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Relation between the length of the internal carotid stenotic segment and ischemic cerebrovascular events as well as white matter lesion load

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ρ	Spearman correlation
A1	First segment of the anterior cerebral artery
A2	Second segment of the anterior cerebral artery
ACST-1	The Asymptomatic Carotid Surgery Trial 1
A.D.	Anno Domini
AF	Atrial fibrillation
AHA/ASA	American Heart Association/American Stroke Association
aICAD	Asymptomatic internal carotid artery disease
ASA	Atrial septal aneurysm
B.C.	Before Christ
CEA	Carotid endarterectomy
CTA	Computed tomography angiogram
CRP	C-reactive protein
D1	The degree of internal carotid artery stenosis on the potentially symptomatic side (D1-PS) or in absence of potentially symptomatic stenosis, the more stenotic side (D1-St)
D1-PS	The degree of internal carotid artery stenosis on the potentially symptomatic side
D2	The degree of internal carotid artery stenosis on the asymptomatic side in patients with bilateral carotid stenosis
DSA	Digital subtraction angiogram
DWI-MRI	Diffusion weighted magnetic resonance imaging
DWM1	Deep white matter lesions ipsilateral to D1
DWM2	Deep white matter lesions contralateral to D1
ECST	European Carotid Surgery Trial
FLAIR	Fluid attenuation inversion recovery
HbA1c	Glycated Hemoglobin A1c
HDL-cholesterol	high density lipoprotein cholesterol
ICA	Internal carotid artery
ICAD	Internal carotid artery disease

IMTV	Intima media thickness variability
IQR	Interquartile range
L1	Length of internal carotid artery stenosis on the potentially symptomatic side or, in absence of potentially symptomatic stenosis, the more stenotic side
L1-PS	length of internal carotid artery stenosis on the potentially symptomatic side
LDL- cholesterol	Low density lipoprotein cholesterol
M1	1st segment of the middle cerebral artery
MCA	Middle cerebral artery
MES	Microembolic signals
MRA	Magnetic resonance angiogram
MRI	Magnetic resonance imaging
N/A	Not available
NASCET	North American Symptomatic Carotid Endarterectomy Trial
NIHSS	National Institutes of Health Stroke Scale
P	Statistical P-Value
PACS	Picture archiving and communication system
PFO	Patent foramen ovale
psICAD	Internal carotid artery disease ipsilateral to the infarction
PV1	Periventricular white matter lesions ipsilateral to D1
PV2	Periventricular white matter lesions contralateral to D1
SCS	Stenotic carotid segment
sICAD	Symptomatic internal carotid artery disease
TIA	Transient ischemic attack
TOAST	Trial of Org 10172 in Acute Stroke Treatment
TWMLL	Total white matter lesion load
USA	United States of America
WHO	World Health Organization
WML	White matter lesions
WMLL	White matter lesion load

I. INTRODUCTION

I. A. Ischemic Cerebrovascular Stroke

I. A1. Epidemiology:

Stroke represents the most common cause of adult disability (1, 2) and the fourth common cause of mortality worldwide (3). In Germany, 243.000-260.000 patients suffer from stroke each year (1), as compared to around 795.000 in the USA (3). Stroke leads to dramatic changes in the lives of sufferers as well as their relatives (2). More than half of stroke survivors regard a major stroke as an event worse than death (2). The incidence of stroke in men is higher than women, but the prevalence among women is higher because women survive longer than men (4). Epidemiological studies revealed that ischemic stroke, hemorrhagic stroke and subarachnoid hemorrhage represent 77%, 15% and 3%, respectively with the rest (5%) being classified as unknown (5).

I. A2. Brief history of stroke:

Stroke was described in a document found in the tomb of the vizier Weshptah related to the 5th dynasty (2455 B.C.) in ancient Egypt and later on by Hippocrates (460-370 B.C.) (6). The term used by Hippocrates was apoplexy (7). Loss of speech was described by Hippocrates as a manifestation of stroke (8). As early as the 4th century BC, animal models of stroke were designed by the Alexandrian anatomists Erasistratus and Herophilus (6). In his Canon of Medicine, Ibn Sina (980–1032 A.D.) described two main causes of stroke; 1. blockage of the cerebral vessels and 2. blockage of the mobile and sensitive spirit (affective spirit) of the brain (6). The former is similar to our current understanding of ischemic stroke and the latter can be viewed as an undetermined etiologic entity which, according to our current distinction of scholarly medicine, may pertain to disciplines of traditional or alternative branches of medicine. The Arabic term used by Ibn Sina to describe stroke was “Sekteh” (6). Ibn Sina also had a primitive description for the circle of Willis; specifically he described it as two main large vessels supplying the brain and ending up in a network around the pituitary gland (9). In 1664, Thomas Willis introduced a detailed description of this macrovascular anastomotic circle in his book “Cerebri Anatome” (9). The first use of the word stroke was most probably in 1689 by William Cole in his work “A Physico-Medical Essay Concerning

the Late Frequencies of Apoplexies" (7). Before Cole, the common term was apoplexy (7).

I. A3. Definition of stroke (7):

In 1970, the World Health Organization (WHO) defined stroke as rapidly developing focal neurological signs, lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin. Since the introduction of advanced neuroimaging modalities, the 24-hour criterion proved to be false because brain infarction can develop, even after very short lasting neurological signs. Nowadays, the differentiation of stroke from transient ischemic attack (TIA) relies mainly on the demonstration of brain infarction in the neuroradiological imaging. Nevertheless, a patient presenting with focal neurological signs of acute onset which disappear after receiving intravenous thrombolysis is still eligible for the diagnosis of stroke, even if magnetic resonance imaging (MRI) is normal (10).

I. A4. Definition of transient ischemic attack (TIA):

Historically, transient ischemic attack (TIA) was defined as: transient focal neurological deficit lasting less than 24 hours. This definition was introduced in the 1960s by Fisher (11), at a time when MRI was not yet introduced. Later on, it was found that 30-50% of patients with TIA do have brain infarctions on MRI (12-14). In 2009, the American Heart Association/American Stroke Association (AHA/ASA) revised the historical TIA definition as follows: a transient episode of neurological dysfunction caused by focal brain, spinal cord, or retinal ischemia, without acute infarction (12). The implementation of this new definition moved a significant proportion of patients from the TIA to the stroke category (15).

I. A5. Risk factors:

Risk factors for stroke (Table 1) can generally be classified into (2, 4)

1. Non-modifiable risk factors:

a. Age: the risk increases with advancing age.

b. Low birth weight: Individuals with low birth weight were found to have a higher risk of stroke later in life and even a higher stroke mortality rate.

c. Race: the stroke risk was found to be higher in Blacks (16) and Hispanic Americans. It remains unclear whether this risk is attributed to environmental factors, genetic factors or both.

d. Genetic factors: positive family history of stroke as well as history of stroke in parents before the age of 65 were found to increase the stroke risk. Family history is even stronger in younger stroke patients. Common variants on chromosome 9p21, adjacent to the tumor suppressor genes CDKN2A and CDKN2B, are associated with large-artery ischemic stroke. Common variants on 4q25 and 16q22, adjacent to genes involved in cardiac development (PITX2 and ZFHX3, respectively), are associated with ischemic stroke, particularly cardioembolic stroke.

2. Modifiable risk factors:

a. Physical inactivity: physically active subjects have around 25-30% lower risk of stroke. Physical activity is a well-established preventive measure of stroke.

b. Dyslipidemia: high serum levels of total cholesterol or of low density lipoprotein (LDL) cholesterol are related to ischemic stroke. The effect of lower levels of high density lipoprotein (HDL) cholesterol and high levels of triglycerides is controversial; some studies showed an increased stroke risk while others did not.

c. Diet: Mediterranean diet as well as low potassium intake are protective against stroke. On the other hand, high salt intake is associated with increased stroke risk. Of note, the observational studies addressing dietary factors were associated with a plenty of methodological weaknesses, especially related to difficulty in dietary assessment methods and recall bias.

d. Hypertension: is a strong, continuous and independent risk factor for stroke. A 10 mmHg increase in the systolic blood pressure was found to increase the stroke risk to 8% in whites and to 24% in African Americans (3).

e. Obesity and body fat distribution: abdominal obesity is a well-established risk factor for stroke.

f. Diabetes mellitus: is another well-established risk factor for stroke.

g. Cigarette smoking: is associated with a 2-fold increase in the risk of stroke. Smoking cessation was found to be associated with a reduction in the stroke risk but not to the levels of individuals who never smoked.

h. Atrial fibrillation (AF): is associated with a 5-fold increase in the risk of stroke (17). The risk of AF-related stroke was found to increase dramatically with age; the risk is 1.5% at the age of 50-59 years and reaches 25.5% at 80-89 years (17). This risk is usually related to the formation of thrombi in the left atrial appendage as a result of disturbance of the laminar blood flow. Paroxysmal and permanent AF are associated with the same risk.

i. Other cardiac diseases: acute myocardial infarction (MI), cardiomyopathy, valvular heart disease, patent foramen ovale (PFO), atrial septal aneurysm (ASA), cardiac tumors and aortic atherosclerosis are all associated with increased risk of stroke.

j. Carotid artery atherosclerosis.

k. Other conditions: sickle cell disease and hypercoagulapathies are also related to stroke.

I. A6. Etiological classification of acute ischemic stroke:

In 1993, the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification was introduced and later became popular, because of its therapeutic implications (18). The TOAST classification is shown in Table 2.

Table 1: Risk factors of ischemic stroke (2, 4)

Non-modifiable	Modifiable
Advancing age	Physical inactivity
Low birth weight	Dyslipidemia
Male sex	Diet: high salt intake
Blacks and Hispanic Americans	Hypertension
Genetic factors	Obesity
	Diabetes mellitus
	Cigarette smoking
	Atrial fibrillation
	Other cardiac diseases e.g. acute myocardial infarction (MI), cardiomyopathy, valvular heart disease, patent foramen ovale (PFO) and atrial septal aneurysm (ASA)
	Carotid artery atherosclerosis.
	Other conditions e.g. sickle cell disease and hypercoagulapathies are also related to stroke

Table 2: Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification (16, 18-20)

Stroke subtypes	Features
1. Large vessel disease (macroangiopathy), nearly 9-12% of all strokes *	≥50 % stenosis or occlusion of an extracranial or intracranial major vessel or branch cerebral artery as shown in duplex or angiographic images
2. Cardioembolic disease 15-28%*	A major cardioembolic source must be detected e.g., atrial fibrillation, mechanical prosthetic valve, left atrial thrombus, left ventricular thrombus,…..etc.
3. Small vessel disease (microangiopathy) or lacunar infarction, nearly 15-27% of all strokes *	CT/MRI shows white matter lesions and/or infarction<1.5 cm in diameter
4. Acute stroke of other determined etiology 2-3%*	Other causes identified like vasculitis, hypercoagulable state or hematologic disorder
5. Stroke of undetermined etiology 24%	a. Two or more causes identified, b. Negative evaluation, c. Incomplete evaluation

* Possible or probable depending on results of ancillary studies.

I. B. Carotid Atherosclerosis

Carotid atherosclerosis is the underlying etiology in around 15-20% of ischemic strokes (20, 21), and is especially common among middle-aged patients (22). The prevalence of asymptomatic carotid artery atherosclerotic plaques ranges from 34-87% (23-26).

Two methods can be used to measure the degree of stenosis: the European Carotid Surgery Trial (ECST) method and the North American Symptomatic Carotid Endarterectomy Trial (NASCET) method (27). Initially, both methods were developed to assess carotid stenosis using DSA. In mathematical terms, the degree of stenosis according to the NASCET method equals (A-B)/A and according to the ECST method (C-B)/C, where A= the diameter of the healthy carotid just above the stenosis, B= minimal diameter of the residual lumen and C= estimated diameter of the carotid bulb (27). In other words, the NASCET criteria are based on the reduction of diameter in relation to the distal lumen, whereas the ECST criteria are based on reduction of the diameter in relation to the estimated diameter of the carotid bulb (28). To change from NASCET to ECST, the following equation can be used: NASCET% = (ECST – 40)%/0.6 (28). From 1986 till 2011 the criteria used in Germany for grading carotid stenosis were based on the ECST method (28). Because the decision for carotid revascularization relies mainly on the degree of stenosis, it is important to standardize the criteria for grading carotid stenosis (28). Therefore, in order to avoid confusion, the NASCET criteria were accepted by the DEGUM (German Society of Ultrasound Medicine) in 2011 as the standard criteria for grading the degree of carotid stenosis and replaced the old ECST criteria (29). Furthermore, the NASCET criteria were also accepted as the standard criteria for grading carotid stenosis in computed tomography angiogram (CTA), magnetic resonance angiogram (MRA) and digital subtraction angiogram (DSA) (28).

I. B1. Symptomatic internal carotid artery disease (sICAD):

According to the findings of large multi-centric studies, the risk of stroke recurrence is related to the degree of the carotid stenosis and therefore, carotid endarterectomy (CEA) is recommended in patients with non-disabling high grade sICAD (>70%) (30-

32). Patients undergoing CEA were even more likely to suffer from periprocedural myocardial infarction, whereas patients≥ 65 years undergoing carotid artery stenting were slightly more likely to develop periprocedural stroke (33). Later on, the small differences in outcome effects after carotid artery stenting were shown to vanish during longer follow-up. In this context, stroke recurrence is either due to microembolisation as a result of plaque rupture (34, 35) or due to hemodynamic compromise, depriving the brain from its blood supply (35). Whether carotid revascularization is indicated in patients with lower stenotic grades remains a matter for further research. In patients with moderate ICAD 50-69%, CEA has a marginal beneficial effect and in patients with low grade ICAD of <50%, it has no protective effect (32). Nevertheless, the occurrence of cerebrovascular events in patients with lower grades of ICAD suggest that also these stenoses should be considered as a potential cause of ischemic stroke (36). Of note, low grade ICAD bearing a high risk of ischemic events was identified in several studies (37-39). For example, echolucent or ulcerated plaques were found to be of higher risk (36, 40). Later on, a risk index combining the degree of ICAD and the degree of plaque echolucency was proposed and was found to be higher among sICAD (41). In 1987, the NASCET started recruiting patients with sICAD to either medical treatment or CEA (42). In February 1991, the NASCET stopped recruiting patients with ICAD ≥ 70% because of marked positive results in favor of CEA. Afterwards, the NASCET continued recruiting patients with ICAD <70%. A total of 2226 eligible patients were enrolled with sICAD <70%. About 38.5% of these patients had ICAD of 50-69% and another 42.4 % had ICAD of 30-49%. Even 19.1 % of these patients presented with a sICAD <30%. The inclusion of these patients in the NASCET study, after excluding other potential stroke etiologies, shadows the importance of the relation between ICAD <70% and the occurrence of ipsilateral ischemic stroke. On the other side, severe grades of ICAD might remain asymptomatic over years and only one fourth of the patients with sICAD >70% will develop stroke under best medical treatment (27), whereas lower grades might cause cerebrovascular events (39, 43-46).

Patients with sICAD presenting with mild stroke or TIA were shown to be at high risk for further ischemic cerebrovascular events within the first days; around 21% of these stroke survivors experienced further ischemic events within the first 72 hours after

the index stroke, 7% between 72 hours and 7 days and 4% at 14 days (47). These findings pose a special clinical significance in the management of those patients.

I. B2. Asymptomatic internal carotid artery stenosis (aICAD):

Asymptomatic internal carotid artery stenosis (aICAD) is defined as the presence of ICAD with no ipsilateral cerebral or retinal symptoms in the past 6 months (48). The yearly incidence of stroke in patients with aICAD is 1.3% (49). Among patients with ICAD 50-99% under best medical treatment, this incidence was found to be 0.93%/year (95% confidence interval (CI): 0.11-3.37) (50). Around 30% of the normal population aged 40-54 years have aICAD (51). The pooled random-effects prevalence of moderate aICAD (50-69%) was found to be 4.2% and among those ≥ 70 years, the prevalence is 12.5% in men and 6.9% in women, whereas the pooled random-effects prevalence of severe asymptomatic ICAD ≥ 70% was found to be 1.7% (23). Up till now, it remains unclear whether patients with aICAD are more likely to benefit from carotid revascularization, either by surgical or endovascular means, or from best medical treatment (52). The Asymptomatic Carotid Surgery Trial 1 (ACST-1) randomized 3120 patients with aICAD ≥70% in a 1:1 ratio either to CEA or to deferral of any procedure (48). Patients were followed up for a median of 9 years or until they died. The authors found that immediate CEA among patients younger than 75 years of age achieved as nearly as 50% stroke risk reduction. However, further medical treatment options for both groups were left to the treating physician in this trial. Specifically, this trial was done before the wide application of statins for cerebrovascular patients (53). Later on, the Ontario study showed that aggressive medical treatment using antiplatelet, statins, smoking cessation, exercise, implementation of a Mediterranean diet and optimal blood pressure management can achieve a significant reduction of cardiovascular events, rate of plaque progression and microembolic signals as shown in the transcranial duplex (TCD) among patients with aICAD ≥60% (53). The authors emphasized the role of high dose statins irrespective of the serum LDL-Cholesterol levels. Based on these results, it was hypothesized that CEA should be mainly done in patients with microembolic signals (MES). The authors recommended CEA for the subgroup of patients with MES, since the risk of a new cerebrovascular event was 10.3%/year in this group. They concluded that, CEA should only be offered to patients without MES if the procedure

bears a procedural risk of <1%, since the stroke risk among those patients receiving aggressive medical treatment remained as low as 1.4%/year. Women were found to be less likely to benefit from surgery. The investigators of the asymptomatic carotid surgery trial (ACST) explained this finding by the higher operative risk in women (54), whereas the investigators of the NASCET study attributed this gender difference to the higher stroke risk in men (42). CEA or endovascular stenting should only be recommended, if it could be achieved with a complication rate of <3% (53). Eventually, in the Asymptomatic Carotid Stenosis trial (ACT), endovascular stenting was shown to be non-inferior to CEA among patients with aICAD 70-99%(55).

A previous study from the 1980's examined patients with aICAD (56). Over a follow up period of seven years, the annual mortality rate was 7% with only 0.6% stroke mortality, while the remaining mortality occurred because of cardiac diseases. Only 0.4% developed stroke without an alarming TIA. Progression of stenosis was the only criteria found to predict poor prognosis and thus was more likely associated with a poor outcome than any other investigated risk factor.

