Biomarkers and antithrombotic treatment in cervical artery dissection
The TREAT-CAD trial

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Statistical analysis plan – dated Dec. 18th 2019

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Methods & Statistics

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This statistical report and analysis plan will be finalized before database closure and will be under version control at the Clinical Trial Unit, University Hospital Basel.

1 Background

Antithrombotic treatment in cervical artery dissection (CAD) is still a matter of debate. Most physicians prescribe anticoagulants for stroke prevention in CAD-patients although this approach is not evidence-based.

This randomized controlled, open labeled multicenter non-inferiority trial with blinded assessment of outcome events compares anticoagulant treatment to antiplatelet treatment.

2 Analysis data sets

The **full analysis set (FAS)** consists of all patients that were randomized. According to the intention-to-treat principle, each patient will be analyzed according to the treatment she/he was randomly allocated to. Patients who withdrew their consent during the course of the study will be analyzed in the FAS including only their baseline data.

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The **per protocol set (PP)** consists of patients in the FAS set without any of the following major protocol deviation:

- no dissection: diagnostic criteria for CAD not met in central MR reading
- violation of an inclusion criterion or having an exclusion criterion (for pregnancy, only a positive pregnancy test will be considered as a major protocol deviation)[1]
- switching between treatment groups (cross-over) or discontinuation of the allocated treatment before follow-up visit 2 for any reason other than as a reaction to a primary outcome-event or an SAE
- MRI at follow-up visit 1 earlier or later than 14 +/- 10 days
- follow-up visit 2 prior to 60 days after randomization [2]

The following deviations will be considered as minor protocol deviations:

- result of pregnancy test too late but negative (ie. confirmed absence of pregnancy after inclusion and randomization)
- randomization or start of treatment allocated not within 72h after initial MRI scan
- changes in treatments within the allocated treatment arm (eg. dosage of ASA 100mg rather than 300 mg) prior to follow-up visit 2

Patients with only minor protocol deviations will still be part of the PP.

Each patient will be analyzed according to the treatment she/he received until follow-up visit 2.

3 Demographic and baseline characteristics

Demographics and relevant baseline variables will be summarized for the PP and the FAS set. All summaries will be broken down by the treatment patients received.

Categorical data will be presented as frequencies and percentages. For continuous variables, the total number of measurements as well as the mean and the standard deviation will be presented. For skewed data the median and the lower and upper quartile will be shown.

4 Primary objective

The primary objective is to demonstrate the non-inferiority of anti-platelet treatment to anti-coagulant treatment in preventing further cerebrovascular ischemic events in patients with cervical artery dissection (CAD).

[1] The protocol does not distinguish between "confirmed absence of pregnancy" and "assumed but unconfirmed absence of pregnancy". We only consider the latter as a major protocol deviation since patients with a late but negative pregnancy test are still part of the intended study population.

[2] In the protocol the window for visit 2 was 90 +-30 days.

4.1 Primary endpoint

The primary endpoint - **C**erebrovascular **I**schemia, major **H**emorrhagic events or **D**eath (**CIHD**) - is a composite endpoint. It is defined as the occurence of at least one of the following events during the treatment period:

1. Cerebral ischemic events (clinical) or surrogate findings for cerebral ischemia:
   (a) Any ischemic stroke, defined as: new symptomatic neurologic deterioration lasting at least 24 hours that was not attributable to a non-ischemic cause, or a new symptomatic neurologic deterioration that was not attributable to a non-ischemic cause and was accompanied by neuroimaging evidence of a new brain infarction. (Amarenco et al. (2018)). This includes Transient Ischemic Attack (TIA) with Diffusion Weighted Imaging (DWI) lesion. Ischemic stroke also includes retinal infarction defined according to (Hayreh & Zimmerman (2005))
   (b) New acute ischemic lesions on DWI MRI since baseline are defined as: (1) those with an unequivocal lack of continuity between new and existing (on baseline imaging) lesions on the same slice as well as on adjacent slices – irrespective of vascular territories and lesion pattern, (Gensicke et al. (2015)) and (2) "acute" being defined as a hyperintense signal alteration on DWI with a corresponding hypointense or isointense signal on ADC (Apparent Diffusion Coefficient) maps (Gensicke et al. (2015)).
2. Hemorrhages (clinical) or surrogate findings:
   (a) any major extracranial hemorrhage: any clinically apparent extracranial bleeding requiring any kind of intervention [including hospitalization or prolongation of hospitalization] or leading to death.
   (b) any symptomatic intracranial hemorrhage defined as: any documented intracranial hemorrhage - i.e. an acute extravasation of blood into the brain parenchyma (Amarenco et al. (2018)) - that was temporally related to any deterioration in the patient's clinical condition.
   (c) any new, asymptomatic micro- or macrobleeds (visible on follow-up brain T2*-MRI or Susceptibility weighted imaging (SWI), which were absent on the baseline MR-scan) defined as: (1) those with an unequivocal lack of continuity between new and existing (on baseline imaging) lesions on the same slice as well as on adjacent slices – irrespective of vascular territories and lesion pattern, (Gensicke et al. (2015)) and lesions being defined as hypointense brain lesions with clear margins on T2*-MRI or SWI.
3. Death of any course: In an adjudication procedure, causes of death will be distinguished as cardio/cerebrovascular (i.e.: fatal acute coronary syndrome, fatal stroke, fatal intracranial hemorrhage, fatal pulmonary embolism, sudden death, and unobserved or unexpected death) (Amarenco et al. (2018)) vs all other causes.

If MR-sequences required to detect micro- or macrobleeds (i.e. T2* or SWI-sequence) were not performed at baseline, any micro- or macrobleeds seen in the follow-up

MRI will be considered as new[3]. The number of patients for whom the baseline MR-sequences required to detect micro- or macrobleeds were not performed and their patient characteristics at baseline will be presented.

4.2 Hypothesis

We will test the following null hypothesis:

$$H_0 : \pi_{\text{anti–platelet treatment}} \geq \pi_{\text{anti–coagulant treatment}} + \delta \quad (1)$$

where δ is the non-inferiority margin. The alternative hypothesis is:

$$H_1 : \pi_{\text{anti–platelet treatment}} < \pi_{\text{anti–coagulant treatment}} + \delta \quad (2)$$

where π is the probability of a CIHD under the indicated treatment.

The non-inferiority margin is set to $\delta = 0.12$ (12 % absolute risk difference).

4.3 Model and method of analysis

The difference in CIHD rate $\pi_{\text{anti–coagulant treatment}} - \pi_{\text{anti–platelet treatment}}$ will be compared with the non-inferiority margin using a two-sided 95 % confidence interval. We will use wilson's method to calculate CI (continuity-corrected modification of the Wilson's score method Newcombe, 1998). The primary analysis will be performed on the PP. Section 5.2 describes the handling of missing values.

4.4 Presentation of results

We provide the point estimate together with it's 95 % confidence interval. This approach will also allow us to assess superiority of either treatment.

4.5 Supportive analyses and sensitivity analyses

The following supportive and sensitivity analyses will be conducted to support the main analysis described above (Section 4.3). The rationale for each sensitivity analysis is indicated in bold. All sensitivity analyses except for the sensitivity analysis nr 2 will be performed on the PP. Missing values will be handled as described in section 5.2 if not defined otherwise.

1. **Missing values:** In case of missing values for the primary endpoint, the main analysis will be repeated performing the following imputations

[3]In the protocol, this was planned as sensitivity analysis. However, given the rather young patient population, we do not expect preexisting micro- or macrobleeds. Therefore the probability of falsely labeling an existing microbleed as new due to missing baseline T2* - or SWI MRI was considered to be small and the aim to preserve the randomization was of overriding importance. Therefore the two analyses were switched.

(a) Missing values in the primary endpoint will be imputed as CIHD events (assuming the worst for patients without follow-up).

(b) Missing values in the primary endpoint will be imputed as no CIHD events (assuming the best for patients without follow-up).

