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Expression of specific matrix proteins in atherosclerotic plaques in patients who underwent carotid artery surgery

Submission date
23/05/2021

Recruitment status
No longer recruiting

Registration date
25/05/2021

Overall study status
Completed

Last Edited
16/09/2022

Condition category
Circulatory System

[_] Prospectively registered
[_] Protocol
[_] Statistical analysis plan
[X] Results
[_] Individual participant data

Plain English summary of protocol

Background and study aims
A stroke is a serious life-threatening medical condition that happens when the blood supply to part of the brain is cut off. Atherosclerosis is a disease in which plaque builds up inside your arteries. Plaque is made up of fat, cholesterol, calcium, and other substances found in the blood. Over time, plaque hardens and narrows your arteries. This limits the flow of oxygen-rich blood to your organs and other parts of your body.
Atherosclerosis is responsible for one-third of all stroke. The rate of death from the first stroke is about 35%, and from the next up to 65%, while as many as 15 - 30% of survivors are permanently incapable of work.
Atherosclerotic plaque formation is a complex process that may be partially mediated by the enzymes matrix metalloproteinase-9 (MMP-9) and cyclooxygenase-2 (COX-2).
Components of a carotid artery (main blood vessel to the head) atherosclerotic plaque can be analyzed by noninvasive preoperative radiological imaging (magnetic resonance imaging, computed tomography and ultrasound), while the expression of matrix metalloproteinase 9 and cyclooxygenase-2, proteins with a role in remodelling of atherosclerotic plaques, can be analyzed immunohistochemically.

The aim of this study is to analyze the characteristics of carotid plaques in symptomatic and asymptomatic patients.

Who can participate?
Participants with an indication for carotid endarterectomy (CEA, a surgical procedure to remove the atheromatous plaque material) of the internal carotid artery will be included in the study.

What does the study involve?
Patients undergoing carotid endarterectomy will be informed of the study and consented to participate.
Preoperatively, participants will undergo a routine clinical examination. Routine blood tests and highly sensitive C-reactive protein will be performed preoperatively.
Participants will undergo preoperative ultrasound-colour Doppler, MRI or MSCT scan of carotid

arteries.
Carotid atherosclerotic plaque will be excised during CEA and preserved for analysis. Pathohistological characteristics and expression of MMP-9 and COX-2 proteins in carotid atherosclerotic plaque after carotid endarterectomy will be analysed.

What are the possible benefits and risks of participating?
All study participants could benefit from more close monitoring than would be possible outside of the study.
Defining the association of preoperative radiological characteristics of carotid atherosclerotic plaque with pathohistological features and biological indicators of carotid atherosclerotic plaque is important in clinical practice due to the possible better identification of patients in whom CEA is indicated with the potential predictability of preoperative diagnostic methods.
There are no potential adverse effects

Where is the study run from?
University Hospital Merkur, Zagreb (Croatia)

When is the study starting and how long is it expected to run for?
July 2013 to July 2021

Who is funding the study?
INEL – medicinska tehnika d.o.o., Zagreb (Croatia)

Who is the main contact?
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Contact information

Type(s)
Scientific

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Additional identifiers

EudraCT/CTIS number

Nil known

IRAS number

ClinicalTrials.gov number
Nil known

Secondary identifying numbers
Nil known

Study information

Scientific Title
The expression of MMP-9 and COX-2 proteins in the analysis of carotid atherosclerotic plaques in patients with carotid endarterectomy

Study objectives
Radiological features of carotid plaque instability analyzed by preoperative noninvasive radiological diagnostics (ultrasound/colour Doppler, MRI and/or MSCT scan) are significantly associated with the expression of MMP-9 and COX-2 proteins in atherosclerotic plaque and are highly prevalent in patients with unstable plaque.

Ethics approval required
Old ethics approval format

Ethics approval(s)
1. Approved 18/07/2013, University Hospital Merkur Ethics Committee (Zajceva ulica 19, Zagreb, 10000, Croatia; +385 (0)1 2431390; no email provided), ref: none
2. Approved 16/11/2015, Clinic for Cardiovascular Diseases - Magdalena Ethics Committee (Ljudevita Gaja 2, Krapinske Toplice, 49217, Croatia; +385 (0)49 244444; no email provided), ref: none
3. Approved 09/10/2020, University Hospital Centre Rijeka Ethics Committee (Kresimirova 42, Rijeka, 51000, Croatia; +385 (0)51 658808; no email provided), ref: 2170-29-02/1-20-2

Study design
Multicenter single-blinded observational cohort study

Primary study design
Observational

Secondary study design
Cohort study

Study setting(s)
Hospital

Study type(s)
Diagnostic

Participant information sheet

See additional file (in Croatian) ISRCTN46536832_PIS (added 01/06/2021)