Previous authors found that, among patients with aICAD, progression of the stenosis to 80% predicts the development of either occlusion or ischemic events (odds ratio (OR): 54, $p<0.001$) (57). Cigarette smoking, diabetes mellitus and age <65 years were found to be the main risk factors predicting disease progression (57).

I. C. Cerebral White Matter Lesions

White matter lesions (WML) are commonly detected in the brains of elderly people (58-60). They are more frequently identified with increasing age (60) and correlate with cognitive changes, gait instability, focal neurological signs as well as bladder and bowel symptoms (61). They are considered as an independent risk factor for the development of further cerebrovascular events (62, 63). MRI is a highly sensitive diagnostic modality for identifying those lesions but not specific as they may be mistaken for inflammatory lesions and remain per se unspecific with regard also to other non-inflammatory etiologies (60, 64). Hypertension was frequently found to be associated with increased white matter lesion load (WMLL) (65, 66), which supports an underlying microvascular disease as an origin for these lesions. Furthermore, several other risk factors were identified including diabetes mellitus, dyslipidemia and smoking (61), all of which would further support a microvascular origin. Nevertheless, the Rotterdam study revealed that carotid atherosclerosis is related to the presence or absence of WML (58), which contradicts the microangiopathic theory. Moreover, increased carotid plaque burden was found to be associated with increased WMLL (67) proposing an embolic origin of those lesions. The embolic origin of those lesions is further supported by the positive association between instability of the carotid plaques and WMLL (68). Intimal media thickness variability (IMTV) was found to correlate positively with WMLL (69). IMTV represents irregularity in the carotid wall and is considered as a marker of instability (69). On the other hand, several studies found no relation between carotid atherosclerosis and WML (70, 71). For example, Potter et al. found no association between ipsi- or contralateral carotid stenosis and WML, before and after adjusting risk factors (70). The contradicting results of studies addressing the relation between WMLL and the ICAD signal the importance of conducting further studies addressing new parameters.

The degree of stenosis was found in multicenter studies to be strongly related to the recurrence of carotid related ischemic cerebrovascular stroke (30-32). To our knowledge, the relation between the length of the stenotic carotid segment (SCS) and the risk of ischemic cerebrovascular events as well as the WMLL has never been studied. Furthermore, it seems that very long segmental carotid stenosis $\geq$ 50%, in comparison to the short segmental counterpart, induces less increment in the local flow

velocity, as demonstrated on ultrasound scans, and hence is more likely to be underestimated in the duplex sonographic studies (28), posing more diagnostic difficulties. We propose that long segmental carotid stenosis, being more bulky, is associated with a higher plaque burden and may hence be more emboligenic (Figure 1). On the other side, short segmental carotid stenosis ≥50% rather induces more increment in the local flow velocity in comparison to the long segmental counterpart. Therefore, the length of SCS might be related to ischemic cerebrovascular events and might be associated with a different WML pattern or extent.

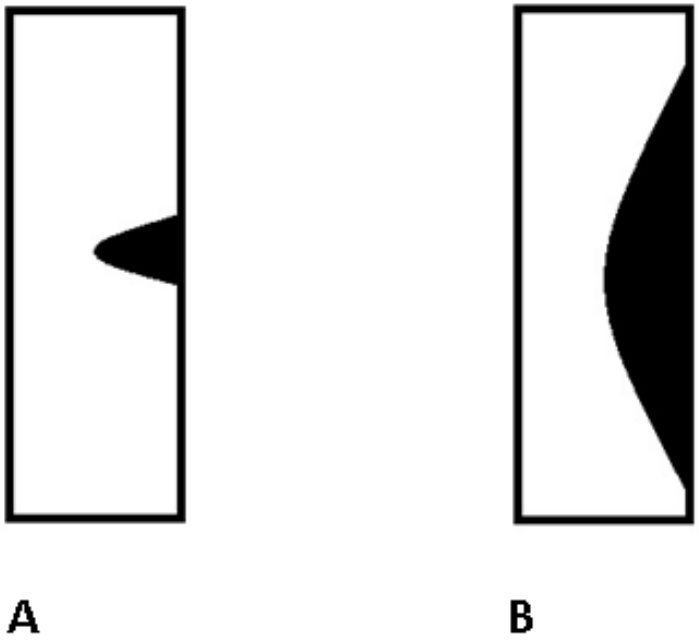

Figure 1: Short segmental (A) vs long segmental (B) 50%-carotid stenosis

Long segmental carotid stenosis, being more bulky, is associated with a higher plaque burden and may hence be more emboligenic

II. AIM OF THE WORK

The aims of this study were:

1. To examine the relation between the length of the carotid stenotic segment and the risk of ischemic cerebrovascular events: a. among internal carotid artery stenosis <70% versus ≥70%, and b. among internal carotid artery stenosis <90% versus 90% (Part I).

2. To examine the relation between the length of the stenotic carotid segment and the cerebral white matter lesion load. (Part II).

III. SUBJECTS AND METHODS

III. A1. Inclusion criteria:

Consecutive patients admitted to the Department of Neurology, University Hospital of Würzburg, with an acute ischemic stroke or transient ischemic attacks (TIA) as well as an extracranial ICAD, either ipsi- or contralateral, were enrolled retrospectively in this study between January 2011 and September 2016. The grading of ICAD was based on duplex ultrasound examination using the hemodynamic criteria of the North American Symptomatic Carotid Endarterectomy Trial (NASCET) (28). We included patients in whom the available duplex sonographic images were of sufficient quality to measure the length of ICAD on at least one side, or those with available MRA and/or DSA examinations. The patients were specifically identified according to the following ICD-10 codes: I65.2 (stenosis or occlusion of the carotid artery) or I65.3 (stenosis or occlusion of more than one vessel or bilateral vessels) plus stroke (I63.0-I63.9) or 164 (stroke, not classified as infarction or bleeding) or TIA (G45.12, G45.13, G45.19, G45.22, G45.23, G45.29, G45.32, G45.33, G45.39, G45.82, G45.83, G45.89, G45.92, G45.93 or G45.99). Stroke was defined if an ischemic lesion was detected on diffusion weighted magnetic resonance imaging (DWI-MRI). In the absence of lesions on MR or CT scans, stroke was defined according to the WHO definition: rapidly developing clinical signs of focal disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin (7). For example, patients with focal neurological signs lasting more than 24 hours and suggestive of subcortical stroke but with no available cerebral MRI or without detectable brain infarction in the CT images were included as stroke patients. TIA was defined according to the American Heart Association/American Stroke Association (AHA/ASA) definition: a transient episode of neurological dysfunction caused by focal brain, spinal cord, or retinal ischemia, without acute infarction (12).

III. A2. Exclusion criteria for part I (association between length of ICAD and risk of cerebrovascular events):

Exclusion criteria:

1. Carotid artery occlusion or pseudo-occlusion because, in this case, the measurement of the stenotic length was not possible. Pseudo-occlusion was diagnosed if the artery was shown in one neurovascular diagnostic modality to

be occluded but revealed as a stenosis in another diagnostic modality. A common example is an ICA considered to be occluded in the MRA but shown to be nearly occluded (i.e., high-grade stenosis) in the ultrasound when using low pulse repetition frequency.

2. Patients with merely common, not internal, carotid artery stenosis. The absence of standardized criteria to grade the stenosis as well as the potential difference between the hemodynamics of the internal and the common carotid arteries was an obstacle to include these patients.

3. ICAD of 10%, measured according to the hemodynamic NASCET criteria (28), because the measurement of the stenotic length in these cases was not accurate. Moreover, a significant proportion of these cases could have been easily missed from our records. Depending on the examiner, these cases were sometimes described as calcifications and in other times as atherosclerosis. Sometimes the word "atherosclerosis" was used as a synonym for "increased intimal medial thickness". Frequently, this term was used to describe atherosclerosis in the carotid bulb, which was used also to refer to the common carotid artery. That is why we restricted our study to patients with ICAD ≥20%.

4. Patients in whom a stroke or TIA occurred ≥14 days before admission to our hospital.

5. Iatrogenic stroke complicating carotid endarterectomy or coronary angiography.

6. Patients in whom TIA was suspected but other differential diagnoses were included in the discharge letter as assessed from the treating neurologist.

7. Patients in whom a definite allocation to symptomatic or asymptomatic ICAD was not possible; a) patients with brain infarctions ipsilateral to ICAD in the presence of a cardioembolic source as defined in the Trial of Org 10172 in Acute Stroke Treatment (TOAST) (18), lacunar infarction or other determined cause of infarction and b) patients with bilateral brain infarctions, with the main infarction bulk located ipsilateral to the ICAD with only minute contralateral brain infarction(s).

8. Patients with no available ultrasound images.

III. A3. Exclusion criteria for part II (association between length of ICAD and WML):

Exclusion criteria for this part were the above mentioned exclusion criteria 1-6.

III. A4. Clinical, laboratory and radiological data:

The following data were collected:

1. Personal data: age and sex.
2. Medical data: history of hypertension, diabetes mellitus, smoking, rheumatological disease, coagulopathy and/or history of atrial flutter or fibrillation. Patients were classified according to the smoking status as: smokers, ex-smokers if the patient stopped smoking >6 months ago or nonsmokers.
3. Time from stroke onset to presentation was obtained and expressed in days. In patients with wake-up stroke or in those with unknown onset of stroke, a time window of 24 hours was supposed.
4. Clinical data: National Institutes of Health Stroke Scale (NIHSS) score on admission was obtained. When the NIHSS score was not directly available, we estimated the value from the data obtained from the neurological examination on presentation.
5. Laboratory data: serum levels of hemoglobin A1c (HbA1c) and low density lipoprotein cholesterol (LDL-Cholesterol).
6. The degree of the ICAD was assessed in the extracranial duplex sonographic studies according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria (28).
7. The ICAD was classified as symptomatic if an ischemic cerebrovascular event occurred ipsilateral to the ICAD. In patients with brain infarctions or TIA contralateral to the ICAD or in the vertebrobasilar territory and in patients with bilateral brain infarctions, the ICAD was classified as asymptomatic. In patients with bilateral brain infarctions, with the main infarction bulk located ipsilateral

to the ICAD with only minute contralateral brain infarction(s), the ICAD was classified as non-specified.

8. The ICAD was classified as potentially symptomatic (psICAD) if the ischemic cerebrovascular event occurred in the territory of the respective internal carotid artery, irrespective of the presence of other potential causes like cardioembolism.
9. The length of the stenotic segment was measured using duplex ultrasound, MRA and/or on DSA images according to the availability as described below.
10. Plaque echolucency was classified by a single observer (A.E.) using the modified classification proposed by Gray-Weale (72): type 1 (predominantly echolucent), type 2 (mixed echolucent/echogenic) and type 3 (predominantly echogenic).
11. The white matter lesions were assessed in the MRI-fluid attenuation inversion recovery (FLAIR) sequence as described below.

III. A5. Measurement of the length of ICAD in the sonographic images (Figure 2):

The longest length of the carotid stenotic segment was measured in the digitalized ultrasound images in our picture archiving and communication system (PACS) from the most proximal to the most distal stenotic end. These two ends were identified according to the following criteria:

1. Visible narrowing of the vascular lumen.

2. Aliasing phenomenon in the proximal end of stenosis. For the distal end, aliasing phenomenon was accepted, only in the presence of corresponding images showing increased systolic flow velocity denoting the presence of stenosis. In absence of corresponding increased systolic flow velocity, criteria 1 and 3 were used to identify the distal end, because the differentiation between aliasing due to stenosis and post stenotic flow disturbance was not possible.

3. The presence of severe calcification causing acoustic shadowing and hence impeding the visualization of the vascular lumen.

III. A6. Measurement of the length of ICAD in the MRA and DSA (Figure 2, panel B):

This measurement was done in the images available in our PACS from the most proximal to the most distal part of the stenotic segment in the best available magnetic resonance angiography (MRA) and/or digital subtraction angiography (DSA). We chose the projection demonstrating the longest stenotic segment.

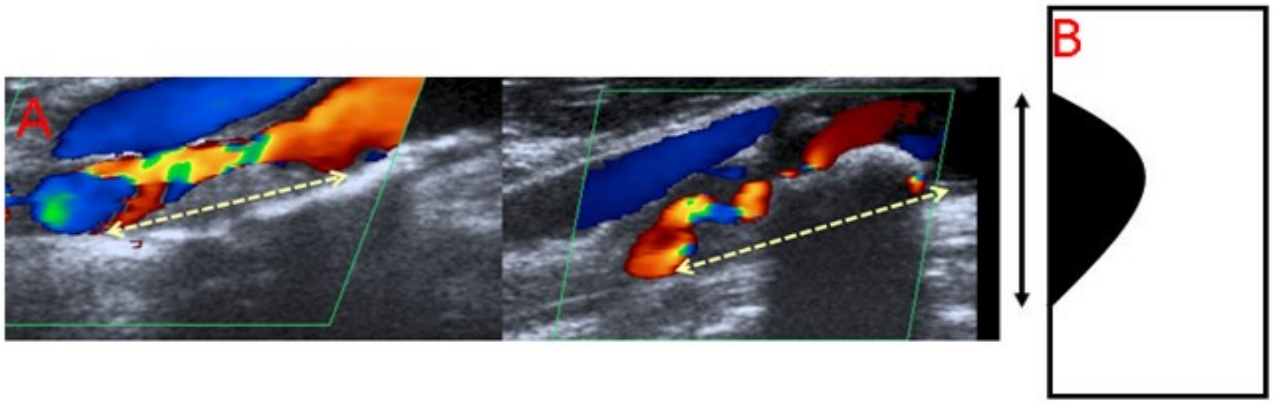

Figure 2: Assessment of the length of ICAD; the length was measured from the most proximal to the most distal stenotic segment

III. A7. Assessment of the white matter lesions:

The periventricular and the deep WML were assessed on FLAIR sequence of cerebral MRI by a non-blinded examiner (AME) using the Fazekas scale (59). The WML were assessed on every side and separately as follows:

1. Periventricular (PV1) and deep white matter lesions (DWM1): ipsilateral to the psICAD or, in absence of psICAD, the more stenotic side.
2. Periventricular (PV2) or deep white matter lesions (DWM2) contralateral to the psICAD or, in absence of psICAD, contralateral to the more stenotic side.
3. Total white matter lesions (TWMLL): the mean of PV1, DWM1, PV2 and DWM2.

III. A8. Ethical approval:

Data were collected within routine clinical care. Therefore, no specific ethics board approval was needed according to the local regulations confirmed by the Ethics Board of the Medical Faculty of the University of Würzburg. Our Ethical Committee waived the need for informed consent because of the retrospective nature of the study.

III. A9. Statistics:

Quantitative data were expressed using median and interquartile ranges, while qualitative data were expressed in absolute and relative frequencies. To check for normality, we used graphical methods (QQ-plot and histogram) and the Shapiro-Wilk test. Univariate statistical tests were conducted using chi-square test for categorical data, and Mann-Whitney U-test as well as Kruskal-Wallis test for continuous data. For the statistical analyses concerning the length of ICAS, the ultrasound-measured length was used, because all included cases had available ultrasound images. We examined the relation between the length of the carotid stenotic segment and the risk of ischemic cerebrovascular events among internal carotid artery stenosis <70% versus ≥70%, and among internal carotid artery stenosis <90% versus 90%. Spearman coefficient and Pearson coefficient were used to analyze correlations. We calculated the sensitivity and specificity of all measured stenotic length values among aICAD and sICAD using an ROC-curve and chose the point with best sensitivity and specificity to represent the cut-off-value for sICAD. Multivariable regression analyses were performed. We used a generalized estimation equation to allow for inclusion of the two stenotic sides in the multivariate regression analysis model in patients with bilateral stenosis. Data were analyzed in SPSS software package version 25 (SPSS, Chicago IL USA). P-values <0.05 were considered statistically significant.

IV. RESULTS

IV. A. Descriptive and Statistical Analysis

Four hundred eighty nine patients were screened and 168 patients with 208 ICAD were included. In 166 patients, duplex images were available, while in two patients the duplex images were missing. In one of the two patients with missing duplex images we had DSA and MRA images and in the other, MRA images were available. MRA and DSA images were available in a total of 88 and 25 patients respectively. In duplex ultrasound, the degree of ICAD could be assessed in 202 arteries. We were able to measure the length of ICAD in 177, 118 and 28 arteries in duplex ultrasound, MRA and in DSA, respectively. One-hundred twenty-one (121) patients met the inclusion and exclusion criteria for assessment of the relation between the length of ICAD and the ischemic cerebrovascular events (Part I) with 95 sICAD and 64 aICAD. In 136 patients, FLAIR sequences were available, and hence the inclusion criteria for part II. Figure 3 shows the included and excluded cases from the current study.

IV. A1. Baseline characteristics of our population (Tables 3 and 4):

The present study cohort included 116 males (69%) and 52 females (31%). Their ages ranged from 45-93 years with a median (IQR) of 74 (66-80) years. There was a tendency for ICAD ≥70% to occur in younger patients (P=0.07).

Table 3: Demographic data

Characteristic	All patients (n=168)
Male sex	116 (69%)
Median (IQR) age (y)	73,5 (66-80)

Data are expressed as absolute values or percentages

Table 4: Demographic data for ICAD <70% vs ≥70%

Characteristic	ICAD <70% (n=107)	ICAD≥70% (n=95)	P-value
Male sex	72 (67.3%)	67.4%	0.99
Median (IQR) age (y)	75 (67-82)	73 (66-77)	0.07

Data are expressed as absolute values or percentages
N.B. This table was based on arteries, not patients, in two patients the degree of ICAD could not be determined.

