Sahlgrenska Carotid Cohort

Research program

Research questions

Defined research questions

- How should symptomatic near-occlusion be managed?
- What is the best medical therapy before surgery?
- Why does the prognosis with and without treatment differ between patients with cerebral and retinal events? Is there a pathophysiological difference?
- What is the impact of novel stroke definitions on the carotid field?
- Do two recent events increase the risk of perioperative stroke?
- How is carotid web diagnosed and managed in routine practice?
- Can blood biomarker analyses improve prognostic or pathophysiological understanding?
- Can assessment of effective reserve improve prognostic understanding in patients with carotid stenosis?
- What affects long-term prognosis for patients with carotid stenosis?

Additional research questions

Various other questions regarding diagnostics, prognostics, pathophysiology and management will be addressed with the gathered material.

Background

Carotid stenosis is a common cause of ischemic stroke and transitory ischemic attack (TIA) [1]. Stenoses that have recently caused a stroke or TIA are defined as "symptomatic". Symptomatic carotid stenoses ≥50% usually undergo surgery, which is well established in several randomized controlled trials (RCT) and all relevant guidelines [1-3]. Despite being a well-studied field there are still many unanswered questions.

How should symptomatic near-occlusion be managed?

Near-occlusion are the most severe stenoses where the artery beyond the stenosis has reduced in size. These, somewhat unexpectedly, have good prognosis even when symptomatic. No benefit with surgery in RCTs and latest guidelines recommend conservative treatment except well-selected cases with repeated symptoms [3,6]. Long-believed to be rare, but recent studies have shown that this is an erroneous belief. Rather, near-occlusions have been misunderstood and systematically underdiagnosed [7-8]. When using diagnostic methods closely resembling those of the RCTs, near-occlusions are common [9]. Due to this long-standing misunderstanding with diagnostics, no study has yet assessed the prognosis if guidelines are followed, i.e. systematically diagnose symptomatic near-occlusions and treat them conservatively. This might show other results than expected as all RCTs have case selection but were also done long ago – we now have better medical treatment. Also, near-occlusion is rather an expert diagnosis that is hard to do well in routine practice [10]. Therefore, it has been challenging to even try to follow guidelines in routine practice. However, very recent developments in phase-contrast MRI have made it possible to reproduce the diagnostics of RCTs in routine practice with very good accuracy [11]. Thus, it is now possible to diagnose near-occlusions and follow guidelines – and the prognostic outcome when doing this should be studied. Further diagnostic refinements are also warranted.

Also, the most severe variant of near-occlusion (with full collapse) has a very poor short-term prognosis and no published cases with reasonably relevant management performed [12]. Management trials are warranted but is beyond the scope of this application given the observational design. However, this affects the design of our planned analyses.

What is the best medical therapy before surgery?

Single anti-platelet therapy (SAPT), such as aspirin (Trombyl®) 75 mg 1x1, has long been the standard therapy for symptomatic carotid stenosis. For patients with ischemic stroke or TIA but

without carotid stenosis (or stenoses where surgery is not planned), it has been shown that dual anti-platelet therapy (DAPT) is superior to SAPT during the first 3 weeks after an event, where DAPT in the form of Clopidogrel + aspirin has foremost been studied [4]. It is unclear whether SAPT or DAPT is preferable for patients with carotid stenosis before surgery. On one hand, the risk of early stroke recurrence is high [5], but on the other hand, there might be an increased risk of life-threatening wound hematoma. Given these uncertainties, latest guidelines have no recommendation [2-3].

Why does the prognosis with and without treatment differ between patients with cerebral and retinal events? Is there a pathophysiological difference?
Patients with symptomatic stenosis due to a cerebral event (stroke or TIA) have worse prognosis than patients with retinal events (amaurosis fugax [Afx] and retinal artery occlusion). This in terms of prognosis before surgery, after surgery, without surgery and perioperative risk [1, 3, 5]. The pathophysiological causes of this are unclear. Imaging techniques that are recently added to routine practice use and good patient history and neurological exam might shed some light on the differences. One example is retinal claudication (severe and prolonged blinding in the ipsilateral eye by a moderate light source), it is almost only described for carotid occlusions, little is known about it in carotid stenoses [13].

What is the impact of novel stroke definitions on the carotid field?
In the coming ICD 11 classifications, the definition of stroke will change to also include cases with transient symptoms and evidence of recent ischemia on brain imaging [14], such cases have thus been considered TIA. If employed, this will likely fundamentally change descriptions in the carotid field without changing what actually happens to the patients as recent ischemia is very common in patients with carotid stenosis and clinical TIA. If the new stroke definition is used as outcome, carotid stenosis becomes will likely seem more dangerous, which is especially problematic if one wants to compare with studies with the old definition. Detailed studies comparing the two approaches for patients with carotid stenosis are warranted.

