline regressors:

- Age (continuous)
- Gender (dichotomous)
- Diabetes (dichotomous)
- Dyslipidemia (dichotomous)
- Renal failure (eGFR < 60 ml/min dichotomous)
- History of CHD (dichotomous)
- History of heart failure (dichotomous)
- History of atrial fibrillation/flutter (dichotomous)
- Haemoglobin (<12 g/dl for women, < 13 g/dl for men, dichotomous)
- Initial NIHSS (continuous)
- Heart rate (> 100/min, dichotomous)
- ECHO core lab: wall motion disturbance (dichotomous)
- ECG core lab findings: ACS typical changes (dichotomous)
- Peak troponin T value (continuous)

In an explorative analysis, an optimized multiple logistic regression model for the prediction of the primary endpoint is derived using the following procedure: in the first step, a multiple logistic regression model is established in a blinded manner, i.e. without taking troponin values into account, including relevant confounding variables into the model. In order to decide which variables are included in the model, clinical and statistical criteria are used, considering the distribution of the variables and their structure of missing values.

In the second step, variable selection is performed using backward selection.

In the last step, an unblinded analysis is performed, i.e. the troponin is included in the prognosis model to predict the primary endpoint. The final choice of variable selection method is based on the technical feasibility of the model, use of performance parameters, and clinical considerations. For the calculation of the ROC-AUC as performance measure, a 10-fold validation procedure will be performed.

The same procedure is applied in order to derive a prognostic model for the prediction of the presence of type 1 myocardial infarction as well as in those patients in whom ACS is present, for the prediction of type 1 vs. type 2 myocardial infarction as adjudicated by the EAC.

5.3.3 Prediction

We will calculate the area under the Receiver-Operating-Characteristic-Curves (ROC-analysis) to evaluate the predictive accuracy of changes in troponin alone, and adjusted for the variables found in the step above, particularly relative changes in the 20% to 50% interval, for the presence of ACS, the presence of type 1 myocardial infarction as well as in those patients in whom ACS is present, for the prediction of type 1 vs. type 2 myocardial infarction in stroke patients using Harrell's c statistic to determine the best cut-off to predict ACS as well as type 1 myocardial infarction.

We will calculate a prediction algorithm that identifies patients with ACS with highest area-under-the-curve (AUC) in a ROC-Curve (ROC-analysis of c-statistics) and which takes the results of pre-defined diagnostic procedures into account.

A gender-specific analysis will be conducted to determine if there are different cut-off values by testing an interaction between gender and troponin change.

5.3.4 Safety analyses

All SAEs will be summarised by numbers and percentages and evaluated in a descriptive manner. In order to assess the safety risk of the study, the DSMB will evaluate all SAEs arising during the course of the study and provide its recommendation regarding the safety risk of the study in a report.

5.4 Statistical software

- STATA 15 or newer
- R 3.4.1 or newer

- SPSS 22.0 or newer

6 References

Bossuyt, P. M., et al. „The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration." *Ann Intern Med*, 2003: 138(1):W1-12.

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7 Appendix

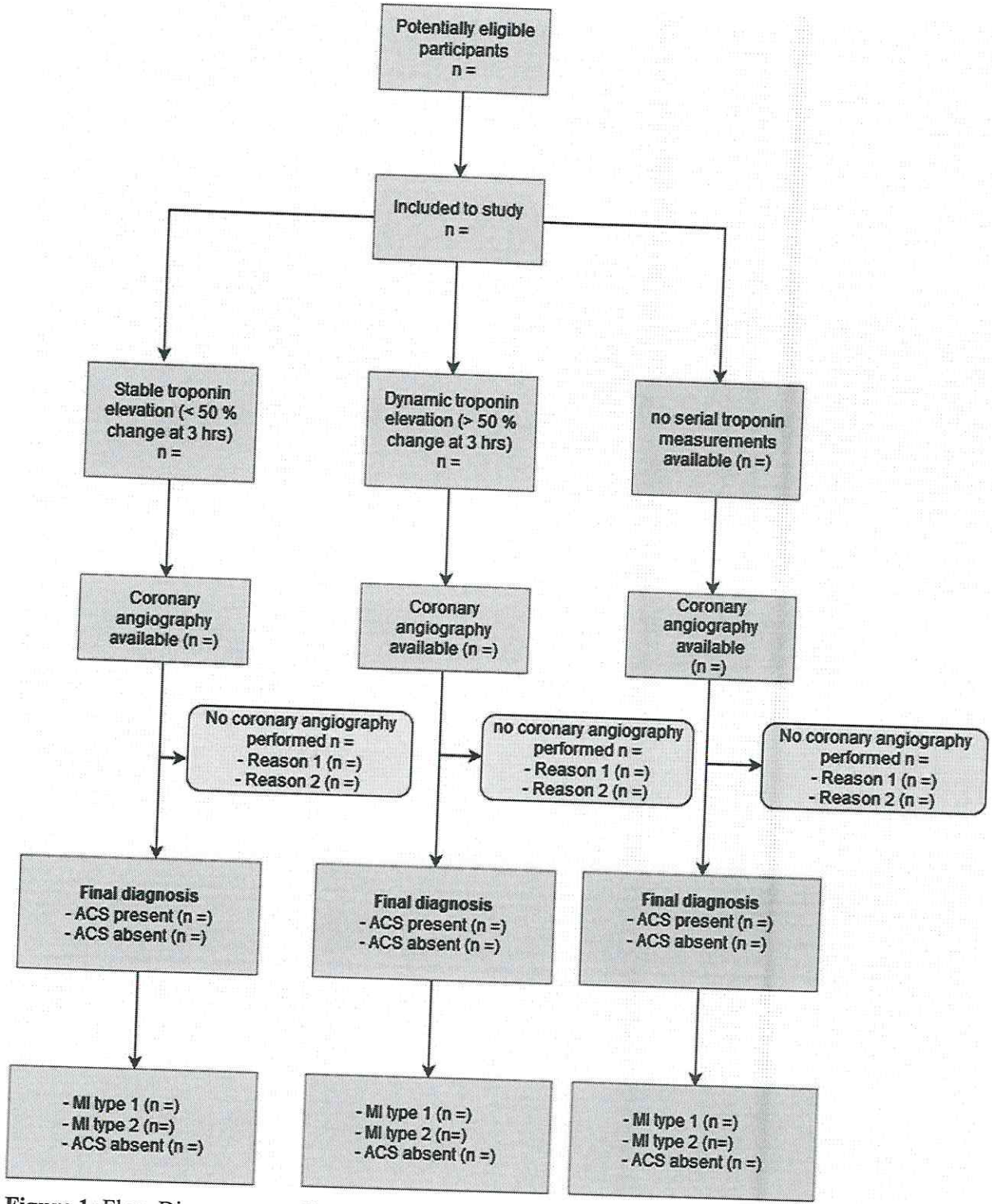

Figure 1: Flow-Diagram according to the STARD Statements