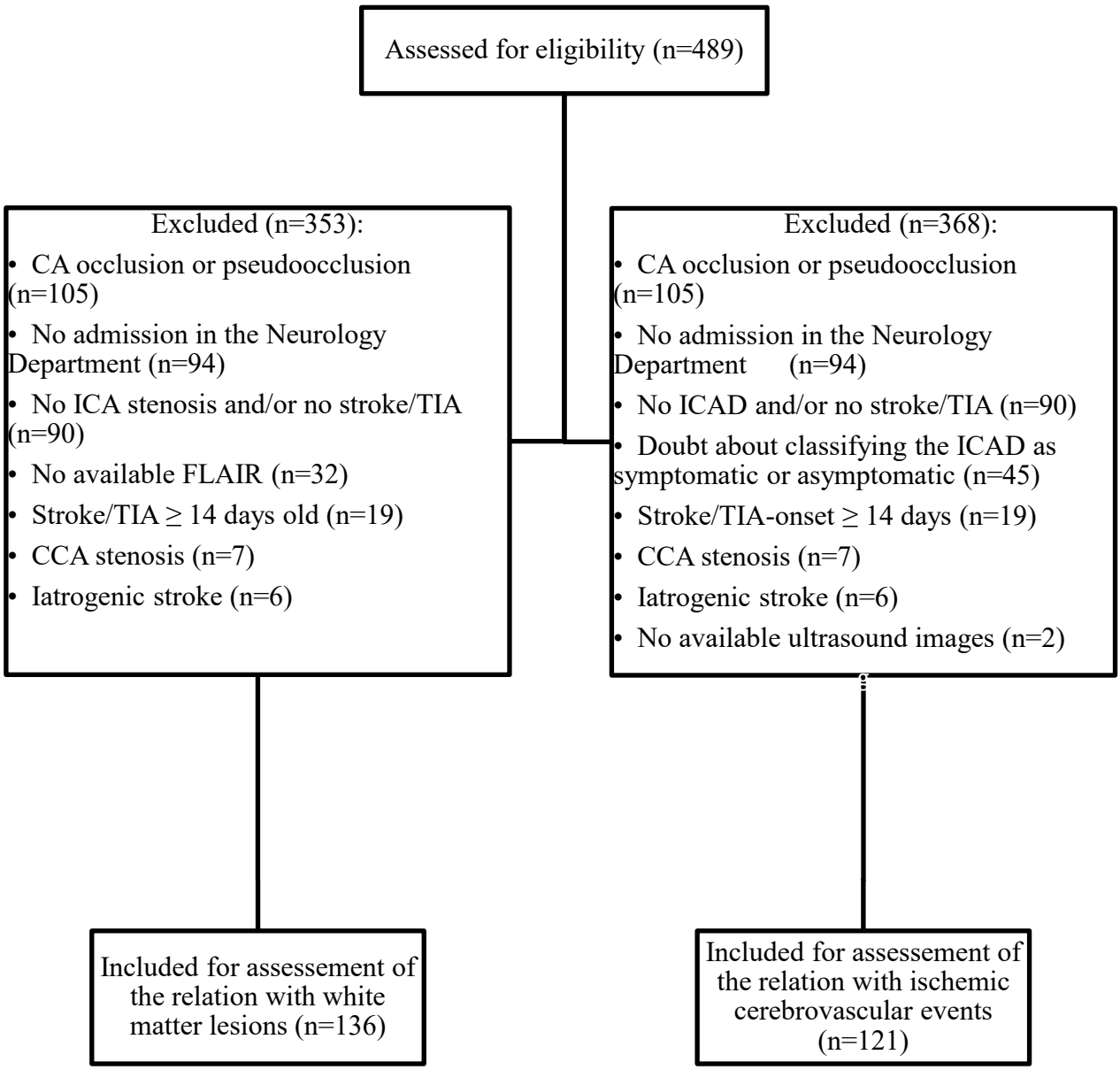

Figure 3: Flow chart showing the included and excluded patients from the current study

CA: carotid artery, CCA: common carotid artery, ICA: internal carotid artery, ICAD: internal carotid artery disease, TIA: transient ischemic attack, iatrogenic stroke (5 cases after carotid endarterectomy, 1 case after coronary angiography). Of the 489 patients screened, only 136 patients met our inclusion and exclusion criteria for assessment of the relation with the white matter lesions and 121 met our inclusion and exclusion criteria for assessment of the relation with ischemic cerebrovascular events.

IV. A2. Risk factors (Tables 5 and 6 as well as Figure 4):

One hundred and fifty-one patients (89.9%) were hypertensive, 57 patients (33.9%) were diabetic, 26 patients (15.5%) had atrial fibrillation (AF) and one patient had atrial flutter. In one patient atrial flutter was diagnosed 3½ years later. Whether this finding is associated with the preceding stroke remains elusive. Regarding smoking, 35 patients (20.8%) were active smokers, 43 patients (25.6%) were ex-smokers and in 2 patients, data regarding smoking status were not available. Seven patients (4.2%) had a known or possible coagulopathy and 6 patients (3.6%) had a possibly relevant rheumatological illness. We found no statistical association between the risk factors and the occurrence of ICAD <70% versus ≥ 70% (Table 6).

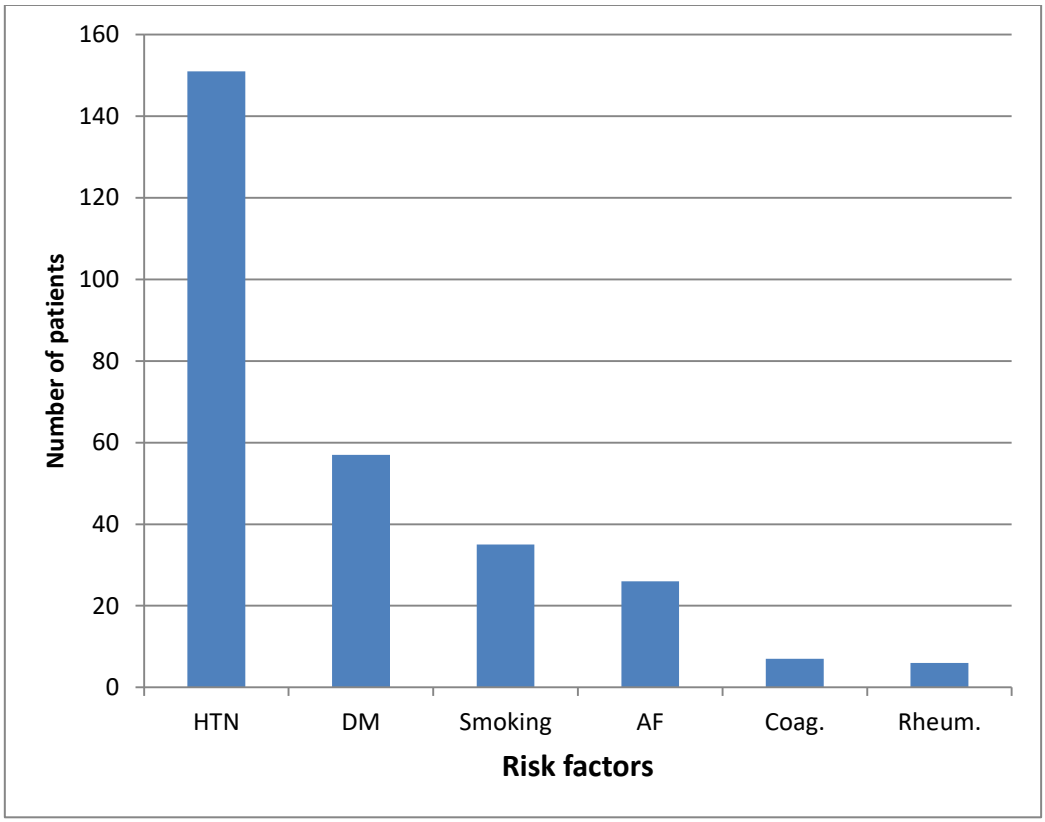

Figure 4: Risk factors

AF: atrial fibrillation, coag.: known or possible coagulopathy, DM: diabetes mellitus, HTN: hypertension, Rheum.: possibly relevant rheumatological illness, smoking: including only active smokers

Table 5: Risk factors

Characteristic	**Number (%)**
Hypertension	151 (89.9%)
Diabetes mellitus	57 (33.9%)
Atrial fibrillation	26 (15.5%)
Atrial flutter	1 (in 1 case the atrial flutter was diagnosed 3½ years later)
Smoking status	
smoker	35 (20.8%)
ex-smoker	43 (25.6%)
non-smoker	88 (52.4%)
NA	2 (1.2%)
Combined risk factors	
hypertensive smoker	29 (17.3%)
hypertensive diabetic	52 (31.0%)
Known or possible coagulopathy	7 (4.2%)
Possibly relevant rheumatological illness	6 (3.6%)

NA: not available

Table 6: Risk factors for ICAD <70% versus ≥70%

Risk factors	ICAD <70% (n=107)	ICAD ≥70% (n=95)	P-value
Hypertension	100 (93.5%)	83 (87.4%)	0.14
Diabetes mellitus	42 (39.3%)	28 (29.5%)	0.15
Atrial fibrillation	21 (19.6%)	14 (14.7%)	0.36
Atrial flutter	2 (1.9%)	in 1 case (1.1%) the atrial flutter was diagnosed 3½ years later	0.632
Smoking status			0.19
smoker	17 (16%)	24 (25.5%	
ex-smoker	30 (28%)	24 (25.5%)	
non-smoker	59 (55.7%)	46 (48.9%)	
NA	1 (0.9%)	1 (1.1%)	
Combined risk factors			
hypertensive smoker	15 (14.2%)	18 (18.9%)	0.34
hypertensive diabetic	38 (35.5%)	27 (28.4%)	0.28
Known or possible coagulopathy	5 (4.7%)	3 (3.2%)	0.17
Possibly relevant rheumatological illness	6 (5.6%)	4 (4.2%)	0.16

Results are expressed as absolute values (percentages)
This table was based on arteries, not patients, in two patients the degree of ICAD could not be determined, ICAD: internal carotid artery disease, NA: not available

IV. A3. Clinical and laboratory data (Tables 7 and 8 as well as Figures 5-8):

Ischemic stroke was diagnosed in 123 patients (73.2%), whereas TIA in 45 patients (26.8%). The median (IQR) NIHSS score on admission (only for stroke patients) was 3 (1-6). The median (IQR) duration of stay in the Department of Neurology was 9 (6-14)

days. The results of NIHSS score are shown in Figure 7. The median (IQR) time from onset of stroke to presentation in our hospital was 1 (1-1) day.

The median (IQR) LDL-cholesterol and HbA1c serum levels were 109 (84.0-136.5) mg/dl and 5.9 (5.6-6.5)%, respectively.

ICAD ≥ 70% was associated with the occurrence of stroke rather than TIA (P=0.01). No statistically significant difference was found between ICAD <70% versus ≥70% regarding NIHSS score for stroke patients, duration of stay in the Department of Neurology, serum levels of LDL-cholesterol or serum levels of HbA1c.

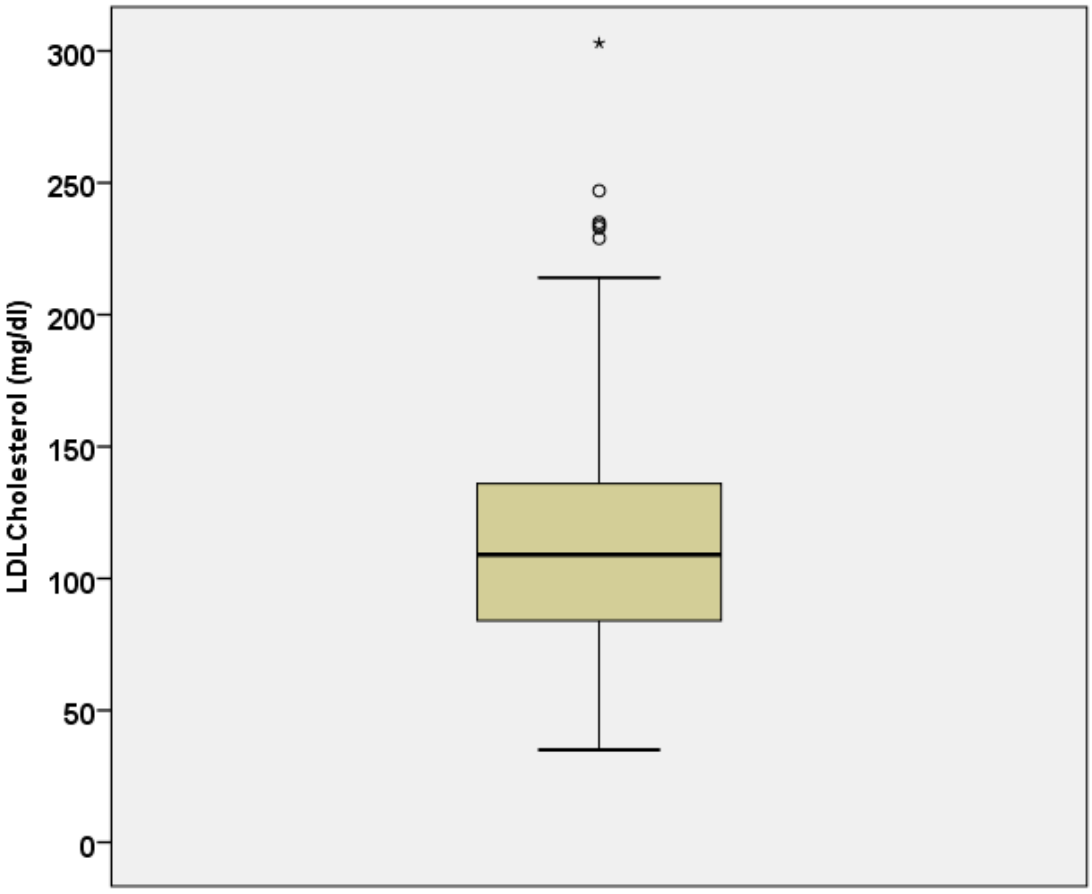

Figure 5: Serum levels of LDL-cholesterol (mg/dl)

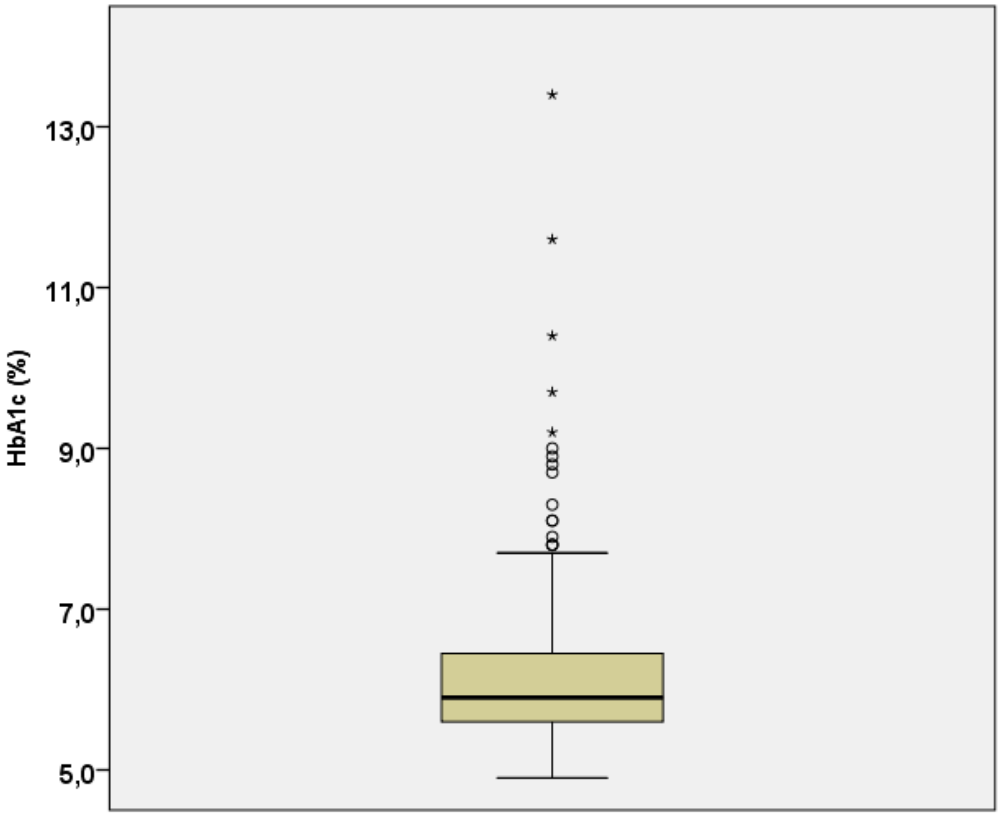

Figure 6: Serum levels of HbA1c (%)