Do two recent events increase the risk of perioperative stroke?
For a decade, there has been a debate about if very early surgery (within 48 hours of presenting event) increases the risk of perioperative stroke [3]. The crux has been that most studies are from registries, and early surgeries are often performed in unstable patients, causing confounding. However, our preliminary results, using data with higher quality than registries (gather for over a decade, why we could reach sufficient power), show that patients with ≥2 events within 7 days of surgery have a high risk (15%) of perioperative stroke. When accounting for this, time since last event had no impact on perioperative risk. These findings should be confirmed, but as current registries don't register data of next-to-last event, a dedicated study is needed.

Can blood biomarker analyses improve prognostic or pathophysiological understanding?
In a still ongoing study, we will assess a previous material of patients with symptomatic carotid stenosis with various blood biomarker assessments with a hypothesis generating approach. This has never before been done at this scale, and smaller scale attempts have not revealed any relevant findings. If we have any findings, validation will be needed. Also, novel biomarker techniques are being developed, why additional research questions can be asked "tomorrow" for the blood we store "today".

Can assessment of effective reserve improve prognostic understanding in patients with carotid stenosis?
The concept of effective Reserve comprises estimating an individual's brain reserve capacity and can be quantified using machine learning-based methods analyzing MRI of the brain [15]. Essentially, brain volume and other metrics in routine MRI brain images are combined. The rationale is that if two patients suffer a stroke with the same severity, the one with a biologically younger/healthier brain than the other will have better prognosis. This is well within the experience of stroke clinicians. The effective Reserve presents an opportunity to more directly and accurately address the brain's capacity to mitigate adverse events, rather than solely registering traditional risk factors for poor outcome. The

formalization of this concept is currently in early development at specialized centers in the US and has not yet been assessed in a cohort with carotid stenosis.

How is carotid web diagnosed and managed in routine practice?
Carotid web is a rare carotid disease where the intimal layer of the artery wall undergoes hyperplasia, creating a structure that look like a single venous valve leaflet with a "web"-like structure [16]. In the "pocket" created directly distal to the web, a thrombus can form. Symptomatic carotid web usually affects young and middle-age adults and has a high risk of recurrence. Best management is unknown, but many undergo surgery. These cases lack diagnostic code and a specific registry, why they are difficult to study and few dedicated prospective studies exist. Thus, while not technically carotid stenosis, it is reasonable to also include and study these patients as they are assessed by the carotid stenosis team at Sahlgrenska.

What affects long-term prognosis for patients with carotid stenosis?
Long-term prognosis, with and without surgery is not well studied in a large clinical cohort of carotid stenosis. Here, the prognosis of symptomatic patients with <50% stenosis, carotid occlusions and >50% stenosis that not treated patients due to some contraindication are especially relevant, to guide future treatment decisions. The impact of vascular medications, imaging and blood biomarkers at baseline on both stroke and other vascular diseases are relevant.

Various other questions regarding diagnostics, prognostics, pathophysiology and management.
The data will gather can be combined to better understand diagnostics, prognostics, pathophysiology and management. Also, over the years, various questions have come up and best practice in terms of management and diagnostics slowly shifts.

Methods

Overall design & infrastructure approach

We will include patients with symptomatic carotid stenosis and carotid web. The study is foremost a study with quality-control methods. That is, we register what happens to the patient in clinical routine practice and do not alter what exams are performed or management. The sole exceptions are that we also draw and store blood for biomarker analyses and improve follow-up assessments.

It is the intention that when we conduct this study, it will serve as an infrastructure for other carotid stenosis studies that goes beyond quality-control methods. That is, all relevant patients are asked to be enrolled into this study where we only observe and store blood. The other studies add exams, changes management or in other ways affects the participants. These other studies are performed using separate ethical approvals and specific consent for the additional aspects. These studies are then combined in joint analyses where this current study is useful as control material and for drop-out analyses in the other studies. It is also the intention to merge the data gathered here with other similar studies when this is required to allow relevant research questions to be answered.

Inclusion criteria

The target group are patients assessed for symptomatic stenosis, occlusion or carotid web. It is intended that cases with borderline symptomatic stenosis should also be included as these are relevant for some analyses. It is also the intention to study cases that do not undergo surgery or stenting, in order to assess the prognosis of such cases.

To be included, a person needs to fulfill either A, B or C, and both D and E

A) Suspicion of symptomatic ≥50% carotid stenosis or occlusion. Here, "suspicion" has two parts:
    - The stenosis was ≥50% on at least one clinical examination, but not necessarily has a final diagnosis of ≥50% after study assessments of all examinations. While symptomatic ≥50% stenosis on study assessments is the main target group, cases with borderline stenosis are relevant to study for prognostic and diagnostic research questions.

    - A clinical event (*insjuknande*) within 6 months that were at least initially suspected to cause the stenosis to be symptomatic. While those with final diagnosis of symptomatic stenosis is the main target group, cases are still included if final diagnosis is that the stenosis is not symptomatic, such as infratentorial location of the ischemia on MRI. The rationale being to be able to study the frequency and details of these changes is symptomatic status.