2. **Choice of analysis population (PP vs. FAS):** The analysis will be repeated on the FAS and compared to the main analysis. According to the intention-to-treat principle, each patient will be analyzed according to the treatment she/he was randomly allocated to. In case of missing values for the primary endpoint, the following imputations will be performed:

   (a) Missing values in the primary endpoint will be imputed as CIHD events (assuming the worst for patients without follow-up).

   (b) Missing values in the primary endpoint will be imputed as no CIHD events (assuming the best for patients without follow-up).

   (c) Patients with missing values in the primary endpoint will be excluded from the analysis. Inverse probability of censuring weights will be used as described for the main analysis in ection 5.2.

3. **Incomplete information about micro- or macrobleeds at baseline:** The main analysis will be repeated excluding patients without baseline information with regards to micro- or macrobleeds.

4. **Effect of failure to preserve the randomization due to major protocol deviations:**[4] We will use stabilized inverse probability weights to assess the impact of patients excluded from the PP analysis set according to (Robins et al., 2000) and (Hernan & Robins, 2006; Austin & Stuart, 2015). The weights will be estimated on the FAS. Thereby we will use the following variables: age, sex, occlusion of dissected artery and National Institutes of Health Stroke Scale (NIHSS). If the patient did not have a stroke, 0 will be imputed for the NIHSS. Thereafter the main analysis will be repeated including the weights. By doing so, patients without deviation who are similar to the patients with deviation will be inflated (compared to and patients without similarities).

5. **Assessing a modelling approach:** we compare the probability of CIHD between trial arms using a logistic regression model. The incidence of CHID will be the dependent variable. Treatment will be used as independent factor. In addition, the model will include the presence of cerebral ischemic events clinically and/or neuradiologically (i.e., clinically defined ischemic stroke, or DWI-lesions at baseline, or both) and occlusion of the dissected artery as covariates to adjust for baseline differences among patients.

6. **Effect of minor protocol deviation:**[5] The main analysis will be repeated excluding patients who were randomized or initiated treatment more than 72h after initial MRI scan, which was considered to be a minor protocol deviation.

[4]This sensitivity analysis was not planned in the protocol. However we think it helps to further assess the potential bias introduced when randomized patients are excluded from the analysis set due to major protocol deviations.

[5]This sensitivity analysis is a consequence of the adaption in section 2 ("major" vs. "minor" protocol deviations).

7. **Effect of imaging endpoint (as compared to clinical endpoints):**[6] The main analysis will be repeated including only the clinical endpoints ICH, stroke, extracranical hemorrhage and death as components of the primary endpoint.

All patients with a primary or secondary outcome event will be listed by the PI. For the primary outcome events, the PI will provide information on type, timepoint of the outcome event and relation to the allocated treatment (antiplatelets vs anticoagulants). Laboratory values quantifying coagulation at the time of the event will be provided if available.

5 Secondary objective

5.1 Secondary endpoints

1. new ischemic strokes (including retinal infarction)
2. new acute lesions on diffusion-weighted MRI
3. any major extracranial hemorrhage
4. any symptomatic intracranial hemorrhage
5. any asymptomatic micro- or macro-bleeds
6. any death
7. any increase in volume of the vessel wall hematoma on follow-up cervical MRI as compared to the baseline MR-scan.
8. modified Rankin scale at 3 and 6 months
9. independence in activity of daily living (i.e. modified Rankin scale 0-2) at 3 months and at 6 months
10. excellent functional outcome (i.e. modified Rankin scale 0 or 1) at 3 and 6 months
11. any TIA (classical definition)
12. recurrent cervical artery dissection

All secondary endpoints will be presented descriptively. The particular events leading to the primary endpoint (1 – 6) will be summarized as frequencies and percentages. If patients died while under observation (endpoint 6), we will provide the number of patients with cerebrovascular death. Patients with more than one event will be listed. Time between treatment initiation and event will be presented. The summaries will be done on the PP as well as on the FAS.

[6]This sensitivity analysis is planned since the significance of DWI lesions is still debatable. It also allows to compare the results with studies assessing only clinical endpoints.