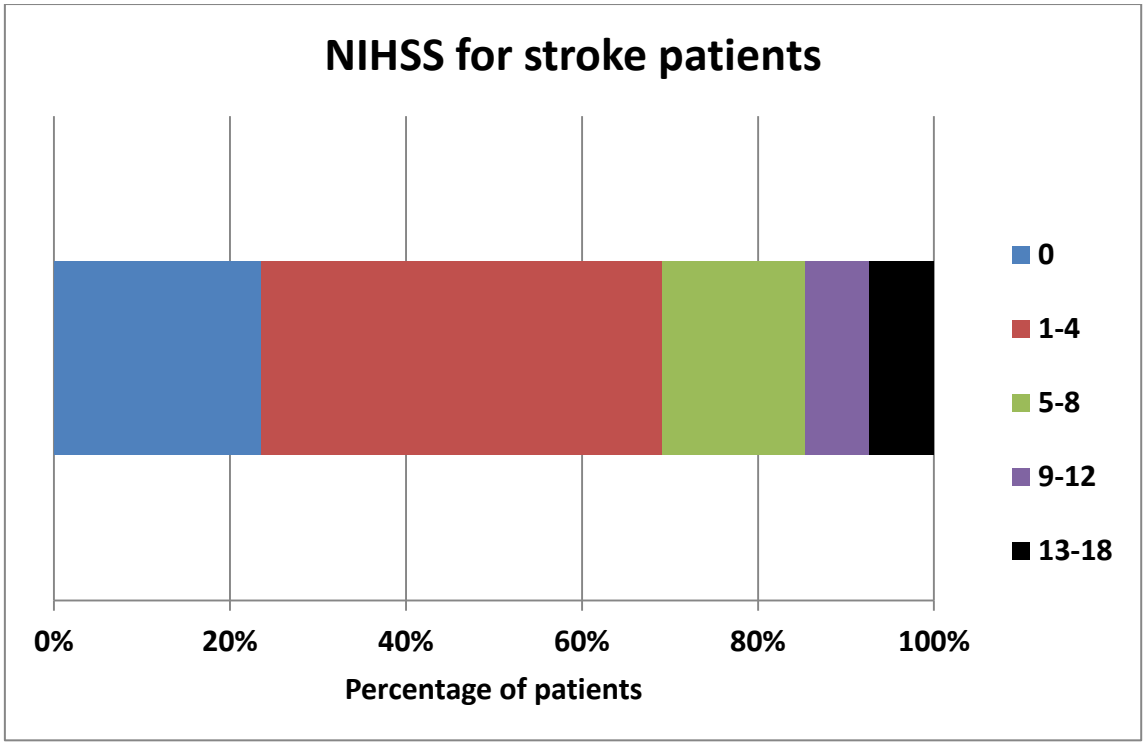

Figure 7: NIHSS score among stroke patients

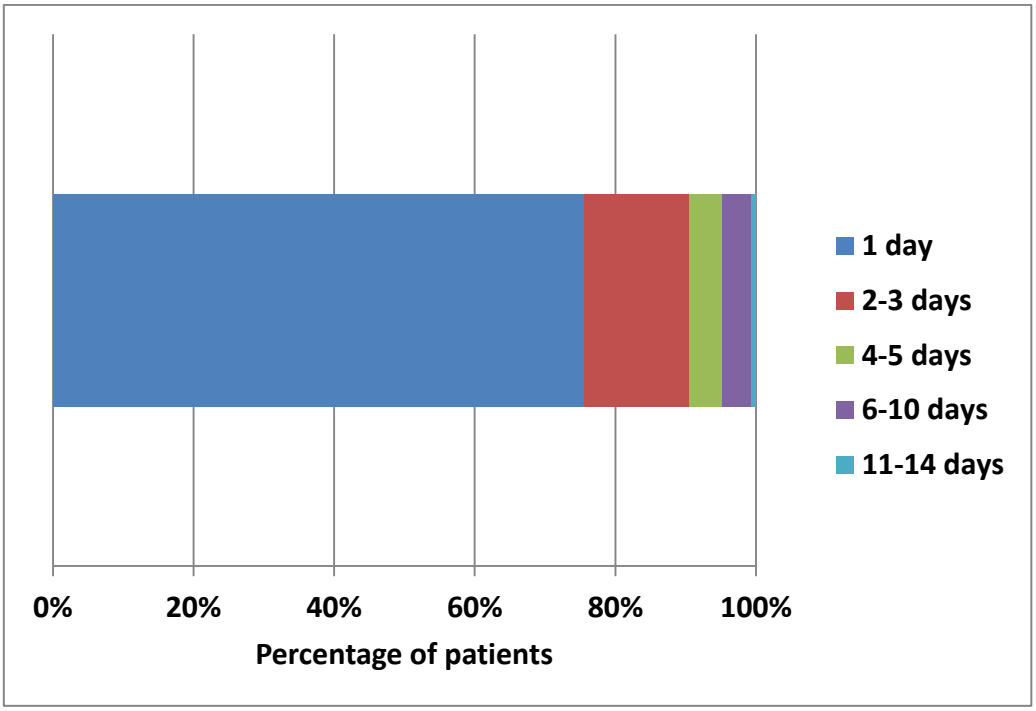

Figure 8: Time from onset to presentation

Table 7: Clinical and laboratory data

Data	**Observation**
Stroke vs. TIA	123 (73.2%) vs. 45 (26.9%)
NIHSS score for stroke patients	3 (1-6)
0	29 (23.6%)
1-4	56 (45.5%)
5-8	20 (16.3%)
9-12	9 (7.3%)
13-18	9 (6.3%)
Duration of stay in the Neurology Department (days)	9 (6-14)
Time from onset to presentation (days)	1 (1-1)
LDL-cholesterol serum levels (mg/dl)	109 (84.0-136.5)
HbA1c serum levels (%)	5.9 (5.6-6.5)

Results are expressed as absolute values (percentages) or median (IQR)
NIHSS: National Institute of Health Stroke Scale, TIA: transient ischemic attack

Table 8: Clinical and laboratory data for ICAD <70% vs ≥70%

Data	**ICAD <70% (n=107)**	**ICAD ≥70% (n=95)**	**P-value***
Stroke vs. TIA	73 (68.2%) vs. 34 (31.8%)	79 (83.2%) vs. 16 (16.8%)	0.01*
NIHSS score for stroke patients	3 (1-7)	2 (0-6)	0.18
0	14 (13.1%)	25 (26.3%)	
1-4	35 (32.7%)	33 (34.7%)	
5-8	13 (12.1%)	10 (10.5%)	
9-12	5 (4.7%)	7 (7.4%)	
13-18	6 (5.6%)	4 (4.2%)	
Duration of stay in the Neurology Department (days)	9 (6-14)	9 (6-14)	0.92
LDL-cholesterol (mg/dl)	107 (84-141)	117 (87-141.8)	0.52
HbA1c serum levels (%)	6 (5.6-6.7%)	5.9 (5.5-6.5)	0.2

Results are expressed as absolute values (percentages) or median (IQR)
N.B. This table was based on arteries, not patients, in two patients the degree of ICAD could not be determined, *statistically significant, ICAD: internal carotid artery disease, IQR: interquartile range, NIHSS: National Institutes of Health Stroke Scale, TIA: transient ischemic attack

IV. A4. Interventional treatment of ICAD (Figure 9)

Seventy-three patients (43.5%) received CEA, 16 patients (9.5%) underwent ICA stenting, one patient received angioplasty for a previous carotid stent and in one patient stenting of the basilar artery was performed. In 11 patients (6.5%), CEA or ICA stenting were recommended but not done and in 66 patients (39.3%) no interventional treatment of the ICAD was indicated.

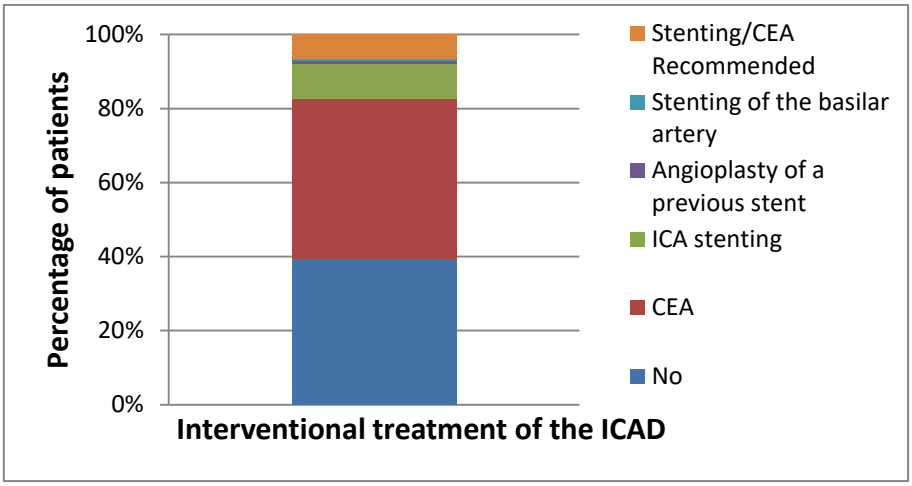

Figure 9: Interventional treatment of the internal carotid artery disease (ICAD) CEA: Carotid endarterectomy, ICA: Internal carotid artery

IV. A5. Degree and length of ICAD (Tables 9 and 10 as well as Figures 10 and 11):

Overall, the median (IQR) degree of ICAD, as measured in duplex ultrasound, was 60 (20-80)%. The median (IQR) length was 17 (12-20) mm, 10.6 (8-14) mm and 11.5 (10-17) mm in duplex sonography, MRA and DSA, respectively (Table 9). We found a statistically significant positive correlation between the measurements of the length in duplex and the two other examination modalities (Table 10). The correlation between the measurements in MRA and DSA was slightly beyond statistical significance (ρ=0.47, p=0.07, N=16).

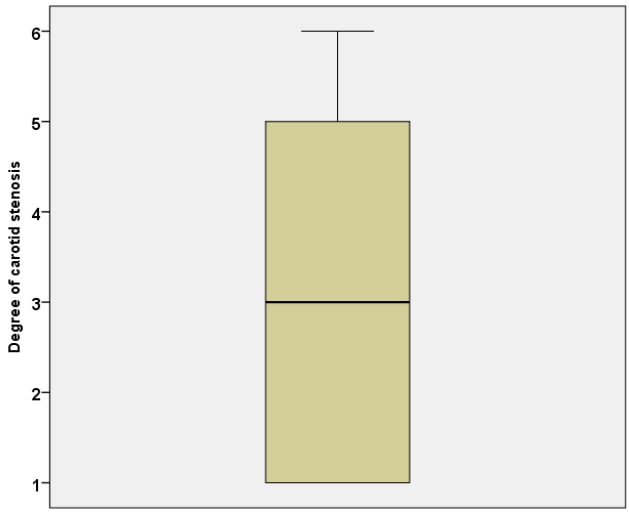

Figure 10: Degree of ICAD in duplex sonographic examination (1=20-40%, 2=50%, 3=60%, 4=70%, 5=80%, 6=90%)

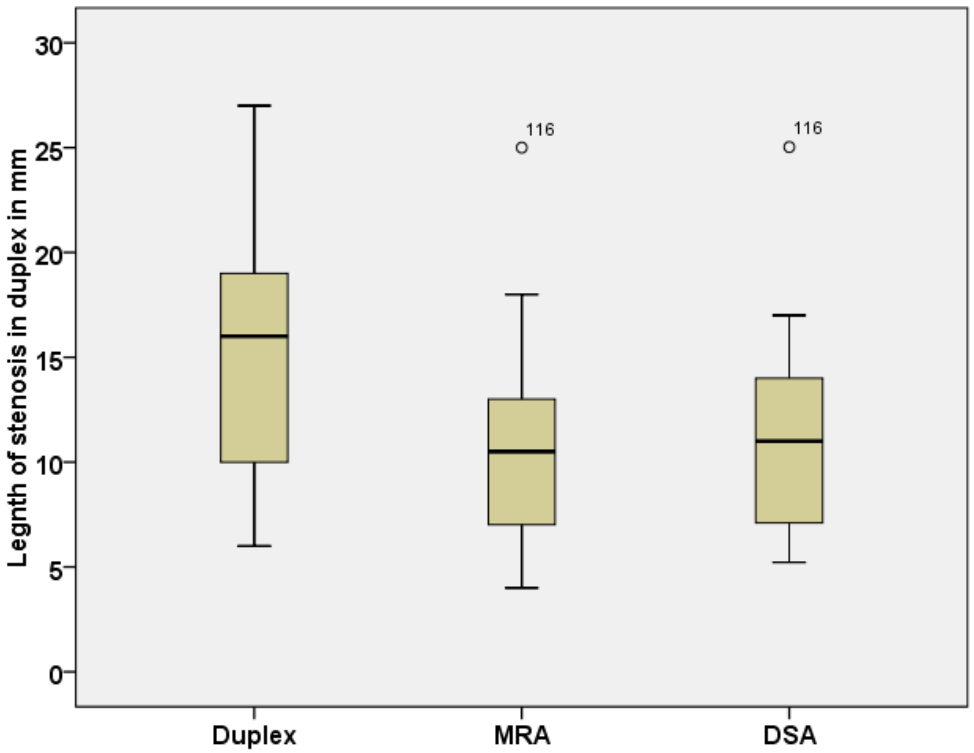

Figure 11: Length of internal carotid artery disease (ICAD) in different examination modalities

Table 9: Degree and length of internal carotid artery disease (ICAD)

ICAD	Median (IQR)
Degree % (duplex) (n=202)	60 (20-80)%
Length (mm)	
Duplex (n=177)	17 (12-20)
MRA (n=118)	10.6 (8.0-14.0)
DSA (n=28)	11.5 (10.0-17.0)

Results are expressed as median (IQR)
DSA: digital subtraction angiogram, IQR: interquartile range, MRA: magnetic resonance angiogram, ICAD: internal carotid artery disease

Table 10: Correlation between the measurement of the stenotic legnth in different examination modalities

Method	Statistical relation
Duplex versus MRA	ρ=0.36*, (p=0.001), n=91
Duplex versus DSA	ρ=0.48, (p=0.04), n=19
DSA versus MRA	ρ=0.47, (p=0.07), n=16

*Spearman correlation, DSA: digital subtraction angiogram, IQR: interquartile range, MRA: magnetic resonance angiogram

IV. A6. Risk factors related to the degree and length of ICAD:

We tested the statistical relationship between the degree of ICAD on the more stenotic side (D) as well as the length of ICAD on the longer side (L) as assessed in duplex, and other cardiovascular risk factors. To sum up, no special risk factor was found to be statistically related to D or L. Specifically, we tested the following risk factors:

- Age: Neither D nor L significantly correlated with age (ρ=-0.08, P=0.28, n=165) and (ρ=0.08, P=0.33, n=152), respectively.

- Sex: Neither a statistically significant relationship was found with D (median for males 70% with n=113 and for females 70% with n=52, P=0.8) nor with L (median for males 17.5 mm with n=106 and for females 17 mm with n=46, P=0.28).

- Diabetes: There was no statistically significant correlation between D and diabetes (median for non-diabetics 70% with n=109 and for diabetics 60% with n=56, P=0.34) nor between L and diabetes (median for non-diabetics 17 mm with n=104 and for diabetics 17 mm with n=48, P=0.27).

- HbA1c serum level: There was no statistically significant correlation between D (ρ=-0.07, n=157, P=0.42) or L (ρ=-0.04, n=144, P=0.61) and HbA1c serum level.

- Hypertension: There was no statistically significant correlation between D (median for hypertensive patients 70% with n=148 and median for non-hypertensive patients 80% with n=17, P=0.30) or L (median for non-hypertensive patients 15 mm with n=16 and median for hypertensive patients 17 mm with n=136, P=0.46) and hypertension.

- Smoking: There was no statistically significant correlation between D (median for non-smokers 70% with n=87 and median for ex-smokers 70% with n=42 and median for currently smokers 80% with n=34, P=0.14) or L (median for non-smokers 17 mm with n=81 and median for ex-smokers 17.5 mm with n=40 and median for currently smokers 16 mm with n=29, P=0.31) and smoking.

- LDL-cholesterol serum level: there was **a statistically insignificant trend for D to increase with increasing the LDL-cholesterol serum level** (ρ=0.10, n=154, P=0.20). This trend was not observed in relation to L (ρ=-0.02, n=141, P=0.81).

We further tested, whether a statistical relationship exists between D or L and combined cardiovascular risk factors:

- Combined hypertension and smoking: There was no statistically significant correlation between D (median for patients with no combined hypertension and smoking 70% with n=135 and for patients with combined hypertension and smoking 80% with n=28, P=0.31) or L (median for patients with no combined hypertension and smoking 17.5 mm with n=126 and for patients with combined hypertension and smoking 15.5 mm with n=24, P=0.19) and combined hypertension and smoking.

- Combined hypertension and diabetes: There was no statistically significant correlation between D (median for patients with no combined hypertension and diabetes 70% with n=113 and for patients with combined hypertension and diabetes 70% with n=52, P=0.5) or L (median for patients with no combined hypertension and diabetes 17 mm with n=108 and for patients with combined hypertension and diabetes 17 mm with n=44, P=0.26) and combined hypertension and diabetes.

IV. A7. Relation between the degree of ICAD and the development of ipsilateral ischemic events (Table 11 and Figure 12):

Significant higher grades of ICAD were found for sICAD with a median of 80 (IQR, 50-90)% compared to 20 (IQR, 20-50)% for aICAD **(P<0.001)**, as assessed in the ultrasound images. Of note, ICAD 90% occurred among 35.1% of sICAD compared to 3.2% among aICAD. However, ICAD 20-40% was found in 19.8% of the sICAD. This degree of stenosis was also the most common in patients with aICAD (54.8%).

Table 11: Relation between the degree of ICAD and the development of ipsilateral ischemic symptoms

Degree of ICAD	aICAD (n=63)	sICAD (n=94)	Statistical significance
20-40%	34 (54.8%)	14 (19.8%)	
50%	15 (24.2%)	10 (9.2%)	
60%	6 (9.7%)	6 (6.1%)	
70%	3 (4.8%)	15 (13.7%)	
80%	2 (3.2%)	13 (16%)	
90%	3 (3.2%)	36 (35.1%)	
Total	63	94	<0.001

Results are expressed as absolute values (percentages)

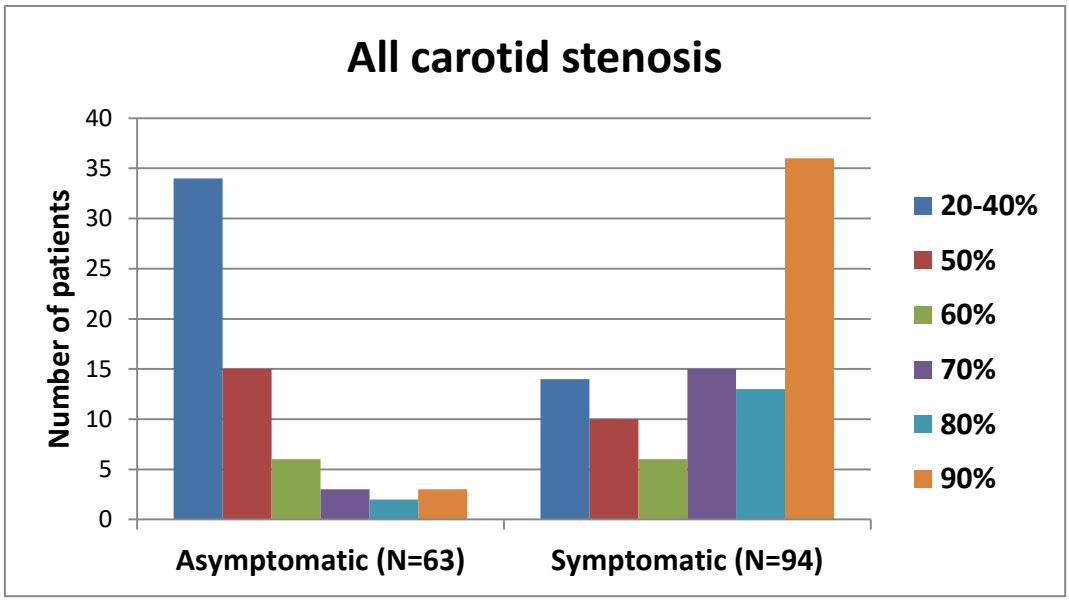

Figure 12: Relation between the degree of ICAD and the development of ipsilateral ischemic symptoms (*P*<0.001)

<u>IV. A8. Relation between the length of ICAD among sICAD and aICAD as well as among different symptomatic and asymptomatic degrees (Table 12 as well as Figures 13-15):</u>

We classified the ICAD into <70% and ≥70%. We found an inverse relationship between the ultrasound-measured degree and the length of sICAD, measured by ultrasound (ρ=- 0.39, *p*<0.001, n=78), MRA (ρ=- 0.24, *p*=0.07, n=60) and DSA (ρ= -0.12, *p*=0.67, n=15). Further analyses yielded only an inverse correlation among

sICAS≥70% (duplex sonography, ρ= - 0.57, p<0.001; MRA, n=51, ρ= - 0.38, p=0.01, n=44 and DSA, ρ= - 0.37, p=0.22, n=13). However, among sICAS<70%, such inverse correlations were not found (duplex sonography, ρ= 0.15, p=0.45, n=27 and MRA, ρ= 0.54, p=0.03, n=16). The relation between the ultrasound-measured length and degree of sICAD is shown in Figure 13.There were only two available DSA images for sICAS>70%; therefore, a measurement of the correlation coefficient was not possible. Regarding aICAD, we found no statistically significant difference between patients with ICAD <70% and those with ICAD ≥70% (median ultrasound-measured length was 15 (IQR 12-19) among aICAD <70% versus 18 (IQR 11-20) mm among aICAD ≥70%, p=0.74) (Table 12 and Figure 14). We found no statistically significant correlation between the ultrasound-measured degree and the length of aICAD, measured by ultrasound, MRA and DSA, respectively (ρ= 0.07, p=0.64, n=54), (ρ= 0.15, p=0.38, n=36) and (ρ= 0.39, p=0.39, n=7).