B) Symptomatic <50% stenosis (on all exams) that is considered for carotid surgery or stenting anyway, usually due to repeated symptoms despite best medical therapy. Here, "considered" is defined as discussed at a carotid stenosis multidisciplinary conference (or undergoing surgery or stenting without such a conference).
C) Carotid web, both symptomatic and asymptomatic
D) Assessed at, or referred to, the neurological clinic at Sahlgrenska. Here, "referred to" are cases that after clinical consideration was not subject to investigation at Sahlgrenska aimed at surgery or stenting despite being likely to have symptomatic carotid stenosis or web. "Referred" includes telephone consultations about whether investigation at Sahlgrenska is reasonable, i.e. not limited to written referrals.
E) Informed consent. Partial consent, such as accepting only some of the aspects of the study, will be granted and adhered to.

For A-C, "symptomatic" is defined as an ischemic event in the distribution area of the carotid stenosis/web within the last 6 months.

There are no exclusion criteria.

Setting
The study takes place at the neurology clinic (the outpatient carotid stenosis unit and the stroke ward) and at all collaboration units (vascular surgery, radiology and clinical physiology) at Sahlgrenska university hospital. Additional collaborators (*huvudmän*) will only analyze data.

Screening log
All persons (patients) that fulfill or nearly fulfill the inclusion criteria will be logged in a screening log. Logged items are where a unique identifier (personnummer), date of being relevant (i.e. assessed at or referred to the carotid stenosis unit), study number (if included), which inclusion criteria were fulfilled and reason for not being included (when not). This will be used for drop-out analyses. The degree of stenosis on different exams and if the stenosis was symptomatic or not will be registered (even for those not included), i.e. screening log data is not strictly limited to "yes/no" for the inclusion criteria, but includes minimal information. The rationale is to enable high-quality drop-out analyses – such as answering the question "How many of the cases with symptomatic near-occlusion did not provide informed consent?".

Consent procedure
Informed consent is obtained from the participants, not next-of-kin or similar. Informed consent is collected and documented by study personnel, such as doctors and nurses, which are usually the same persons involved in the health care of the patient. As often as possible, consent will be gathered during a face-to-face meeting during the preoperative evaluation. Given the acute nature of the disease and that some patients will not be sent for assessments, the inclusion can be done face-to-face post-operatively and/or by letter/telephone. When consent is taken by letter/telephone, the study information is sent to the person along with a letter explaining the situation and that he/she will be contacted via telephone. 1-2 weeks after sending the letter, the person is contacted via telephone and given oral information and chance to post questions. Consent can then be gathered. Here, blood sampling will not be possible, why a special variant of the study information without the blood sampling information will be used. For all consent procedures, written consent is preferred but a documented oral consent is accepted.

Patients that die after the event of relevance but before a consent procedure can be administered will be included in the project without consent. By workflow design, no blood sampling is possible. Here, death must occur within 6 months of the event of relevance.

In some instances, communication with the possible participant can be challenging due to aphasia and other cognitive difficulties. The study personnel are well-acquainted with these situations as consent should also be gathered for the clinical decision of performing surgery. For the study, consent will only be accepted if the study personnel deem the consent to be an actual informed consent.

Participants that withdraw consent will be asked if already collected data can be used (including already performed analyses on blood), if already drawn blood samples can be further analyzed (or should be destroyed) and if we can follow them in their medical records. The answer given will be adhered to, such as some of these aspects might be accepted. If no reply is given despite at least 4 attempts by telephone and 1 by mail (or 4 telephone or 2 mail when the other is not possible), we will continue all study procedures that does not require further contact. This is because it is our experience from this patient group that this non-reply situation is because of a wish to avoid hazel, not a wish to not participate in studies.

As a separate part (a separate page) of the consent procedure, information about the national databases for medical records (NPÖ) and medications (Pascal) will be given, and a separate consent will be gathered for the study group to use this as a tool when gathering data. The rationale is that use of these databases requires specific consent, whey we gather it separately.

Time frame & study size
Starting in 2024, the study continuously includes participants as long as there are defined research questions to be answered. To answer the defined research questions, 1000 participants will be included which should take 7 years.

Types of data collected
All relevant aspects of carotid stenosis, cerebrovascular disease and atherosclerosis will be assessed.

As part of clinical routine
We will gather data on all previous vascular diseases, diseases with connection to vascular diseases (such rheumatoid arthritis), vascular risk factors, all vascular medications (and those interacting with vascular medications), previous/current cerebrovascular events, intraoperative measurements (such as blood-pressure, stump-pressure and near-infrared spectroscopy (NIRS) with INVOS®) and post-operative outcomes until a 30-day visit. All relevant exams performed as part of clinical routine, such as CT brain, CT angiography, CT perfusion, carotid ultrasound, transcranial ultrasound, MRI (with various protocols) will be assessed. If/when new methods are introduced as part of clinical routine management of the carotid stenosis during the course of the study, these will also be assessed. This includes things such as photon-counting CT technique and flow ultrasound techniques. However, exams for other diseases/conditions (non-relevant exams) will not be assessed. Exams done during the preoperative evaluation is foremost of relevance, but similar exams done as part of clinical routine before or after this evaluation will also be assessed, such as changes in degree of stenosis over time and after surgery.