The effect of treatment (anti-platelet vs. anti-coagulation) on mRS at three months (endpoint 8) will be assessed in a proportional odds model (also called ordinal logistic regression or shift-model). "Independence in activity" and "excellent functional outcome" at three months (endpoints 9 and 10) will be modelled in logistic regression models. Presence of cerebral ischemic events clinically and/or neuradiologically (i.e., clinically defined ischemic stroke, or DWI-lesions at baseline, or both), occlusion of the dissection arteries and mRS before dissection will be included as covariates to adjust for baseline differences among patients. The models will be fit on the PP.

The analyses of other endpoints, described as tertiary endpoints in the study protocol, will be described elsewhere. This also includes the interaction tests of antiplatelet versus anti-coagulant treatment and MMP9/TIMP2 ratio or other biomarker signatures.

All analyses of the secondary endpoints are explorative. Results have to be interpreted as hypothesis generating and not as confirmatory. P-values should be interpreted as continuous measure of evidence against the corresponding null-hypothesis (i.e. no association with predictor) and not as confirmatory ("significant" vs. "non-significant"). No correction for multiple testing will be performed.

5.2 Missing values

Primary endpoint In patients with missing information on micro- or macrobleeds at baseline, any lesion detected on follow-up T2*- or SWI MRI will be considered as new lesion (section 4.1 and sensitivity analysis 3). For missing values in the primary endpoint (e.g. due to missing follow-up), inverse probability of censuring weights will be estimated. Thereby stabilized inverse probability of weights for missingness of the primary endpoint will be estimated for each treatment arm according to (Robins et al., 2000) and (Hernan & Robins, 2006; Austin & Stuart, 2015). The weights will be estimated on the PP except for the sensitivity analysis 2, where the FAS will be used. The following variables will be used to estimate the weights: age, sex, occlusion of dissected artery, and National Institutes of Health Stroke Scale (NIHSS). If the patient did not have a stroke, 0 will be imputed for the NIHSS. Missing values will be further investigated in sensitivity analyses (see sensitivity analysis 1).

Covariates Missing values in covariates used to estimate the inverse probability of censuring weights will be imputed. If the number of missing values does not exceed 5 % in each variable of interest the median (continuous variables) or the mode (= most frequent category, categorical variables) will be used. If there is one covariate with more than 5 % missing values, 5 multiple imputations will be done as described in van Buuren & Groothuis-Oudshoorn (2011). To this end we will generate 5 imputed data sets. In a second step we will estimate the weights for each imputed data set. Thereafter, we will stabilize and average the weights over the 5 imputations. In case of severe variability of the stabilized weights between imputations, we will increase number of imputations. Thereby the weights will be considered to have "severe variability between imputations" if for any patient the inter-weight variability divided by the number of imputations (the amount the variance would be increased in order to correct for the uncertainty of the imputations when using Rubin's rule, Barnard & Rubin (1999)) is larger than 0.01.

Secondary endpoints: The number of missing values will be presented. No imputations will be performed. Secondary analyses will be complete case analyses.

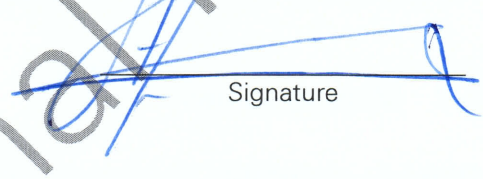
Centralized data control: Imaging characteristics are checked centrally and corrected if necessary. If the required images for central control cannot be provided, the evaluations of the centers are used. The proportion of measurements not checked centrally is indicated.

6 Safety analyses

The primary endpoint is also the most relevant safety endpoint. No further safety analyses are planned.

Principal Investigator:

Basel, 18-12-19
Place/Date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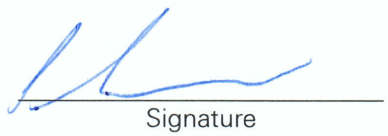
Trial Statistician:

Basel, 18-12-2019
Place/Date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