The ultrasound-measured length of sICAD <70% was longer than that of sICAD ≥70%; median 17 (IQR 15-20) mm versus 15 (IQR 12-19) mm, respectively. However, this difference achieved only a borderline statistical significance (p=0.06; Figure 14).

Moreover, we found that a cut-off length of ≥16 mm yielded a sensitivity and specificity of 74.1% and 51.1%, to discriminate between a sICAD and aICAD among ICAD <70%.

As regarding ICAD <90%, we found a statistically significant difference between the ultrasound-measured length of sICAD and aICAD with a median of 16 (IQR, 12-19) versus 18 (IQR, 15-21) mm, respectively (p=0.03) (Figure 15). Moreover, we observed a statistically significant difference between the length of sICAD <90% and sICAD 90% with a median of 18 (IQR 15-21) mm and 13 (IQR 10-16) mm, respectively p<0.001 (Figure 15).

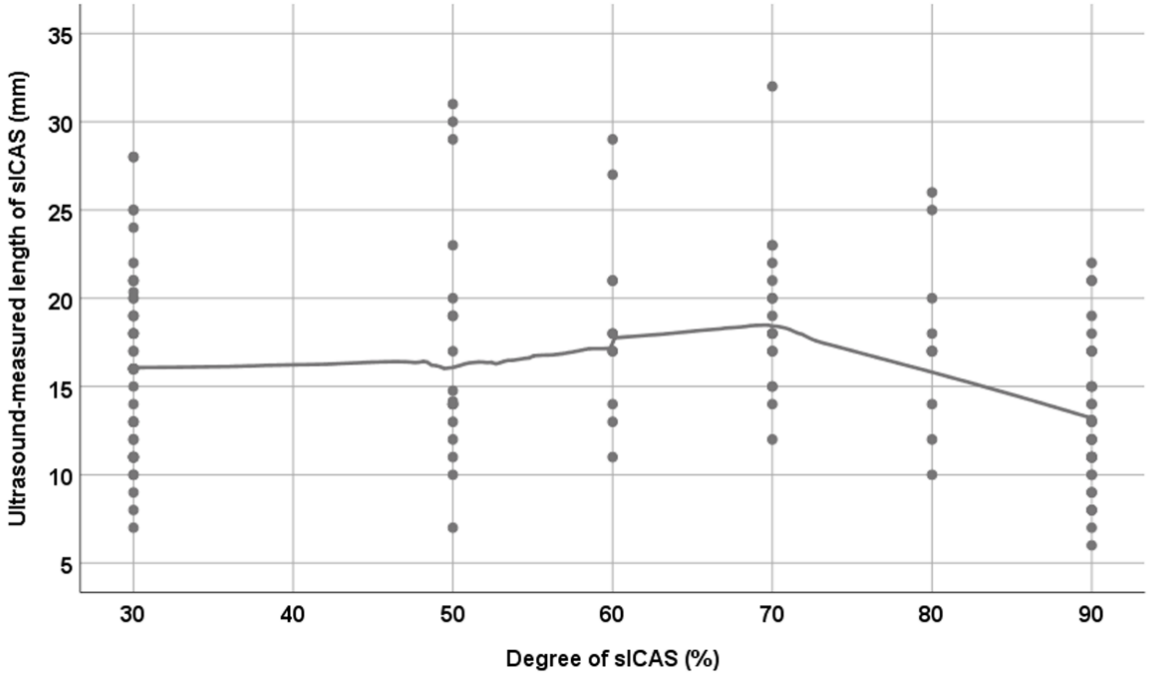

Figure 13: The relation between the ultrasound-measured degree and length of sICAS (symptomatic internal carotid artery stenosis). LOESS regression with a smoothing parameter of 0.6 was used to produce the curve. N.b. For technical reasons, 30% was used to represent 20-40% internal carotid stenosis; the NASCET hemodynamic criteria does not differentiate between 20%, 30%, and 40% internal carotid stenosis)

Table 12: Relation between the ultrasound-measured length of sICAD vs. aICAD among ICAD <70% vs. ≥70% as well as <90% vs. 90%

Degree of ICAD	Length of aICAD (mm)	Length of sICAD (mm)	P-Value
ICAD <70%	15 (12 -19)	17 (15-20)	0.14
ICAD ≥70%	18.0 (11-20)	15.0 (12-19)	0.52
ICAD <90%	18 (15.25-21)	16 (12-19)	0.03
ICAD 90%	11 , 21***	13 (10-16)	***

Results are expressed as median (IQR), aICAD: asymptomatic internal carotid artery disease, sICAD: symptomatic internal carotid artery disease, *Mann-Whitney test was applied to assess the statistical association between the length of ICAD among symptomatic and asymptomatic arteries, *** we had only two available arteries with asymptomatic 90% ICAD, hence statistical analysis was not possible

Table 13: Relation between the ultrasound-measured length of sICAD vs. aICAD among different stenotic grades

Degree of ICAD	Length of aICAD (mm)	Length of sICAD (mm)	P-value
20-40%	16 (11-19)	16.5 (14.75-20.51)	0.33
50%	14 (12.25-27.5)	17 (14-19)	0.7
60%	17 (12-19.5)	19.5 (16.25-27.5)	0.18
70%	Only 3 arteries: 18, 18, 20	19 (16-22.5)	§
80%	Only 2 arteries: 10, 17	17.5 (14.75-23.75)	§
90%	Only 2 arteries: 11, 21	12.5 (10-15.5)	§

Results are expressed as median (IQR), aICAD: asymptomatic internal carotid artery disease, sICAD: symptomatic internal carotid artery disease, § no statistical testing was done because of the small number of the available cases among aICAS

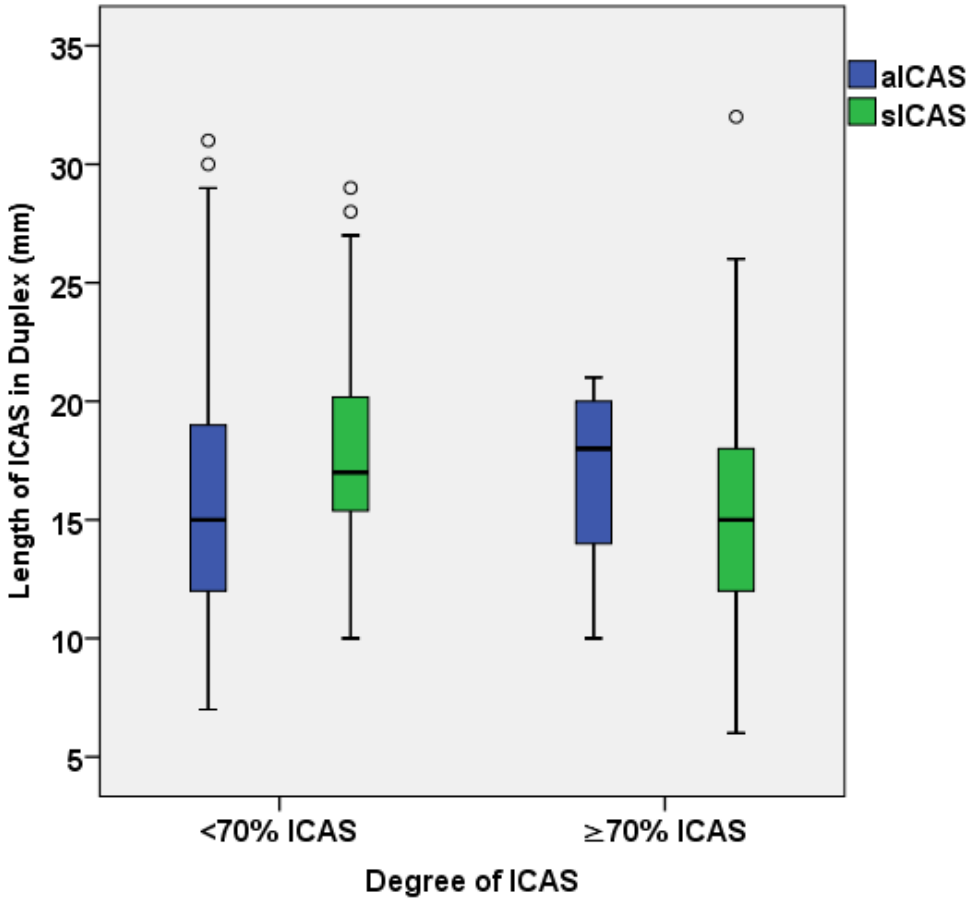

Figure 14: Difference between length of carotid stenosis among symptomatic and asymptomatic arteries in patients with ICAD <70% versus ≥70% (aICAS: asymptomatic internal carotid artery stenosis, sICAS: asymptomatic internal carotid artery stenosis)

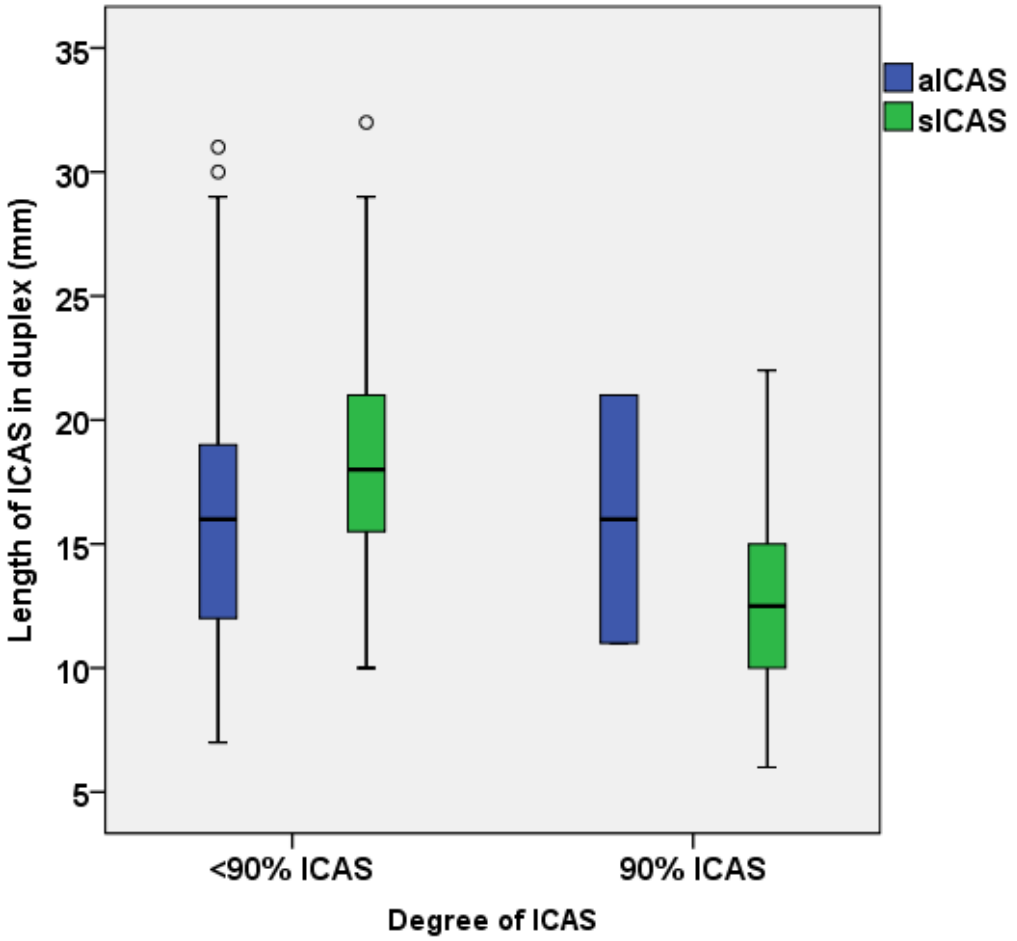

Figure 15: Difference between length of carotid stenosis among symptomatic and asymptomatic arteries in patients with ICAD <90% versus 90% (aICAS: asymptomatic internal carotid artery stenosis, sICAS: asymptomatic internal carotid artery stenosis)

IV. A9. Relation between plaque echolucency among sICAD and aICAD as well as among ICAD ≥70% and <70% (Table 14 and Figure 16):

Irrespective of the stenotic degree, we found that sICAD was more frequently echolucent compared to aICAD (43.2% vs 24.6%, respectively, *p*=0.02) (Table 14 and Figure 16). An association was observed between echogenic plaques and aICAD (symptomatic vs asymptomatic side: 40.9% versus 67.2%, respectively, p=0.002) (Table 14). Among ICAD <70%, echolucent plaques were found in 41.4% of sICAD versus 23.1% of aICAD (*p*=0.08) and echogenic plaques, however, were significantly more frequently observed in aICAD than in sICAD (34.5% versus 69.2%, respectively, *p*=0.002).

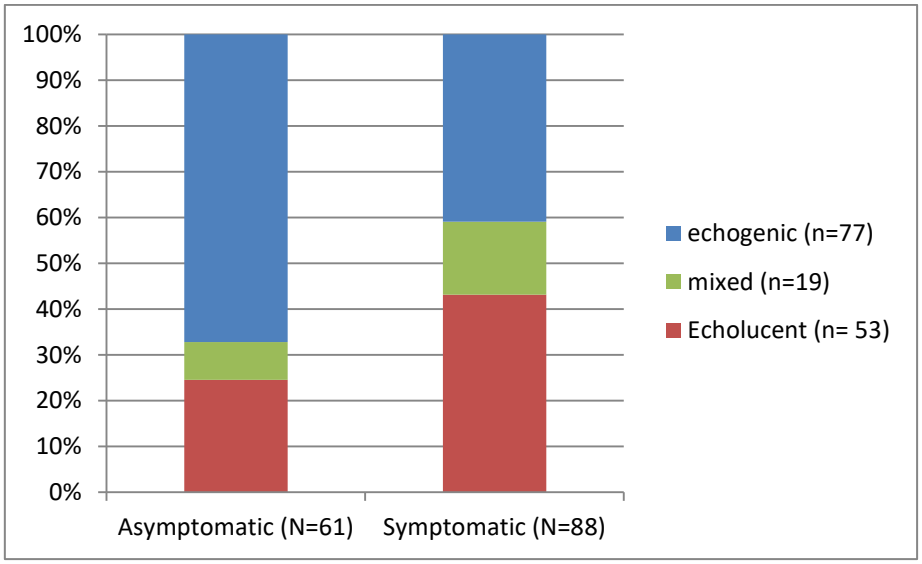

Figure 16: Plaque morphology among sICAD versus aICAD

Table 14: Plaque Morphology among aICAD and sICAD

Plaque morphology	aICAD	sICAD	Statistical significance
echolucent	15 (24.6%)	38 (43.2%)	0.02*+
mixed	5 (8.2%)	14 (15.9%)	0.17+
echogenic	41 (67.2%)	36 (40.9%)	0.002*+

Results are expressed in absolute values (percentages), *statistically significant results, + Statistical analysis for a difference between the mentioned type versus both other types, aICAD: asymptomatic internal carotid artery disease, sICAD: symptomatic internal carotid artery disease

IV. A10. Summary of important high risk plaque characteristics (Table 15):

To sum up, sICAD were more likely to be of higher grades (***P*<0.001**) and were more likely to be echolucent (***P*=0.02**). Among patients with ICAD <70%, the stenosis was more likely to be longer but did not reach statistical significance (*P*=0.14) (Table 15).

IV. A11. Logistic regression:

In a multivariable logistic regression model with a generalized estimation equation including ipsilateral symptoms as the outcome measure, ICAD ≥70% and echolucent plaques favored the development of ipsilateral ischemic events but the stenotic length was not related to the occurrence of ipsilateral ischemic events (≥70% vs. <70%: OR

6,60, 95% CI 2,48—17.57, p = <0.001, hypoechoic vs. echogenic; OR 2.16, 95% CI 1.07-4.34, p = 0.03, length OR 1.03, 95% CI 0.97-1.09, p = 0.29).

Table 15: Relation between different charachterestics of ICAD and the development of symptoms

Characteristic	aICAD	sICAD	*P* value
Degree of stenosis	20 (20-50)%	80 (50-90)%	<0.001*
Echolucent plaque	15 (24.6% of asymptomatic cases)	38 (43.2% of symptomatic cases)	0.02*
Stenotic length (mm) among ICAD <70%	15 (12-19)	17 (15-20)	0.14
Stenotic length (mm) among ICAD ≥70%	18.0 (11-20)	15.0 (12-19)	0.52

Data are absolute numbers of stenoses (%) or median (IQR), *statistically significant results, aICAD: asymptomatic internal carotid artery disease, sICAD: symptomatic internal carotid artery disease

IV. A12. Relation between the TWMLL and risk factors, demographic as well as clinical findings:

- We found a statistically significant relationship between TWMLL and the following parameters:

1. Age **(**ρ=0.41, ***P*<0.001**, n=136).
2. Severity of stroke as assessed by NIHSS score on admission (ρ=0.23, ***P*=0.02**, n=100).
3. Duration of stay in the Department of Neurology (ρ=0.19, ***P*=0.03**, n=136).
4. HbA1c serum levels (ρ=0.26, ***P*=0.003**, n=131). Moreover, a statistically significant relation was found between diabetes mellitus (median for diabetics 2 with n=46 and for non-diabetics 1.25 with n=90) and TWMLL (***P*=0.006**, n=136).