In addition to clinical routine
We will draw up to 50 ml of blood. These will be collected with several tube variants and processes in several ways, including EDTA plasma, buffy coat and preparation of RNA. The samples will be stored at Biobank Väst and used for study analyses. This blood is collected at one time point, which is usually during the preoperative evaluation, but can be during a clinical post-operative visit. However, participants will not be summoned for a visit only blood samples.

We will gather long-term follow-up beyond what is done in clinical routine:

- By telephone after 3 months, functional neurological outcome is assessed on the 7-point modified Rankin Scale (briefly: 0 no symptoms, 1-2 symptoms but can do all activities of daily life, 3-5 symptoms affecting activities of daily life, 6 death). Events (listed in next point) are also collected.
- By medical chart review as often as annually (or less often depending on resources) for 10 years: All-type vascular events, death and causes of death. Whenever possible, the national databased for medical records (NPÖ) and medications (Pascal) will be used, but only in participants providing specific consent for this.
- By registry data for at least 10 years:
    - Riksstroke (limited to if and when a stroke or TIA has occurred, its subtype and reporting hospital)
    - Swedvasc (limited to if and when a vascular operation has occurred, type, complications and reporting hospital)
    - EVAS (limited to if and when a stroke thrombectomy has occurred and reporting hospital)
    - National Board of Health and Welfare's (Socialstyrelsen) registries for
        - Hospital diagnoses (limited to vascular diseases)
        - Medical treatments (limited vascular medications and medications with known/possible interactions with vascular medications)
        - Causes of death

When collecting registry data, connection to unique identifier (personnummer) will be kept in order to allow for data validation in medical records. Interim-searches in registries, such as after 5 years might be undertaken as well. "At least 10 years" intends that if a proportion of participants has >10 years follow-up at the time of registry search, the follow-up will not be capped at 10 years. However, follow-up will stop when the last participant has been followed for 10 years.

Data collection strategy

State-of-the-art clinical assessments

The whole carotid management team (neurology, vascular surgery, radiology and clinical physiology) at Sahlgrenska are involved in the study. The main data inclusion strategy is that each part of the team makes and document state-of-the-art assessments as a part of the normal clinical routine. Thus, when an assessment can be done and/or documented well or less well within the normal borders of clinical routine, we will do it well. This is documented in the participants' medical records in text and in assessments forms. Examples:

- When the neurologist describes recent events in the preoperative evaluation, instead of a brief summary, all recent events by type and date are listed. Also, symptom variants are clearly specified, such as if only part or the whole eye was affected in an amaurosis fugax and if a symptom occurred under orthostatic conditions or not.
- When the radiologist assesses degree of stenosis on CT-angiography, instead of just documenting the degree of stenosis (a ratio between stenosis lumen diameter and distal artery diameter), the measured diameters are also reported.
- When performing conventional angiography, adding a length calibration instrument to the images to allow for more detailed assessments.
- Routine perioperative assessments such as stump pressure, shunt use, NIRS-recordings and flow after plaque removal are noted on a specific form during the procedure, not by memory in the surgical notes after the procedure.

Additional data collection

In addition to the assessments of exams done as part of clinical routine, the exams will be re-analyzed for study purposes.

The stored blood will be used for various blood biomarker analyses, including various "omics" and DNA and RNA analyses. To do this, the exams and blood will be sent to other centers and laboratories

in Sweden, EU/EES and outside EU/EES. The only prespecified analysis is a genetic assessment of Clopidogrel resistance.

Blinded endpoint assessments
All stroke events after presenting event until end of follow-up will be assessed in a blinded manner. As this is done for the whole cohort, regardless of early or in late follow-up, with and without near-occlusion, this allows for a true blinded assessments of the main outcomes in the medical therapy and near-occlusion analyses.

Scientific methods and data analysis plan – near-occlusion prognosis
In a pragmatic single-arm trial, we will assess the prognosis with conservative treatment (only best medical therapy) for symptomatic near-occlusions. Practically, this a detailed analysis plan for a subset of the included participants. Just as for all participants, best management is always provided and done as part of clinical routine, not as part of the study. However, as we want to add the safety provided by interim analyses, it is reasonable to call it a trial.

