Statistical Analysis Plan (SAP) for the 2nd European Carotid Surgery Trial (ECST-2)

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Introduction

Carotid endarterectomy is currently recommended for patients with recently symptomatic carotid stenosis ≥50%, based on randomised trials conducted 30 years ago. A risk model based on factors that influenced the risk-benefit ratio of carotid revascularization (e.g., sex, age, timing from index event, carotid plaque morphology and patient comorbidities) was derived from first European Carotid Surgery Trial (ECST-1)[1] and validated in North American Symptomatic Carotid Endarterectomy Trial (NASCET)[2]. This model predicts the 5-year risk of ipsilateral stroke on the side of the stenotic carotid artery in patients treated with optimised medical therapy (OMT) alone, and allows patients to be stratified into low, intermediate or high risk. Since the original trials, medical treatment has improved significantly. The ECST-2 trial was designed to compare the safety and efficacy of immediate revascularisation plus OMT versus OMT alone asymptomatic or symptomatic carotid stenosis ≥50% with an estimated 5-year risk of stroke of <20%.

Study design

Detailed description of the conduct of the trial, including the study population, treatments and follow-up schedule are published in the study rationale and protocol.[3] Briefly, ECST-2 is designed as a multicentre, prospective, randomised, controlled, open, multi-centre, non-inferiority clinical trial with blinded outcome adjudication of the primary outcome. Patients with asymptomatic or symptomatic carotid stenosis ≥50% with an estimated 5-year risk of stroke of <20% are identified as suitable for inclusion in ECST-2 using the Carotid Artery Risk (CAR) score. The CAR score is calculated based on the following patient characteristics: sex, age, diabetes, myocardial infarction, peripheral vascular disease, hypertension, percentage of carotid stenosis, the presence of a near carotid occlusion, plaque ulceration, time in days from the last ischaemic event to expected day of randomisation, and type of event (major stroke/multiple or single transient ischaemic attack (TIA)/monocular event). Patients are randomly allocated in a 1:1 ratio balanced by minimization to be treated either by immediate carotid revascularisation plus OMT or by OMT alone.

Interventions

Optimised Medical Therapy

OMT will be applied to both treatment groups immediately after randomisation (if not started beforehand) and in general includes antihypertensive medication and cholesterol-lowering medication, adjusted to maintain pre-specified targets. The targets are specified by the local investigator for each patient in line with European, national or local guidelines. Antiplatelet therapy is prescribed according to local guidelines. Anticoagulation is used as an alternative if indicated. Further details regarding OMT are described in the protocol paper.[3]

Revascularisation
Patients allocated to revascularisation will have carotid endarterectomy (CEA), or in selected cases carotid artery stenting (CAS), as soon as possible after randomisation and not later than 2 weeks for symptomatic patients or 4 weeks for asymptomatic patients, in addition to OMT. It is anticipated that the majority of patients allocated to revascularisation will be treated by CEA.

General hypothesis
Our hypothesis is that patients with carotid stenosis ≥50% associated with a low to intermediate risk of stroke will not benefit from additional carotid revascularisation when treated with OMT.

Objective
To evaluate whether immediate revascularisation plus OMT is comparable to OMT alone in the combined risk of peri-procedural death, any stroke, myocardial infarction (MI), or new cerebral infarction assessed on MRI scan in patients with carotid stenosis ≥50% associated with a low to intermediate risk of stroke.

Secondary outcome measures
Secondary outcome measures include individual components of the primary outcome, the composite of peri-procedural death, MI and stroke, the composite of peri-procedural death and stroke, subtypes of stroke (e.g. ischaemic/haemorrhagic, ipsilateral/contralateral) cardiovascular death, hyperperfusion syndrome, new-onset epileptic seizure, any hospitalisation for vascular disease, carotid revascularisation during follow-up other than that allocated at randomisation, cranial nerve palsy attributed to revascularisation, haematoma caused by treatment requiring surgery, transfusion or prolonging hospital stay, other adverse events attributed to medical treatment or revascularisation, stenosis progression (defined as recurrent stenosis of the randomised artery after revascularisation, or progression in severity of stenosis in a non-revascularised artery), further revascularisation of the randomised artery after the initial attempt, decline in cognitive function assessed by the Montreal Cognitive Assessment (MoCA) and decline in functional status as assessed by an increase in the modified Rankin score (mRS). In addition, measures will be reported relating to the quality of life and health status, health service use (e.g. length of stay in hospital, surgery, medications) and health service costs.

Analysis populations
The primary analysis by intention-to-treat; i.e. patients will be analysed by their randomly allocated group despite what treatment they actually receive.
In the per-protocol analysis, cross-overs to revascularisation without relevant symptoms within the first 6 weeks of randomisation, and patients in whom there are delays of more than 6 weeks in performing CEA or CAS, will be excluded, while patients allocated OMT alone who receive carotid revascularisation more than 6 weeks after randomisation will be censored at the time of onset of the revascularisation procedure. All analyses will be adjusted for centre and predetermined risk factors and for type of revascularisation procedure and time to revascularisation procedure.