5. Female sex: the TWMLL was **significantly increased among females** (Median for males 1.25, n=97 and for females 1.75, n=39, *P*=0.049).

6. Hypertension: the TWMLL was **significantly increased among hypertensive patients** (Median for non-hypertensive patients 1.25 with n=16 and for hypertensive patients 1.5 with n=120, *P*=0.048).

- **A statistically significant inverse relation was observed between smoking status and TWMLL** (median for non-smokers 1.75 with n=67, for ex-smokers 1.50 with n=35 and for smokers 1 with n=32, P=0.04). This relation can be explained by the strong inverse relation between smoking and age in our cohort (median age for non-smokers 77 years with n=88, for ex-smokers 73 years with n=43 and for smokers 63 years with n=35, P<0.001).

- No correlation was found between the TWMLL and LDL-cholesterol serum levels (ρ=0.03, P=0.77, n=131).

IV. A13. Correlation between the length and degree of ICAD with WMLL (Tables 16 and 17):

In all examination modalities (ultrasound, MRA and DSA), we did not find any statistically significant correlation between the length of ICAD and WML. Moreover, we found no correlation between the degree of ICAD and WMLL. All calculated correlations are shown in Tables 15 and 16. We found **a statistically insignificant trend for PV1 and DWM1 to increase with increasing L1-PS** as measured in all examination modalities **and for TWMLL to increase with increasing the length of the longer stenotic side** as measured in MRA and DSA. Furthermore, **another statistically insignificant trend was observed for increasing DWM1 with increasing L1** as measured in MRA. This trend was not observed for the correlation between PV2 or DWM2 with L2 as measured by duplex or in MRA, which can be explained by the insufficient number of patients. On the other hand, when performing these correlations with the degree of ICAD, none of the above mentioned trends was observed.

Table 16: Correlation between the length of ICAD with white matter lesion load (WMLL)

Exam. method	L1 with PV1	L1-PS with PV1	L1 with DWM1	L1-PS with DWM1	L2 with PV2	L2 with DWM2	L1 with PV2	L1with DWM2	TWMLL with longer side
Duplex	ρ=0.08* (*P*=0.40) n=116	ρ=0.14 (*P*=0.19) n=88	ρ=0.07 (*P*=0.45) n=116	ρ=0.16 (*P*=0.14) n=88	ρ=0.03 (*P*=0.90) n=25	ρ=0.01 (*P*=0.96) n=25	ρ=0.03 (*P*=0.77) n=116	ρ=-0.004 (*P*=0.97) n=116	ρ= 0.07 (*P*=0.42) n=122
MRA	ρ= 0.13 (*P*=0.24) n=82	ρ=0.16 (*P*=0.20) n=71	ρ= 0.18 (*P*=0.11) n=82	ρ=0.20 (*P*=0.11) n=71	ρ=-0.34 (*P*=0.08) n=28	ρ=-0.07 (*P*=0.73) n=28	ρ=0.11 (*P*=0.31) n=82	ρ=0.12 (*P*=0.31) n=82	ρ=0.19 (*P*=0.09) n=83
DSA	ρ=0.38 (*P*=0.09) n=21	ρ=0.35 (*P*=0.16) n=18	ρ=0.21 (*P*=0.36) n=21	ρ=0.15 (*P*=0.54) n=18	ρ=0.5 (*P*=0.67) n=3	ρ=0.87 (*P*=0.33) n=3	ρ=0.25 (*P*=0.28) n=21	ρ=0.30 (*P*=0.19) n=21	ρ=0.31 (*P*=0.18) n=22

*Spearman correlation, **L1:** the length of internal carotid artery stenosis on the potentially symptomatic side or, in absence of potentially symptomatic stenosis, the more stenotic side, **L1-PS:** the length of internal carotid artery stenosis on the potentially symptomatic side, **L2:** the length of internal carotid artery stenosis on the asymptomatic side in patients with bilateral carotid stenosis, **PV1 and DWM1:** periventricular and deep white matter lesions, respectively ipsilateral to L1, **PV2 and DWM2:** periventricular and deep white matter lesions respectively contralateral to L1, TWMLL: total white matter lesion load, DSA: digital subtraction angiography MRA: magnetic resonance angiogram.

Table 17: Correlation between the degree of ICAD with white matter lesion load (WMLL)

Exam. method	D1 with PV1	D1-PS with PV1	D1 with DWM1	D1-PS with DWM1	D2 with PV2	D2 with DWM2	D1 with PV2	D1 with DWM2	TWMLL with more stenotic side
Duplex	ρ=0.003* (*P*=0.98) n=134	ρ=0.09 (*P*=0.38) n=103	ρ=0.04 (*P*=0.64) n=134	ρ=0.07 (*P*=0.47) n=103	ρ=-0.19 (*P*=0.31) n=30	ρ=-0.02 (*P*=0.92) n=30	ρ=0.05 (*P*=0.56) n=134	ρ=0.08 (*P*=0.34) n=134	ρ=0.05 (*P*=0.55) n=134

*Spearman correlation, **D1:** the degree of internal carotid artery stenosis on the potentially symptomatic side or, in absence of potentially symptomatic stenosis, the more stenotic side, **D1-PS:** the degree of internal carotid artery stenosis on the potentially symptomatic side, **D2:** the degree of internal carotid artery stenosis on the asymptomatic side in patients with bilateral carotid stenosis, **PV1 and DWM1:** periventricular and deep white matter lesions respectively ipsilateral to D1, **PV2 and DWM2:** periventricular and deep white matter lesions, respectively contralateral to D1, TWMLL: total white matter lesion load.

IV. B. Case examples

IV. B1. Patient 66 (Figure 17):

A 72-year-old male patient presented with persistent hemihypesthesia, transient hemiparesis on the left side as well as transient non-specified vertigo. Ultrasound examination showed ICAD of 20-40%, with a length of 20 mm on the right side (Figure 17). PFO was detected in the transesophageal echocardiography (TEE). MRI examination was not done, because the patient had a cardiac pacemaker. Figure 17 shows an example for measuring the length of ICAD.

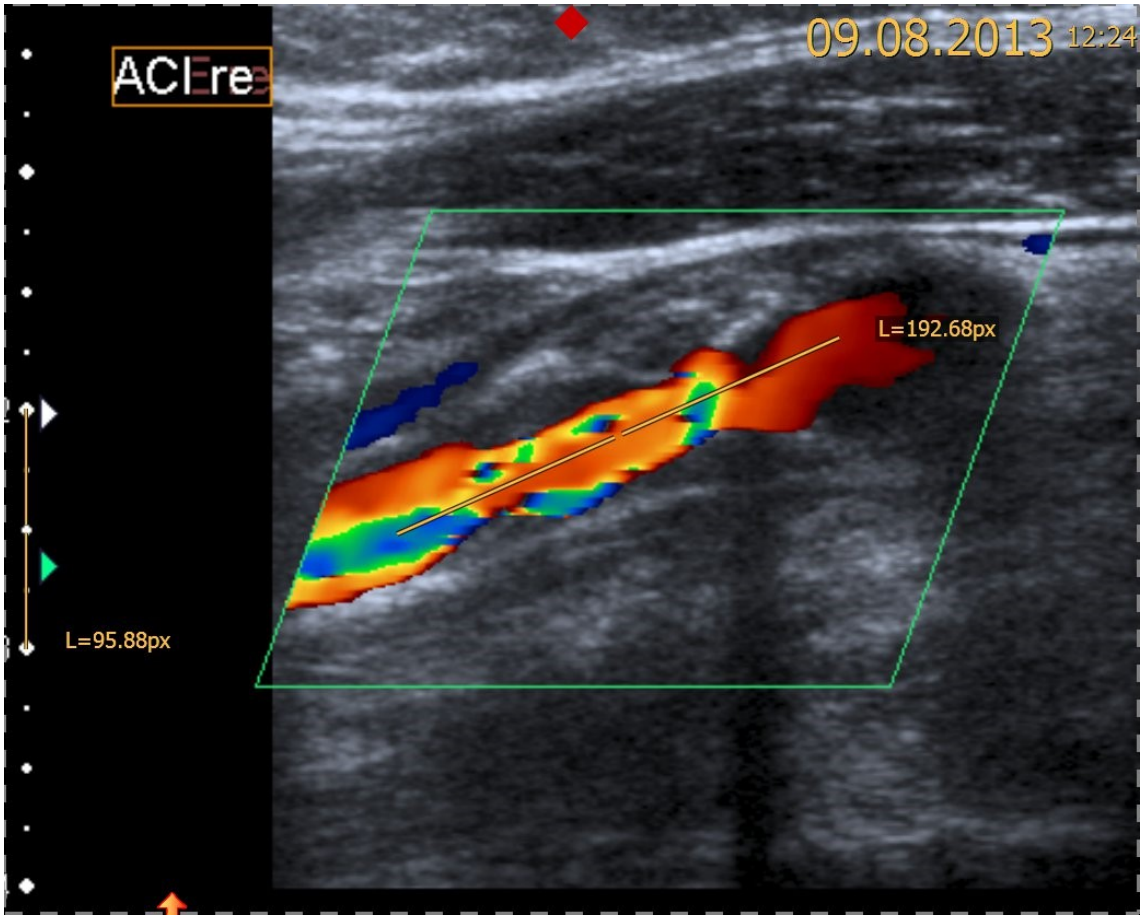

Figure 17: Ultrasound examination (patient 66) showing a stenosis of 20-40%, with a length of 20 mm on the right side

IV. B2. Patient 77 (Figure 18 and 19):

An 80-year-old male patient presented with mild left brachiofacial paralysis (NIHSS score of 2 on admission). Ultrasound examination showed a large plaque causing ICAD of 70% on the right side with a length of 14 mm (Figure 18). In the MRA, the ICAD

was slightly shorter, i.e. 11 mm long (Figure 19, panel A). An embolic infarction was seen in the territory of the corresponding right MCA on the MRI scans (Figure 19, panel B). Of note, sICAD≥70% were found in this study to be shorter in length than aICAD <70%.

IV. B3. Patient 79 (Figure 20 and 21):

A 77-year-old male patient presented with fine motor dysfunction of the left hand (NIHSS score of 0). Ultrasound examination showed a calcified plaque of 21 mm in length causing an ICAD of 90% on the right side (Figure 20). The ICAD was shorter in the MRA; 16 mm (Figure 21, panel A) and in the DSA; 18 mm (Figure 21, panel B). The MRI examination showed right MCA infarctions (Figure 21, panel C).

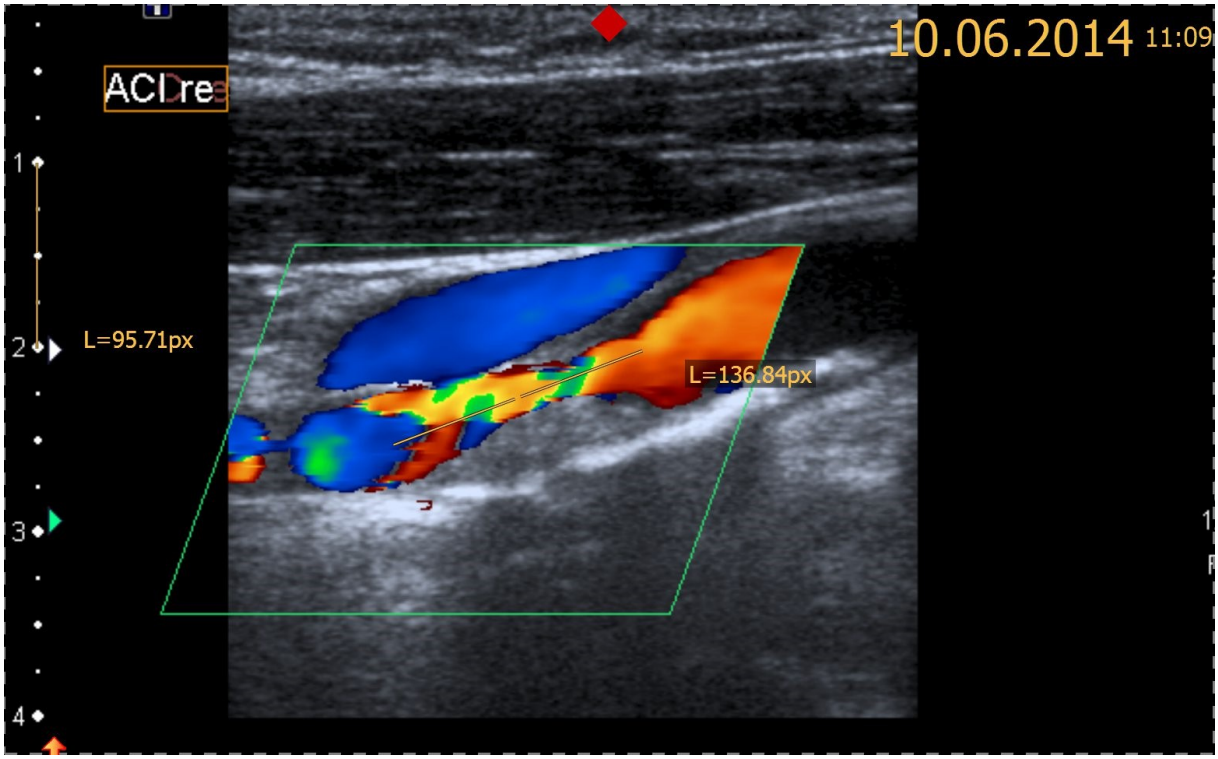

Figure 18: Ultrasound examination (patient 77) showing a large plaque causing an ICAD of 70%, with a length of 14 mm on the right side

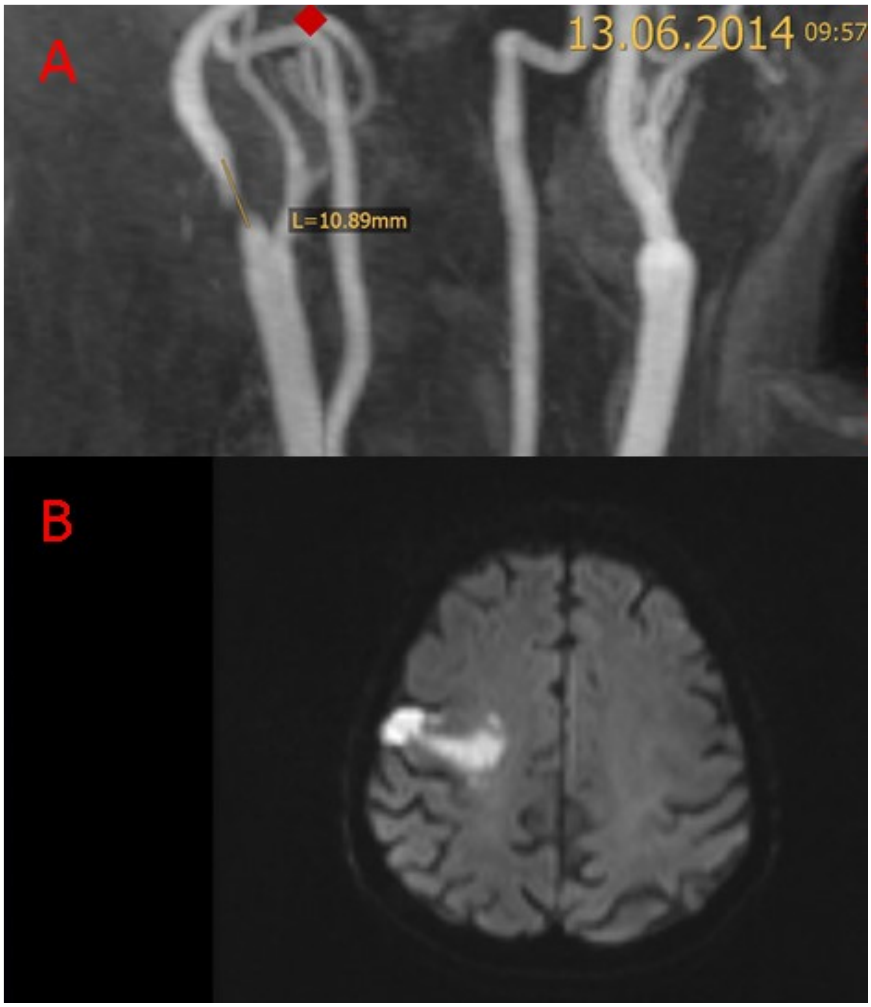

Figure 19: A. MRA (patient 77) showing an ICA stenosis with a length of 11 mm, B. DWI-MRI showing an embolic infarction in the corresponding MCA territory

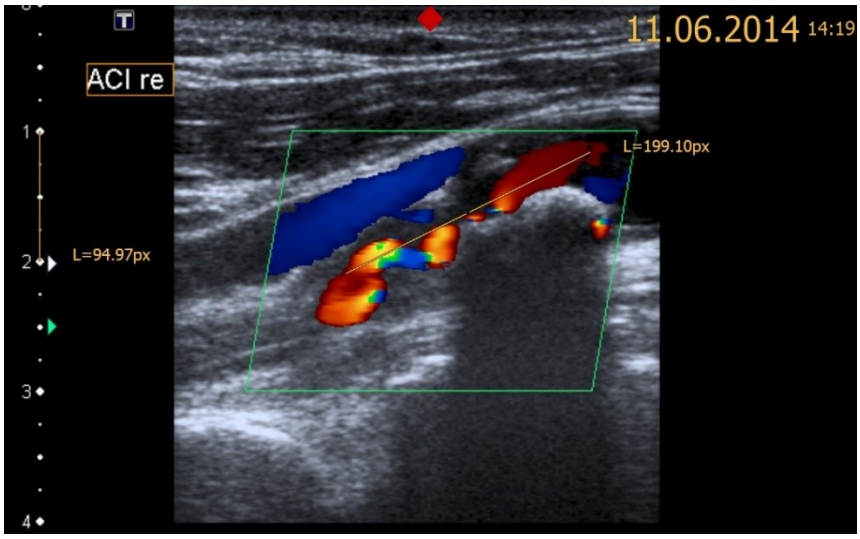

Figure 20: Ultrasound examination (patient 79) showing a calcified plaque with a length of 21 mm causing an ICAD of 90% on the right side