Inclusion criteria for this analysis are (4 of 4)
- Fulfills the inclusion criteria of the main study.
- Symptomatic near-occlusion
    - Near-occlusion is defined as ICA-CBF[1] flow rate ratio ≤22.5% on phase-contrast MRI [11]. Measurement-based criteria from CT-angiography and/or carotid ultrasound that are ≥99% specific compared to ICA-CBF flow rate ratio ≤22.5% will also be accepted, if and when such are made available[2].
    - Symptomatic is defined as an ischemic stroke/TIA/RAO/Afx in the distribution area of the stenosis within 3 months of index date (not 6 months as for main study).
    - Index date is defined as the day after management decision is taken. It is the day after management decision (not the same day) to account for a short delay between decision and when surgery/stenting could have been performed if that had been the management decision.
- Eligible for surgery/stenting.
    - Defined as would have been recommended to undergo surgery/stenting if the stenosis had been 80% instead of near-occlusion. This is a holistic assessment that includes items such as premorbid status, co-morbidities, timing since last event and technical difficulties. Here, "recommended" mean that it would have been done unless patient refused, but patient refusal cannot be truly gathered unless the treatment is actually offered.
    - Surgical/stenting technical difficulties arising specifically from the degree stenosis will not be accounted for (such cases will still be included), such as difficulty visualizing the distal end of the stenosis. The rationale being that such technical aspects might be overcome in the future, hence they should be studied, and these issues will be specifically noted and presented.
- Conservative treatment as first treatment decision.
    - The treatment decision is done as part of clinical management, not as part of the study.
    - The clinical routine is conservative management. Surgery/stenting can be considered in selected cases such as free-floating thrombus attached to the stenosis or repeated symptoms despite best medical therapy. Such cases are not to be included if their first treatment decision is to recommend surgery/stenting. However, if a second decision is made during the course of the trial, changing conservative to surgical/stenting, this does not affect inclusion in the analysis (intention-to-treat by first decision).

Exclusion criteria for the analysis
- Near-occlusion with full collapse and ≤7 days since last event at the time of index date.

[1] ICA flow divided by CBF where CBF is the flow in both ICAs + mid Basilar.
[2] Such criteria are being developed in our research group and will be validated in this study (see below).

- Full collapse is defined as ≤2.0 mm distal ICA diameter and or ≤0.42 ICA ratio (side-to-side) on CT-angiography [3, 12]. For those that cannot/do not undergo CT-angiography, full collapse can be defined if either is positive:
  - On ultrasound as a very severe stenosis on B-mode, <1.5 m/s peak systolic velocity in the stenosis and low distal ICA velocity (not specified, but usually <0.3 m/s, used to ensure separation from <50% stenosis) [8, 17].
  - On phase-contrast MRI as no visible flow in distal extracranial ICA (but proven flow in another modality) [11].
- If >7 days has passed at the time of index date, full collapse is not an exclusion criterion.
- If a participant fulfills the inclusion criteria later (such as on day 8 after last event) and have not been treated in clinic or enrolled in a treatment trial by then, they will be included with the day of fulfilling the criteria set as index date (day 8 after last event is the index date).
- The rationale for this exclusion criterion is that near-occlusion with full collapse causes a very high short-term risk during the 2-4 first days after presenting event [12]. This risk increase should not be included in this analysis. Rather, such patients are planned for separate treatment trials. Participants that are not relevant for such trials (such as too long time has passed) should be assessed for long-term prognosis – hence to be included.

Outcomes
Main outcome is combination of ipsilateral ischemic stroke, ipsilateral RAO and any stroke/death within 30 days of trial surgery. Here, trial surgery is defined as ipsilateral carotid surgery or stenting (included participants can be subject to this as a second treatment decision). Secondary outcomes are all parts of the main outcome separately, any ipsilateral ischemic event (stroke, TIA, RAO, Afx), all-cause stroke and all-cause mortality.

Planned analyses
The main statistical analysis is survival analysis by intention-to-treat approach when the last participants has been followed for 5 years. Death, lost-to-follow up and last follow-up are censors except when death is within 30 days of trial surgery (then it is an outcome). Events between index-date and censoring will be assessed. Secondary analyses will include comparison with historical controls from previous studies and for all secondary outcomes. Secondary analyses will also include assessments where index date is changed to presenting event.

Pre-specified subgroup analyses for main outcome (and presented for both with and without operative risk) are age (<65, 65-74, ≥75 years), sex, type of presenting event (stroke, TIA, retinal), time between last event and index date (quartiles), >1 event within 30 days of index date (yes/no), free-floating thrombus on CTA (yes/no) and severity of the collapse in 3 ways: 1) CTA-based distal ICA diameter ratio (quartiles, not available with contralateral occlusion), 2) ICA-CBF-flow rate ratio (quartiles, not available for many historical controls), 3) full collapse per exclusion criteria definition.

Sample size
Main analysis is descriptive, why it is difficult to assess power, largest studies have to-date included 100-150 near-occlusions in the medical arm [1, 18]. For secondary analyses, an expected sample of 100-200 participants will be required, based on two power calculations (below). Assuming that 20% of annual carotid cases can be included in this trial, 100 participants will take approximately 5 years to include. Therefore, 100 participants is the target if this study is conducted as single-center (current application). However, if additional centers can be recruited (with separate ethics approval), the target will be 200 participants.