Health condition(s) or problem(s) studied
Carotid artery stenosis

Interventions
Participants with an indication for carotid endarterectomy (CEA) of the internal carotid artery will be included in the study.
The indication for surgery will be set according to the recommendations of multicenter randomized studies (NASCET, ECST, ACAS, ACST and ESVS). Both symptomatic and asymptomatic patients will be enrolled.
The identity of participants will be protected by the identification number of the protocol of radiological diagnostic examination and biopsy of carotid atherosclerotic plaque after carotid endarterectomy.
Preoperatively, participants will undergo a clinical examination. Routine blood tests and highly sensitive C-reactive protein will be performed preoperatively.
Participants will undergo preoperative ultrasound-colour Doppler, MRI or MSCT scan of carotid arteries.
The stenosis of carotid artery will be determined according to NASCET criteria, and then the plaque cross-section at the site of greatest narrowing will be analyzed. Carotid atherosclerotic plaque will be qualified as a stable or unstable plaque.
The characteristics of carotid atherosclerotic plaque will be determined: degree of carotid artery stenosis, expression and size of lipid nucleus, bleeding, calcification, neovascularization, surface ulceration and plaque thrombosis according to the most recent modified AHA classification.
The images will be independently analyzed by two researchers.
Carotid atherosclerotic plaque will be excised during CEA respecting the rules of atraumatic technique with minimal sample manipulation. The distal end of the plaque sample will be marked and processed by a standard procedure to obtain pathohistological specimen that includes tissue fixation in 10% buffered formalin, embedding in paraffin blocks, cutting to a thickness of 4 µm, dewaxing and standard hemalaun-eosin staining, staining by a Masson method to detect collagen which will be semiquantitatively analyzed.
Immunohistochemical analysis of MMP-9, COX-2 expression will be performed.
Primary antibodies will be used in the study: MMP-9 (Leica Mikrosysteme Vertieb GMBH, UK, monoclonal, clone 15W2, 1:50 dilution), COX-2 (Abcam, USA, monoclonal, clone SP21, pre-diluted for use), CD68 PG-M1 (DakoCytomation, Denmark, monoclonal, clone PG-M1, pre-diluted for use), SMA (DakoCytomation, Denmark, monoclonal, clone 1A4, pre-diluted for use), CD34 (DakoCytomation, Denmark, monoclonal, clone QBend 10, pre-diluted for use).

Intervention Type
Other

Primary outcome measure
Measured at a single time point:
1. The characteristics and type of carotid atherosclerotic plaque measured by non-invasive radiological diagnostics (ultrasound/colour Doppler, MRI and/or MSCT scan) before surgery
2. Pathohistological characteristics and type of carotid atherosclerotic plaque measured using patohistological analysis after surgery
3. Expression of MMP-9 and COX-2 proteins in carotid atherosclerotic plaque after carotid endarterectomy measured using immunohistochemistry analysis after surgery

Secondary outcome measures

Measured at a single time point:
1. Clinical and laboratory characteristics of patients measured by reviewing patient notes before and after surgery
2. Semiquantitative analysis of macrophages, smooth muscle cells and microvascular density in carotid atherosclerotic plaque and the histological type of bleeding within the plaque measured using patohistological analysis after surgery

Overall study start date
15/07/2013

Completion date
15/07/2021

Eligibility

Key inclusion criteria
Participants with an indication for carotid endarterectomy (CEA) of the internal carotid artery

Participant type(s)
Patient

Age group
Adult

Sex
Both

Target number of participants
Planned sample size: 30

Total final enrolment
15

Key exclusion criteria
Patients with a contraindication for MRI scan (presence of a ferromagnetic foreign body, claustrophobia)

Date of first enrolment
26/09/2013

Date of final enrolment
30/06/2021

Locations

Countries of recruitment
Croatia

Study participating centre
University Hospital Merkur
Zajceva ulica 19
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Croatia
10000

Study participating centre
Clinic for Cardiovascular Diseases - Magdalena
Ljudevita Gaja 2
Krapinske Toplice
Croatia
49217

Study participating centre
University Hospital Centre Rijeka
Kresimirova 42
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51000

Sponsor information

Organisation
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Sponsor type
University/education

Website
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ROR
https://ror.org/01b6d9h22

Funder(s)

Funder type
Industry

Funder Name
INEL – medicinska tehnika d.o.o., Zagreb, Croatia, EU

Results and Publications

Publication and dissemination plan
Planned publication in a high-impact peer-reviewed journal.

Intention to publish date
01/07/2022

Individual participant data (IPD) sharing plan
The datasets generated during and/or analysed during the current study are/will be available upon request from (Dr Davorin Sef, davorin.sef@gmail.com; type of data - database in Microsoft Excel, data will become available following the publication of the study and for 12 months afterwards, by reasonable request data will be shared with medical professionals or researchers, for purpose of descriptive and/or statistical analyses, and by formal request to corresponding author, consent from participants was obtained using only anonymous data, ethical or legal restrictions will be provided by the journal in which the results will be published)

IPD sharing plan summary
Available on request

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Participant information sheet			01/06/2021	No	Yes
Results article		22/12/2021	21/03/2022	Yes	No
Results article	Immunohistochemical analysis of MMP-9 and COX-2 expression in carotid atherosclerotic plaques	05/09/2022	09/09/2022	Yes	No