Analysis of 2-years combined main outcome paper
It was specified in the protocol that a preliminary analysis would be conducted at 2 years after randomisation. For this analysis, the primary outcome will be analysed as a hierarchical outcome of any clinically manifest peri-procedural death, stroke, new cerebral infarct on MRI or MI occurring before 2 years after randomisation analysed in the intention to treat population. The precise definition and order of components in the hierarchical outcome follows later.

We chose to define a hierarchical outcome at 2 years rather than a time-to-event outcome for two reasons:

I. the timing of new cerebral infarctions on MRI cannot be established

II. the clinical consequences of the components of our primary outcome vary greatly (from procedural death to silent infarction). Using a hierarchical outcome allows more clinically important outcomes to contribute more towards the final result, whereas in time-to-first event analyses component events contribute equally

III. We expect a clear violation of the proportional hazards assumption, i.e. a risk approximately constant in time, so that Cox proportional hazards models may be inappropriate. We expect rates to be initially higher in the immediate revascularisation plus OMT group (due to peri-procedural events), with the trend reducing or reversing later in follow-up.

Definition main outcome variables:

- *Periprocedural death:*
  Death within 90 days after randomisation.

- *Stroke:*
  Stroke is defined as an acute disturbance of focal neurological function, with symptoms lasting more than 24 hours or relevant cerebral infarction on imaging resulting from intracranial vascular disturbance. It must be established whether the cause is infarction or haemorrhage (intracerebral or subarachnoid). In patients without imaging evidence of acute cerebral infarction whose ischaemic symptoms lasted less than 24 hours after receiving thrombolysis (or thrombectomy) are classified as stroke, not as transient ischaemic attack.
  Visual loss resulting from embolic or haemodynamic retinal ischaemia with symptoms or signs lasting more than 24 hours will be included within the category of ischaemic stroke. Subdural haemorrhage and cerebral haemorrhage secondary to trauma will not be classified as stroke. Stroke will be classified as fatal stroke if the patient dies within 30 days of onset of the stroke and the death is attributed to the consequences of the stroke.
  Ipsilateral stroke is classified as the side of the randomised carotid artery and includes anterior cerebral artery and middle cerebral artery territories on that side. All other territories are classified as non-ipsilateral or contralateral.

  - *Transient ischaemic attack (TIA)*
    TIA is defined as an acute disturbance of focal neurological function with symptoms lasting less than 24 hours attributed to cerebrovascular disease. If imaging at the time of a clinical TIA shows evidence of relevant acute infarction, the event will be included in the category of ischaemic stroke.
    In the absence of imaging confirmation, events will be classified as probable TIA if they satisfy the following criteria:
    I) The patient must have had a documented sudden onset focal neurological deficit, either observed or the symptoms recorded by a doctor in the patient's medical record in sufficient detail to be certain about their nature.
    II) The symptoms must have been typical, such as transient blindness on one side, dysphasic speech deficit or transient weakness on one side without a march of symptoms.
    Isolated brain stem symptoms e.g. vertigo, dizziness or diplopia will not be classified as probable TIA. Events that are adjudicated as a possible TIA will not be included in the analysis

- *New cerebral infarction assessed on MRI scan:*
  Any new hyperintense lesion on the fluid attenuated inversion recovery (FLAIR) sequence seen on the 2-year or 5-year follow-up scan that was not present on the baseline scan, and that is consistent with the diagnosis of ischaemic infarction. For the 2-year analysis, we will include all scans that occur within 18 months and 30 months (2 years +/- 6 months) after randomisation. Scans occurring outside of this window will be

discarded as being unreliable for ascertaining new cerebral infarction at 2 years, but will be included in the 5-year analysis.

- *Myocardial infarction:*
  To be counted as an MI, an event will have to fulfil one of the following three criteria:
  1. Detection of rise and/or fall of cardiac biomarkers (preferably troponin) with at least one value above the 99th percentile of the upper reference limit (URL) together with evidence of myocardial ischaemia with at least one of the following:
     - Symptoms of ischaemia;
     - ECG changes indicative of new ischaemia [new ST-T changes or new left bundle branch block (LBBB)];
     - Development of pathological Q waves in the ECG
     - Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.
  2. Sudden unexpected cardiac death, involving cardiac arrest, often with symptoms suggestive of myocardial ischaemia, and accompanied by presumably new ST elevation, or new LBBB, and/or evidence of thresh thrombus by coronary angiography and/or at autopsy, but death occurring before blood samples could be obtained, or at a time before the appearance of cardiac biomarkers in the blood.
  3. Pathological findings of acute myocardial infarction.