Power analyses for secondary analyses with historical controls were done with 80% power, α=0.05 and a 1-sided approach (non-inferiority). The expected outcome could be 6.2% risk at 5 years, being double that of asymptomatic stenosis [19], or it could be 8.5% at 5 years, given risks seen in a smaller

2-year study of symptomatic CNO with modern medical treatment [18]. The 5-year surgical risk (11.8%) assumption is based on the outcome 5-year surgical risk for near-occlusions in the NASCET and ECST trials (148 patients, 16.9%) [1] and assuming a 30% relative risk reduction by improvements in medical treatments since then. To this 11.8% we added 20% as the non-inferiority margin (14.2%). Comparing 6.2% and 8.5% with 14.2% results in 95 and 202 participants, that is approximately 100-200.

Interim analyses
Interim analyses are conducted after 50, 100 and every 100 patient-years thereafter.

The trial will be terminated if the medical group (current cases) has an excessive risk – unless the subgroup of particular importance issue is the cause (see below). Excessive risk is defined by a Kaplan-Meier curve analysis at the day of current mean follow-up time (not at the end to avoid statistical anomalies with small samples). Excessive risk is defined as the lower border of 2-sided 95%CI is above a threshold. The threshold is based on current mean follow-up time:

1. Day 0-365, 0.01973% risk per day. This is a linear description of 7.2% risk during the first year, from the medical arm of symptomatic near-occlusions in NASCET+ECST [1]. This does not account for improvements in medical treatment as it is not clearly relevant the first year.
2. Day 366 and beyond: 7.2% + 0.00315% risk per day. This is a linear description for reaching 11.8% at 5 years (1.15% risk/year) after reaching 7.2% the first year.

Cases bordering conventional stenosis is an interim analysis of a subgroup of particular importance. As presented elsewhere [10], it is particularly relevant to assess the medical risk of cases close to the border of conventional stenosis. If the risk is too high, the inclusion criteria will be changed. Therefore, in every interim analysis, the ICA-CBF flow rate ratio values of the medical treatment (current study) cases will be divided into quartiles. If the highest quartile (closest to conventional) has higher risk than the three other quartiles combined, the inclusion criteria will be modified accordingly (lowering the inclusion criteria to the 75^{th} percentile of the interim analysis, i.e. excluding the highest quartile from further inclusion). This is assessed by Kaplan-Meier truncated at current mean follow-up, log rank $p<0.05$ is positive. If this happens, all trial termination assessments will exclude the cases in the excessive risk subgroup, including if trial termination was triggered at the same interim analysis. I.e. if the study show high risk because of a subgroup that will no longer be included, the trial will continue for remaining cases.

In broader context
This will be a study where we assess the outcome of following guidelines which in turn are based on the results of large RCTs. Hence, all will be treated with best known management. The reason why this is a research question is that it has never done systematically before due to confusion surrounding definition of diagnostics of near-occlusion in RCTs. This is an area where the study group has significant expertise in diagnostics and prognostics. Phase-contrast MRI has recently been revealed to have excellent diagnostic accuracy for near-occlusion. We will implement this method into routine practice and study the effect of this implementation in terms of prognosis. Improvements in other diagnostic methods are likely to be discovered and implemented.

Scientific methods and data analysis plan – all other study aspects
Management of near-occlusion – non-trial aspects
We will make comparisons between various ways to assess near-occlusion with CT-angiography, phase-contrast MRI and carotid ultrasound. The main emphasis will be on validation of diagnostic criteria that are being developed from a previous material when the study starts. The diagnostic criteria are aimed reproducing the findings with PC-MRI (such as the 99% specificity required for trial inclusion as an alternative to PC-MRI). All exams are done as part of clinical routine. In cases where conventional angiography or CT-perfusion is available, additional analyses for diagnostics and pathophysiology are possible – but few participants are expected to be examined with these modalities.

Sahlgrenska Carotid Cohort

What is the best medical therapy before surgery?
This is a short-term observational cluster group comparison. All participants are given best medical therapy, which at Sahlgrenska is defined as DAPT except for cases with indication for anticoagulant (such as atrial fibrillation). However, in a previous study performed in Umeå, best medical therapy was defined as SAPT. Outcomes are assessed in the same way in both studies. By combining the studies, a comparative analysis can be performed. Inclusion criteria in the analysis is symptomatic ≥50% stenosis.

Main analysis will be a cluster comparison by intention to treat, i.e. the groups are Umeå participants Vs Sahlgrenska participants. Primary outcome is the combination of preoperative recurrence and post-operative complications: Here, preoperative recurrence is ipsilateral ischemic stroke or retinal artery occlusion (retinal stroke) after presenting event until surgery/stenting or until 90 days if no surgery/stenting was performed. Post-operative complications are stroke or death within 30 days of surgery/stenting. Secondary outcomes are both parts of primary outcome separately, recurrent pre- or postoperative TIA/Afx, surgery length and reoperation for wound hematoma. Relevant subgroups are degree of stenosis, type of presenting event, number of preoperative events and timing between preoperative events and surgery [5, 12]. Genetic Clopidogrel resistance will be assessed by stored blood samples unless done in routine practice (which might start in the near future). The medications actually taken/administered during the management period will be registered for descriptive purposes.