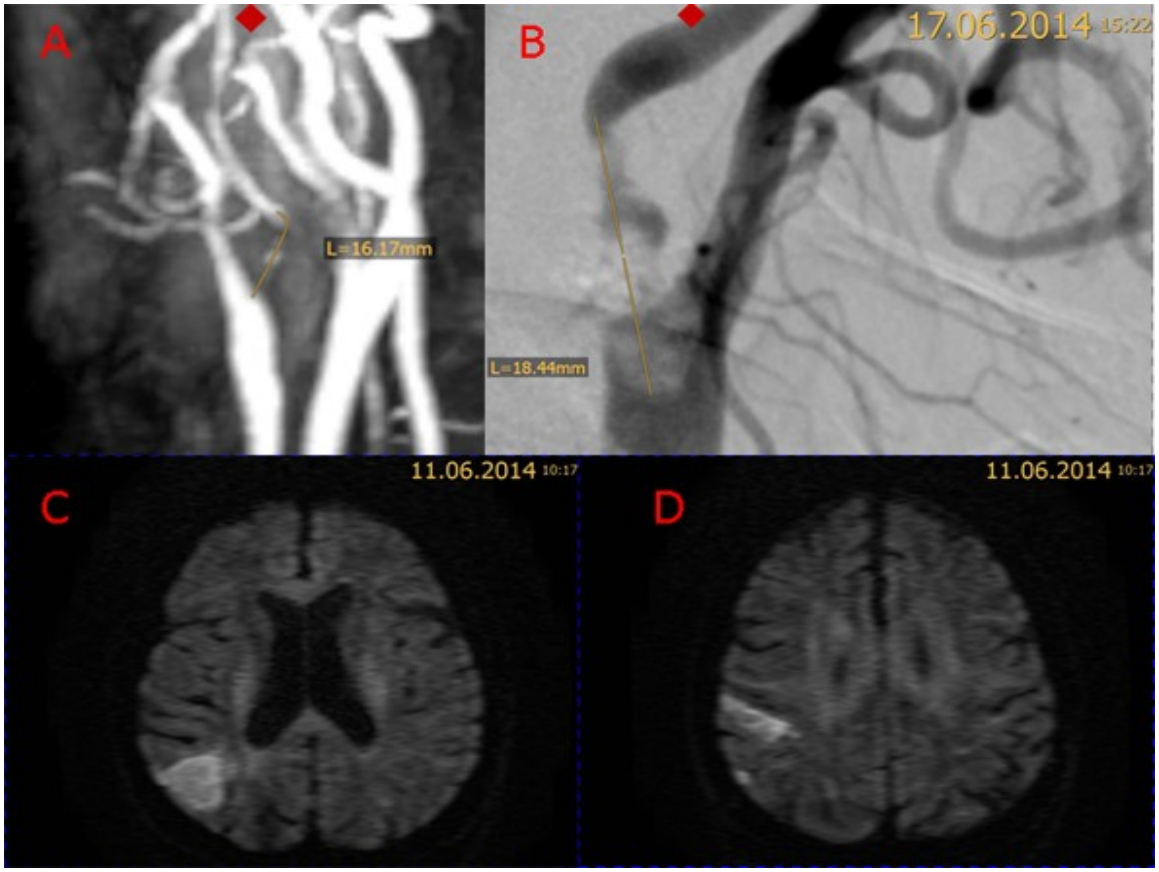

Figure 21: A. MRA (patient 79) showing an ICAD with a length of 16 mm on the right side, B. DSA showing an ICAD with a length of 18 mm, C and D. DWI-MRI showing infarctions within the territory of the MCA

IV. B4. Patient 81 (Figure 22 and 23):

An 82 year-old male patient presented with a right hemiparesis and aphasia (NIHSS score of 16). Ultrasound examination showed a left-sided ICAD of 20-40%, with a length of 16 mm (Figure 22). The stenosis was found to be shorter in the MRA, namely 11 mm (Figure 23, panel A). MRI scans showed multiple small infarctions (Figure 23, panel B). In the current study, sICAD of 20-40% was found in 19.8% of the patients. Among arteries with <70% ICAD, a length of ≥16 mm, measured in ultrasound, yielded a sensitivity and specificity of 74.1% and 51.1% respectively, to discriminate between a sICAD and aICAD.

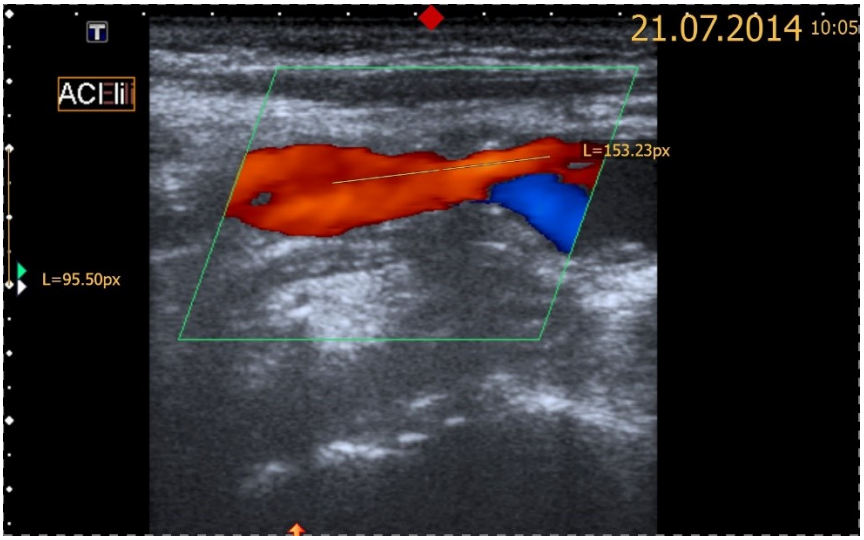

Figure 22: Ultrasound examination (patient 81) showing large plaque on the left side causing an ICAD of 20-40%, 16 mm long

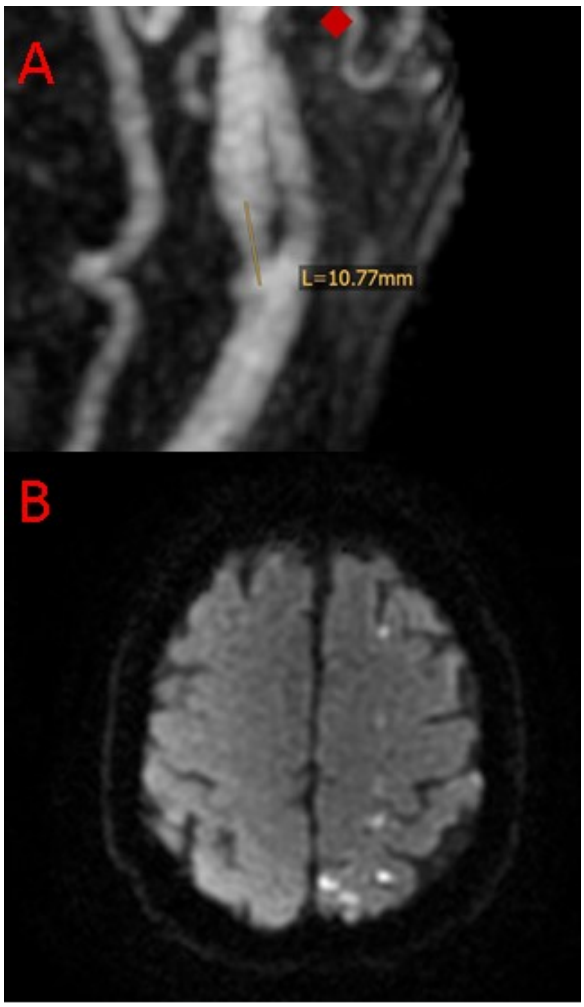

Figure 23: A. MRA (patient 81) showing an ICAD with a length of 11 mm, B. DWI-MRI showing multiple small infarctions

IV. B5. Patient 88 (Figure 24):

A 65-year-old male patient presented with mild dysarthria, transient right brachiofacial paresis and transient aphasia. NIHSS score on admission was 2. Ultrasound examination showed a large plaque causing an ICAD of 90% with a length of 19 mm on the left side (Figure 24, panel A). The ICAD was slightly shorter in the MRA, i.e., 18 mm (Figure 24, panel B) and in the DSA 17 mm (Figure 24, panel C). The MRI scans revealed multiple embolic infarctions in the territory of the left MCA.

IV. B6. Patient 93 (Figure 25):

A 72-year-old male patient presented with right amaurosis fugax. The MRA images showed an ICAD with a length of 8 mm (Figure 25, panel A). No white matter lesions were found on the MRI scans (Figure 25, panel B).

IV. B7. Patient 95 (Figure 26):

A 67-year-old male patient presented with aphasia and mild right hemiparesis. Ultrasound examination showed a 23 mm long echogenic plaque causing an ICAD of 70% on the left side (Figure 26, panel B) and another ICAD of 60% with a length of 11 mm on the right side (Figure 26, panel A). In the MRA, these stenoses had a length of 20 mm (Figure 26, panel D) and 12 mm (Figure 26, panel C), respectively. The MRI showed an infarction of the left MCA-territory (images not shown).

IV. B8. Patient 102 (Figure 27):

A 77 year-old female patient presented with recurrent transient right sided weakness. NIHSS score on presentation was 0. Ultrasound examination showed an ICAD of 90% and a soft plaque with a length of13 mm on the left side (Figure 27, panel A). The ICAD was slightly shorter in the MRA, namely 11 mm (Figure 27, panel B). On MR scans, a small infarction was seen in the left centrum semiovale (Figure 27, panel C). In this study, we showed that sICAD ≥70% were slightly shorter in length than sICAD<70%.

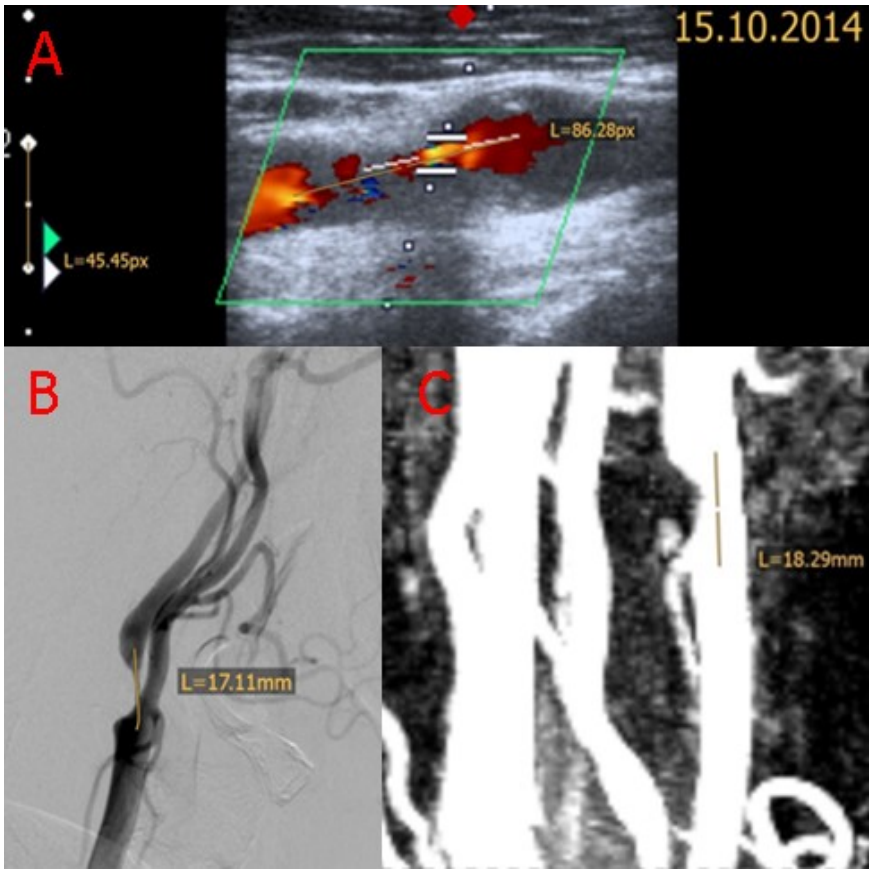

Figure 24: Illustration how the legnth of ICAD (patient 88) was measured in the three examination modalities; A. Ultrasound examination showing a plaque causing a left-sided ICAD of 90%, with a length of 19 mm, B. MRA showing a 18 mm long ICAD, C. DSA showing a 17 mm long ICAD

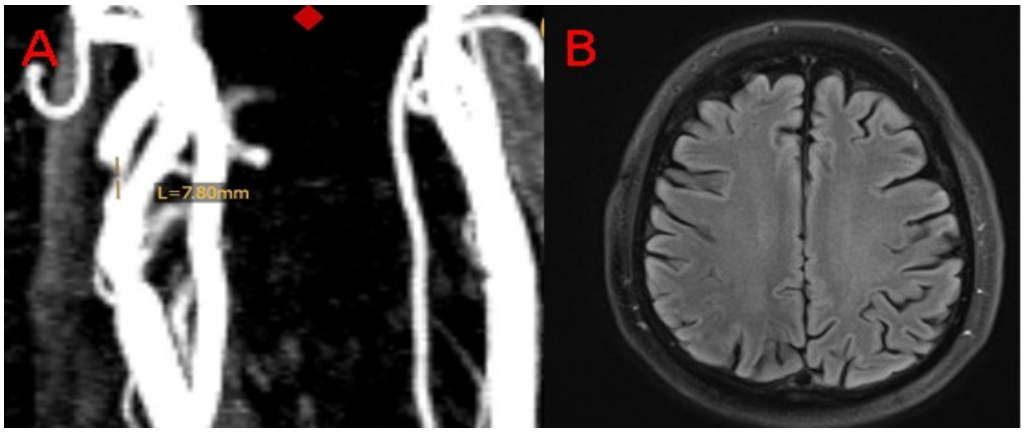

Figure 25: A. MRA (patient 93) showing an ICAD with a length of 8 mm, B. MRI-FLAIR sequence showing no white matter lesions

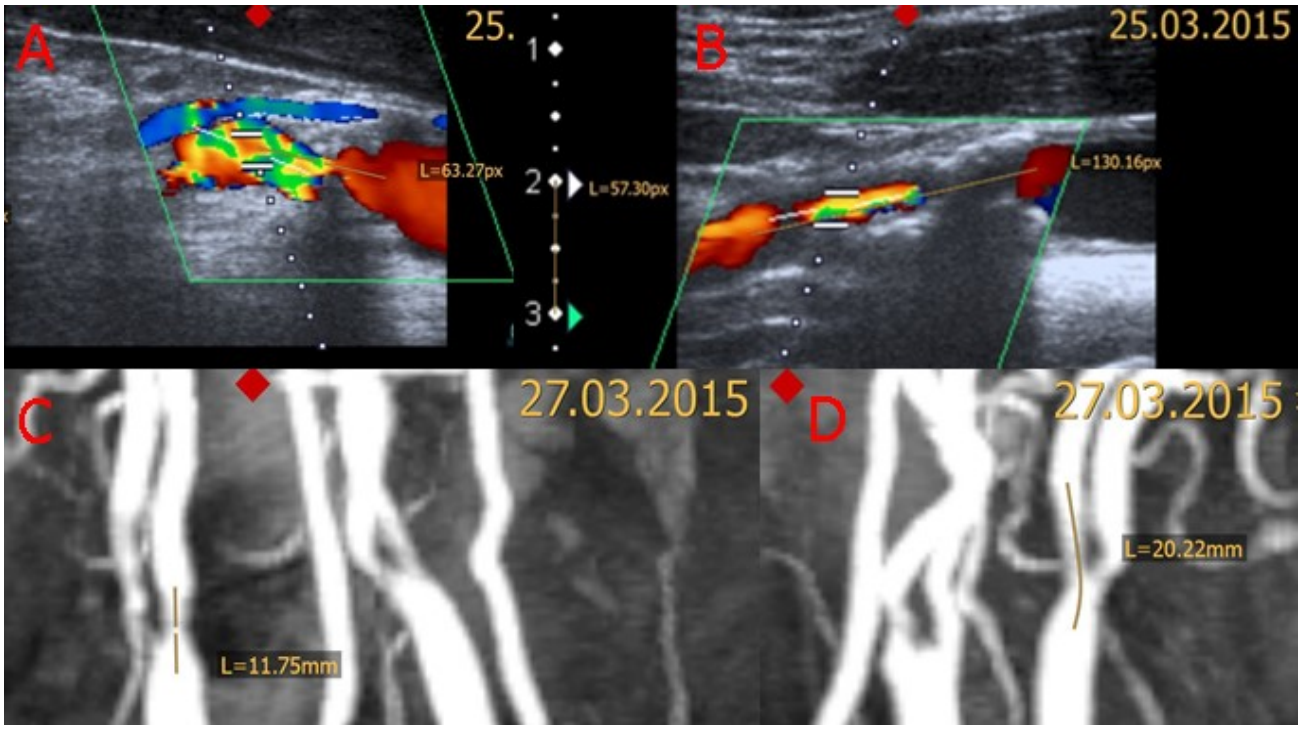

Figure 26: Ultrasound examination (patient 95) showing an ICAD of 60% with a length of 11 mm and an echogenic plaque on the asymptomatic right side (A); on the contralateral side, an ICAD of 70% with a length of 23 mm and an echogenic plaque was detected, which caused the ischemic infarction (B), MRA showing a 12 mm long ICAD on the asymptomatic right side (C) and a 20 mm long ICAD on the symptomatic left side (D)

IV. B9. Patient 147 (Figure 28):

A 49-year-old-male patient presented with recurrent attacks of left amaurosis fugax in the last 4 days. On the day of admission, the patient suffered from 6 attacks. Duplex examination showed an ipsilateral ICAD of 20-40% with a length of 20 mm (Figure 28). CEA was done. MRI was unremarkable. This is another example for a symptomatic ICAD of 20-40%. The length of ICAD was greater than the cut-off value (16 mm) proposed by our study to differentiate between low grade sICAD and aICAD.

IV. B10. Patient 154 (Figure 29):

A 74 year-old male patient presented with transient sensory changes on the left side. On the asymptomatic side, an ICAD of 50%, with a length of 14 mm and a soft plaque, was detected (Figure 29). This example demonstrates a case of asymptomatic ICAD <70% with a length being shorter than our proposed cut-off value.