Primary analysis of the primary outcome: Win ratio analysis
With respect to the primary outcome, we will analyse the combined primary outcome in a hierarchical order using the unmatched win ratio.[4] Using the unmatched win ratio we first form all possible pairs of patients. We then compare pairs of patients, comparing them on outcomes in a set order and stopping once the patient are untied (i.e. no need to evaluate components further down the hierarchy if there is a difference in a more important outcome).
The hierarchy of importance for the individual components of the composite outcome are:

1. Time to peri-operative death, fatal stroke, fatal MI
2. Time to non-fatal stroke
3. Time to non-fatal MI (excluding silent infarcts)
4. New silent cerebral infarction on MRI (Patients pairs where one or more patients has a missing scan (or a scan outside of the window to qualify for the primary outcome) will be considered a tie)

For each possible patient pair they are classified as a win (better outcome on treatment), a loss (better outcome on control) or a tie. Where both patients within a pair experience an event at the same level of the hierarchy, it will be considered a win if the patient who has the longest time from randomisation to the event is in the treated arm, a loss if the patient is in the control arm. If patients experience the event on the same day post-randomisation one continues to evaluate that pair for any remaining components in the hierarchy. Patients are only compared during their shared follow-up time (i.e. during the time that neither patient is withdrawn or lost to follow-up) and are censored at 730 days after randomisation. The win ratio will be calculated by dividing the number of patient pairs with a better outcome in the treated (OMT alone) group (wins) by the number of patients with a better outcome in the control group (losses) (OMT alone + revascularisation). A 95% confidence interval together with a p-value (calculated using the method of Finkelstein-Schoenfeld) will be presented. We will additionally present the win benefit (% wins - % losses) and it's 95% confidence interval. We will present the number of wins and losses at each level of the hierarchy.

Secondary analysis of the primary outcome
We will calculate the proportion of patients at 2 years who had any of peri-operative death, stroke, MI or **known** silent cerebral infarction on MRI (i.e. as a conventional binary outcome at 2 years where all components count equally). Since some patients will have incomplete 2 year follow-up data, we will impute data for the composite of peri-operative death, stroke or MI for

patients who are censored before 2 years using Kaplan Meier estimates from the same treatment arm (using the method of Jackson et al[5].). This will yield a binary outcome for peri-operative death, stroke or MI at 2 years. This will be combined with **observed** data on silent cerebral infarctions on MRI to get a binary outcome for the entire composite at 2 years. We will combine estimates across imputations using Rubin's rules in the usual way. We will report the proportion with the outcome by treatment group, its difference and the 95% confidence interval for the difference. 50 imputations will be used with a random seed of 01012022.

Exploratory analysis of the primary outcome
As an exploratory analysis for the primary outcome we will repeat the secondary analysis of the primary outcome, but also impute missing scans on 2-year MRI scans.

Analyses of secondary outcomes
Secondary outcomes will be analysed up to and including 730 days from randomisation. Within each group we will report the number of events in each group (E_{OMT} and E_R), the Kaplan Meier estimator at 2 years ($KM_{OMT,}$ KM_R) and it's estimated variance ($\hat{\sigma}^2_{OMT}, \hat{\sigma}^2_R$) calculated using the method of Greenwood.[6] We will report the difference between the KM estimators at 2 years, together with it's 95% confidence interval (formed at KM_{OMT}-$KM_R \pm 1.96\sqrt{\hat{\sigma}^2_{OMT} + \hat{\sigma}^2_R}$) and a 2-sided p-value calculated by referring the test statistic Z to a standard normal distribution where: $Z= \frac{\mathrm{KM}_{OMT}-\mathrm{KM}_R}{\sqrt{\hat{\sigma}^2_{OMT}+ \hat{\sigma}^2_R}}$

We will also calculate hazard ratios and 95% confidence intervals.

The exception is for continuous outcomes (e.g. quality of life measures and cognitive measures) which will be analysed using mixed models for repeated measures.

Subgroup analyses
The rate of the primary and key secondary outcomes will be displayed separately by the following characteristics:

- Presence or absence of intraplaque haemorrhage
- Symptomatic vs. asymptomatic disease
- Age (< 70 years vs > 70 years)
- Sex
- CAR score
- Diabetes
- Hypertension
- Severity of stenosis
- Contralateral stenosis or occlusion
- Type of most recent event
- Multiple symptoms
- Centre recruitment

To test whether the rates treatment effect (expressed as a difference in 2-year rates between the revascularisaition and OMT group) differs according to these characteristics, a p-value will be calculated using Cohrane's Q test.

<u>Analysis of 5-years outcomes</u>
Analyses of 5-years outcomes will use methods analogous to those used for the 2-year primary and secondary outcomes, with the obvious exception that censoring will occur at 5 years (5*365 days) rather than 2 years.

Analyses of clinical outcomes for ESOC 2023 presentation

1. Baseline table
2. Kaplan Meier curve 1. primary endpoint (any stroke, MI or periprocedural death)
3. Kaplan Meier curve 2. any stroke
4. Kaplan Meier curve 3. periprocedural death
5. Kaplan Meier curve 4. MI
6. Table with main outcome measures (absolute numbers and percentages per group, HR, Risk diff, p-value; see example ICSS paper 2010); ITT
    a. Primary composite endpoint (any stroke, MI or periprocedural death)
    b. Any stroke (ischaemic and haemorrhagic)
    c. Periprocedural death
    d. MI
    e. Ipsilateral stroke
    f. Ischaemic stroke
7. Forest plot with subgroups → focus om CAS <10 vs 10-20

Others:

    a. Age (< or > 70 years)
    b. Sex
    c. Symptomatic vs asymptomatic
    d. Type of most recent event (stroke, TIA, ocular)

Literature

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