With 80% power, α=0.05, a 33% relative risk reduction (15% to 10%) of preoperative stroke, 686 participants are required in each group. This is available from the Umeå studies. 686 DAPT-treated participants will take approximately 7 years to gather at Sahlgrenska. As this is the longest duration of all relevant analyses, it defines overall study size. During the study period, approximately 850 patients with final diagnosis will be assessed (approximately 160 patients not treated with DAPT, such as those treated with anticoagulation for atrial fibrillation). During the same time, approximately 150 additional patients that also fulfill the inclusion criteria are estimated to be enrolled (such as those not having symptomatic stenosis). Thus, 1000 participants are expected in the entire study.

Why does the prognosis with and without treatment differ between patients with cerebral and retinal events? Is there a pathophysiological difference?
Thus far, detailed assessments of patient history and neurological examination (nervstatus) are lacking for visual symptoms in the carotid stenosis field. To systematically gather and categorize symptoms might lead to new insights. This includes comparing onset placement, spread pattern, nadir (worst) pattern, regression pattern and color description of the visual symptoms between transient (amaurosis fugax) and permanent (retinal artery occlusion) symptoms. This is relevant as the former has no proven mechanism, but the latter has an identified embolus. Example, it is our anecdotal experience that few with amaurosis fugax present with a quadrant pattern at nadir, but many with retinal artery occlusion do so. Comparing with TIA and stroke (often embolic mechanism and have higher risk of stroke) will be assessed. Are there differences in previous vascular disease burden? Can imaging or blood biomarker findings explain differences? Collateral flow patterns of phase contrast MRI? Is retinal claudication associated with symptom types or prognosis? What are the risk factors for stroke recurrence among patients with retinal events?

What is the impact of novel stroke definitions on the carotid field?
When analyzing baseline events and prognostic data, will assess the participants with both the traditional and the novel stroke definition and compare the differences.

Do two recent events increase the risk of perioperative stroke?
All preoperative events are cataloged. The association between repeated preoperative events and risk of perioperative stroke will be assessed.

How is carotid web diagnosed and managed in routine practice?
Descriptive approach. How carotid web should be managed in routine practice is unclear. In the study we observe what is done in routine practice and describe outcomes during follow-up.

Can blood biomarker analyses improve prognostic or pathophysiological understanding?
This is a broad comparison between the blood samples stored in the study and the prognostic/pathophysiological data we gather in the study. One planned analysis is validation of a hypothesis generating study examined with Olink by using the same platform again or targeted analyses of specific proteins. We will also assess genetic Clopidogrel resistance. There will be joint analyses with other studies – but these are then legally/technically performed in each study separately (by separate ethics approvals – our approval cover only our samples). There are no known relevant biomarkers in carotid stenosis, but the intention is to validate those that might appear. See below for ethical considerations, especially what will not be studied.

Can assessment of effective reserve improve prognostic understanding in patients with carotid stenosis?
The MRI brain scans done as part of clinical routine will be assessed for effective reserve capacity and compared with the prognostic data we collet, to predict outcome in patients with carotid stenosis.

What affects long-term prognosis for patients with carotid stenosis?
Long-term prognosis is compared with various baseline data including imaging markers and blood biomarkers are particularly relevant. Here, we expect improvements in how the exams we do as part of clinical routine can be analyzed, such as how to categorize calcifications, how to define free-floating thrombus and advanced software to assess plaque content. We will not only study stroke outcomes but all vascular endpoints as the carotid stenosis is not only a risk factor for stroke but also a marker for how advanced a person's atherosclerotic burden is. We will take the medical treatment taken during follow-up into account.

Additional research questions
Any relevant comparison of collected data will be performed, including re-analyses of performed exams. This includes comparing different exams for diagnostic research questions and imaging markers with pathophysiological relevance such as emboli seen in acute CT-angiography, orthostatic symptoms and retinal claudication, and assessing possible novel imaging makers. See below for ethical considerations, especially what will not be studied.

Data storage
Study database will be RedCap, which requires 2-factor authentication for login. Contributors will only see the forms relevant to them, only the core study group will have access to all data. Study documents, such as consent forms, will be stored in a locked cabinet in a locked room. Study exams will be pseudonymized when exported from clinical radiology servers and stored on hard drives similar to the study documents. When exams or blood is sent to other centers for analyses, only coded data will be sent. When analyzing data for publications, coded data will be used. Code key for all aspects except the blood will be held by the PI. Code key for the blood will be held by Biobank Väst.

Ethical and other regulatory considerations
This is foremost a low-stakes observational study using quality assurance methodology. However, as we also draw blood and do extra follow-up, informed consent is required and will be gathered. Approvals from the national ethics regulation and for biobank will be obtained. The ethical issues of the management analyses of near-occlusion and medical treatment is specifically addressed in the ethics application. Adherence to all regulations about personal data will be followed, such as storage, use and transfers to other centers. Access to NPÖ and Pascal will require specific consent, which we will gather. The entire study will be registered at clinicaltrials.gov, as well will the phase III trial on near-occlusions (separately).

To adhere to ethical standards a norm, these limitations will be imposed on the data collection and analyses:

- While we set out to do various additional analyses, it is not ethical to gather data just for possible future studies. Therefore, the study will stop recruitment when the participants

required to address the defined research questions have been included. However, we expect new research questions to be added over time in ethics addendums, which then may require a larger total sample – which will then be specified in the same addendum. Here, the "additional research questions" only allows for analyses on already gathered material. Hence, these analyses will not affect sample size.

- Beyond what blood samples and follow-up, all participants will receive best management and all exams are done as part of clinical routine. The techniques for exams and treatments used in clinical routine changes over time. When introduced as part of clinical practice, this study will also assess these (we will study all relevant exams and management). Beyond this study, we will likely do additional studies where non-routine management is attempted and exams are done for research purposes – but this will be done with separate ethical approval and separate consent.
- We will only study research questions about carotid stenosis, cerebrovascular disease and/or atherosclerosis. This limitation is particularly important for the biomarker and the additional research question analyses. This is clearly specified in the information given to participants during the consent procedure. To ensure adherence to this, additional analyses of the stored blood will require ethical addendum applications.

Study group, roles and international collaboration

Principal Investigator

Elias Johanson, associate professor (*docent*), specialist in neurology, head of the carotid stenosis research group and responsible for the multiprofessional carotid stenosis management team at Sahlgrenska

Local senior research collaborators for major study parts

- Sofia Strömberg, PhD, consultant (*överläkare)* in vascular surgery, head of the vascular surgery clinic at Sahlgrenska
- Joakim Nordanstig, associate professor, consultant in vascular surgery, head of research and development at the vascular surgery clinic at Sahlgrenska.
- Annika Nordanstig, PhD, consultant in neurology, head of the neurology clinic at Sahlgrenska
- Alexandros Rentzos, PhD, consultant in radiology, head of the neurointervention lab and responsible for vascular aspects of neuroradiology at Sahlgrenska

Local senior research collaborators for minor study parts

- Kerstin Lagerstrand, associate professor, senior physicist, specialist in phase-contrast MRI.
- Christina Jern, professor, consultant in neurology, specialist in biomarker analyses in stroke.

Local senior clinical collaborators

- Anna Molinder, MD, consultant in neuroradiology, responsible for CT neuroradiology at Sahlgrenska
- Miroslav Malac, MD, consultant in neuroradiology, responsible for MRI neuroradiology at Sahlgrenska
- Kim Colliander, MD, consultant in clinical physiology, responsible for carotid ultrasound at Sahlgrenska
- Margareta Abrahamsson, MD, specialist in neurology, part of the carotid stenosis unit
- Brynhildur Hafsteinsdottir, MD, specialist in neurology, part of the carotid stenosis unit
- Mikael Jerndal, MD, specialist in neurology, part of the carotid stenosis unit

Local junior research collaborators

- Johan Skoog, post-doc, resident in Clinical physiology. Focus on carotid ultrasound and pathophysiology.
- Erik Lindgren, post-doc, resident in Neurology. Focus on effective reserve.
- Mahia Aivaz Ihari, PhD-student, specialist in vascular surgery. Focus on impact of medical treatment.

- Tim Unnerstall, possible PhD-student, MD, specialist in neuroradiology. Focus on CT angiography.
- Ameer Naeem, possible PhD-student, MD, AT-läkare. Focus on retinal events.

Other collaborators (all assess data, do not interact with the participants)
Swedish centers, i.e. additional "huvudmän"[3]:

- Group Hedin, KI. Collaborations on novel plaque assessments with CT-angiography
- Group Eklund, Umeå. Collaborations on pathophysiology, especially with phase-contrast MRI.
- Scilife lab Uppsala

International collaborators:

- Group Garcia-Pastor, Madrid, Spain. Collaborations on prognosis in carotid near-occlusion.
- Group Saba, Cagliari, Italy. Collaboration on novel plaque classification systems in general and calcifications in particular
- Group Liu, Philadelphia, US. Collaboration on calcifications in CT-angiography
- Allan J Fox, Toronto, Canada. Professor emeritus, senior advisor in near-occlusion issues. Collaboration in near-occlusion assessments of CT-angiography.

Significance

This project will improve our understanding of carotid stenosis disease. The three most important aspects are: 1) The 1-armed phase III trial of carotid near-occlusion. 2) The assessment om medical treatment before carotid surgery. 3) The study serves as a platform to which other studies can (with separate approvals and consent) collaborate with so that additional research questions that are beyond observation (with blood samples and good follow-up) are easy to perform.

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