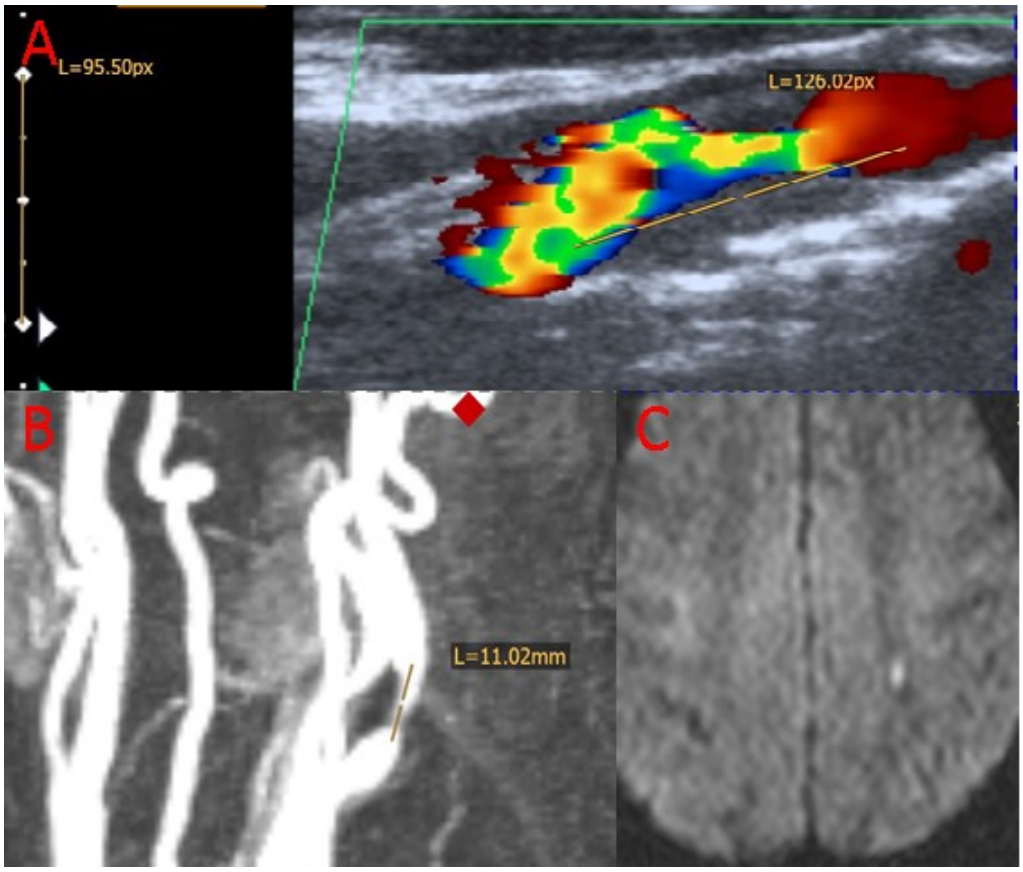

Figure 27: A. Ultrasound examination (patient 102) showing soft plaques causing an ICAD of 90% with a length of 13 mm on the left side, B. MRA revealing an 11 mm long ICAD on the left side, C. DWI-MRI showing a small infarction in the left centrum semioval center

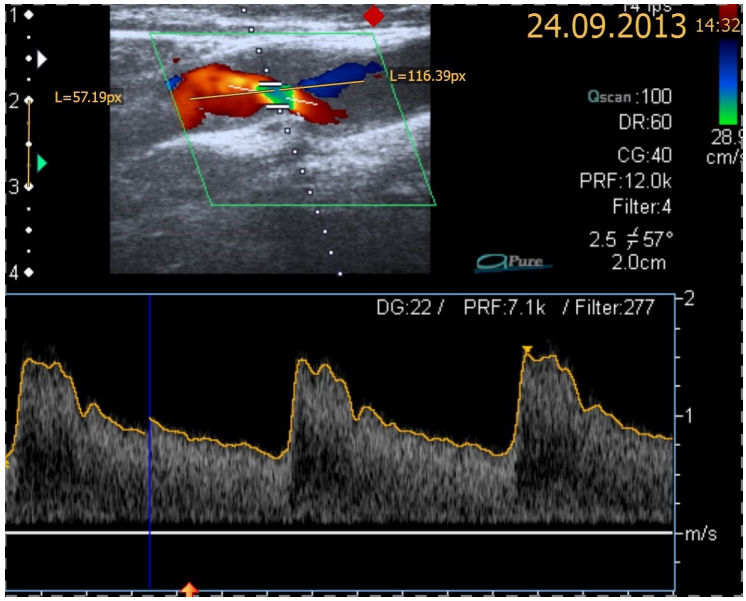

Figure 28: Duplex examination (patient 147) showing an ICAD of 20-40%, with a length of 20 mm on the left side

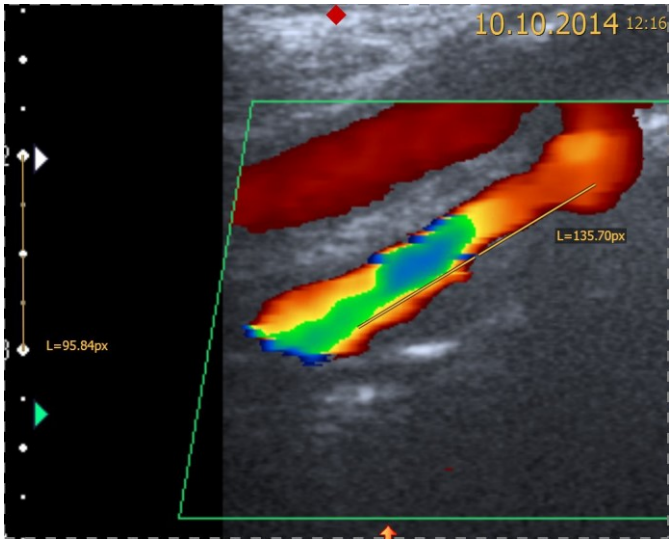

Figure 29: Ultrasound examination (patient 154) showing aICAD of 50% on the right side

V. DISCUSSION

V. A1. Baseline data:

Our population consisted of 116 (69%) males with a median (IQR) age of 74 (66-80) years. The sex distribution in the current study was similar to that of the NASCET study, which assessed patients with sICAD, where women comprised around 28-34% of this cohort (73). In the surgical arm of this study, the mean age of the 1415 patients was 65.4 years, with 70.5% men and 29.5% women (74). In the American Vascular Association National Screening Program, the prevalence of carotid stenosis was 6% in women and 8% in men (***P*=0.03**) (73). Jung et al. (75) examined 167 stroke patients with either cardioembolic etiology or large artery atherosclerosis (i.e. ICA stenosis) etiology. The authors found that stroke patients with large artery atherosclerosis were older (69.9 ± 9.0 vs. 65.8 ± 12.0 years; ***P*=0.002**), with more male predominance (74.5% versus 56.7%; ***P*= 0.033**).

Hypertension was observed among 89.9% of our population and hence was the most predominant risk factor, followed by diabetes in 33.9% of the patients. The risk factors were previously well controlled in our population with a median (IQR) LDL-cholesterol serum level of 109 (84.0-136.5) mg/dl and a median HbA1c serum level of 5.9 (5.6-6.5)%. In a large epidemiological study, elevated blood pressure in the emergency department was found in 69% of patients with acute stroke (76). Another study reported an elevated blood pressure in 84% of patients with acute stroke in the first 24 hours with spontaneous reduction of the blood pressure in the next few days without any blood pressure medications. Only one third of the patients in this cohort showed an increased blood pressure ten days after stroke onset suggesting that the increased blood pressure was only a physiological response (77). Many hypertensive patients in our cohort could have been misclassified, as many stroke patients present with hypertension in the acute stressful phase and normalize in the next few days (77). This may explain the high prevalence of hypertension among our patients.

Our stroke patients showed rather few symptoms on admission with a median (IQR) NIHSS score of 3 (1-6). The good NIHSS-scores on admission in this cohort may be related to a selection bias due to inclusion of functionally good patients. The patients with worse NIHSS-scores were more likely to be admitted to the intensive care unit and

to receive their duplex examination under difficult conditions, sometimes with a central venous line interfering with the visualization of the ICAD. Those patients, with poor general condition, were also less likely to receive complete stroke work-up including neurovascular studies like DSA- or MRA-examinations and hence many of them, with only poor duplex images, did not fulfil our inclusion criteria. This finding is in line with a study involving 47 patients with carotid stroke, which also showed relatively low NIHSS scores with a mean of 4.96 ± 4.50 (78). As mentioned previously, Jung et. al. found better initial NIHSS scores after stroke due to carotid stenosis than those related to cardioembolism (75). On the other hand, Muscari et al found that ICAD ≥60% ipsi- or contralateral to the ischemic stroke was more likely associated with poor functional outcome as indicated by a modified Rankin scale >2 at 9 months (79). The authors found that patients with lacunar strokes have a better prognosis than stroke due to large artery atherosclerosis, while cardioembolic stroke had the worst prognosis. However, the study of Muscari et al. was longitudinal, which cannot be compared with the current cross sectional study.

To increase our study sample, we included patients with onset of symptoms up to 14 days. On one side, this time period was not related to the statistics regarding WML. On the other side, the small number of patients (n=16) with onset of symptoms between 4 and 14 days is unlikely to affect the statistics regarding the relation between ICAD and severity of cerebrovascular stroke. Especially, eight patients presented within 4-5 days from symptom onset and another 8 patients within 6-14 days, while the vast majority (152 patients) presented within 1-3 days from onset of symptoms. As a tertiary stroke center, some of our patients with stroke or TIA due to large artery atherosclerosis were referred from nearby hospitals or even outpatient clinics for further interventional treatment and hence presented several days after onset of symptoms.

V. A2. Risk factors related to the degree and length of ICAD:

The median (IQR) length of ICAD among all examined arteries was 17 (12-20) mm, 10.6 (8.0-14.0) mm and 11.5 (10.0-17.0) mm measured by duplex sonography, MRA and DSA, respectively. To our knowledge, this is the first report addressing this issue. The difference in the length between different examination modalities can be explained

by the expected under-estimation of the length in MRA and visualization of the inner vascular lumen and hence the whole length in ultrasound. We found a statistically significant positive correlation between the measurements of the length in duplex and the two other examination modalities. The median (IQR) degree of ICAD was 60 (20-80)%.

We found no special risk factor related to the degree or length of ICAD. The absence of any statistically significant association between any risk factor and the length or the degree of ICAD may be, to some extent, explained by the good control of risk factors among our patients with a median LDL-cholesterol serum levels of 109 (84.0-136.5) and HbA1c serum levels of 5.9 (5.6-6.5)%, respectively. In other words, most of these values were already within the normal levels, which means we compared whether a difference between values mostly in the upper or lower normal range influences the degree and length of ICAD as well as the TWMLL. Furthermore, the good control of risk factors in our cohort might have abolished any potential role affecting the degree or length of ICAD. Of note, we studied the association between the cardiovascular risk factors and the degree or length of ICAD. However, most of the previous studies compared the relation between those risk factors and the presence or absence of atherosclerosis. In a systemic review, the prevalence of moderate aICAD (50-69%) increased with age and was more often detected in men (23). In a large study involving 2025 patients, older age, high serum levels of LDL-cholesterol, fasting blood glucose, male gender, diabetes, hypertension and smoking were all found to be independent risk factors for carotid plaques (80). A case-control study assessed the prevalence of smoking, hypertension and diabetes among 221 patients with carotid stenosis and compared them with two sex and age matched control groups (81). The authors found that 27.6% of the patients were hypertensive smokers as compared to the two control groups with 9.5% and 17.2 % (***P*<0.01 and *P*=0.016,** respectively). Specifically, among patients with carotid stenosis, 22.6% were hypertensive, 20.4% were smokers (including patients who smoked for more than 1 year and stopped) and only 1.8% were diabetic. Other authors identified male sex, hypoechoic or ulcerated plaque, smoking and contralateral severe carotid artery stenosis or occlusion as predictors of progression in patients with moderate ICAD (82).

V. A3. Relation between the degree and length of ICAD and ischemic cerebrovascular events:

As expected, a strong relation was found between the degree of ICAD and the development of ipsilateral symptoms. As aforementioned mentioned, the risk of stroke recurrence was found in large multicenter studies to be related to the degree of the ICAD (30-32). In the current work, ICAD of 20-40% was found among 19.8% of patients with sICAD. However, ICAD of 20-40% was also the most common group (54.8%) among aICAD. These results may indicate that ICAD of 20-40% is usually associated with a benign prognosis but could also be of high risk and cause with cerebrovascular ischemic manifestations. We hypothesize, that the risk of ipsilateral symptoms in patients with low-grade ICAD could be explained by plaque immaturity or a non-organized plaque in the development phase. Our study lacks a longitudinal or prospective phase, which means that our results cannot identify the risk of recurrence in sICAD of 20-40%, a fact which should be took in consideration, when discussing the indication for carotid revascularization procedures. Low-grade ICAD of high risk was identified in several studies (37-39). In the NASCET study, 2226 patients were recruited with sICAD <70%. Of these patients, 38.5% had ICAD of 50-69%, 42.4% had ICAD of 30-49% and even 19.1 % showed a sICAD <30% (42). In those patients, CEA was not shown to be superior compared to best medical treatment.

In the current work, sICAD <70% were longer than sICAD ≥70%; median 17 (IQR 15-20) mm versus 15 (IQR 12-19) mm, respectively. However, this difference did only achieve a “borderline” statistical significance. This relation was more prominent and reached a statistical significance among patients with sICAD 90% versus aICAD <90%; median (IQR) for sICAD 90% was 13 mm (10-16) and for aICAD <90% was 18 (15-21) mm. Among sICAD ≥70%, we found a statistically significant inverse correlation between the ultrasound-measured degree and length. On the other side, among sICAD <70% as well as among aICAD, no statistically significant correlation was observed between those two variables. The inverse relation between the length and degree of sICAD ≥70% might be attributed to the early development of ischemic cerebrovascular events in severe ICAD at even a shorter length. Perhaps longer plaques give rise to emboli of atheromatous debris, while more severe stenosis, causing more severe flow

disturbances, might give rise to emboli of platelet aggregates. It might be postulated that different pathological processes affect the degree and length of ICAD.

Future studies addressing the relation between the length of ICAD and ischemic cerebrovascular events should take the degree of stenosis in consideration. In other words, we recommend further examination of the relation between the length of different stenotic grades and the occurrence of ischemic cerebrovascular events. The aforementioned correlations between the length and degree among sICAD and aICAD as well as our regression line (Figure 13) clarify this point.

Buon et al. (83) studied ICAD <50% in young patients. In line with our findings he found that the plaques of sICAD were significantly longer and more often echolucent compared to aICAD. Of note, he found that a cut-off length of 12 mm can discriminate between a symptomatic and asymptomatic carotid artery with a sensitivity and specificity of 86% and 73%, respectively. In contrast, the cut-off-value calculated in our cohort was higher (i.e. 16mm) with a lower sensitivity (74%) and specificity (51%). However, Buon et al. studied younger stroke patients with low-grade stenosis and hence a comparison with our data should be considered with caution.

Carotid revascularization has been frequently proved to be the treatment of choice in patients with sICAD ≥70% to decrease the risk of recurrence of ischemic cerebrovascular events (30-32). Regarding moderate sICAD (i.e., 50-69%), the benefit of CEA was found to be questionable and for low-grade carotid stenosis (i.e. 30-49%) CEA was not superior to best medical treatment (32). We recommend that future studies addressing sICAD <70% should also take the length of stenosis in consideration. Furthermore, it seems to be reasonable, to measure the length, when considering the management plan of those patients.

Up till now, best treatment for patients with aICAD is still under debate; whether best medical treatment nor carotid revascularization has been shown to be superior for patients with aICAD (52). Older studies have revealed that CEA is superior to aspirin or deferral of any procedure (48, 84). Later on, best medical treatment using antiplatelets, statins, smoking cessation, exercise, implementation of a Mediterranean diet and optimal blood pressure management proved to significantly reduce the recurrence risk of cardiovascular events, rate of plaque progression and microembolic signals as shown

in the transcranial duplex among patients with aICAD ≥60% (53). In the current work, ICAD ≥70% was found to be related to ischemic cerebrovascular manifestations with even a short length. Additionally, ICAD <70% was found to be insignificantly related to ischemic events after growing in length. Hence, the length of ICAD seems to be an emerging parameter, which might be studied in large interventional trials to answer the question, whether best medical treatment or invasive procedures would best suit these patients.

ICAD of severe grades might remain asymptomatic over years (27). Among patients with aICAD of 50-69%, progression of the stenosis was found to be related to the development of ipsilateral ischemic cerebrovascular events (85). Whether the length of stenosis can predict the long-term prognosis, remains a matter of future research. Furthermore, follow-up of the length of ICAD may be a subject for further studies.

In this study, the length of ICAD was found to be longer when measured by ultrasonography followed by DSA, whereas the length was shorter when measured on MRA scans. This difference can be attributed to the expected underestimation of the length in MRA and better visualization of the whole length in ultrasound. The latter can be ascribed to the ability of ultrasound examinations to visualize the inner vascular wall demonstrating the whole stenotic plaque. We found a statistically significant positive correlation between the length of ICAD in ultrasonography and in the two other examination modalities; MRA and DSA. Furthermore, a trend for a positive correlation was found between MRA and DSA, which is probably related to the small number (n=16).

The Carotid and Vertebral Artery Transluminal Angioplasty Study (CAVATAS) was a randomized open multicenter trial which recruited patients either to endovascular treatment or to CEA (86) on the basis of DSA. An analysis from this study has shown that a carotid stenosis which is longer than 0.65 of the common carotid artery diameter is statistically significantly associated with periprocedural occurrence of new stroke or death after endovascular treatment (OR: 2.79 (1.17–6.65), $\boldsymbol{P = 0.02}$) and CEA (OR: 2.43 (1.03–5.73), $\boldsymbol{P = 0.04}$). The authors further found, that long carotid stenoses were associated with an increased long-term risk of restenosis (hazard ratio 1.68 (1.12–2.53), $\boldsymbol{P = 0.01}$) after endovascular treatment but not after CEA. In this study, the degree of carotid stenosis was found not to be related to periprocedural strokes or death. The

authors concluded that the length of stenosis cannot be used to determine whether endovascular treatment or CEA is more advisable to these patients. Furthermore, the authors noted that the results regarding the endovascular arm should be interpreted cautiously because the majority of patients in this arm were treated using balloon angioplasty rather than stenting. They also recommended that further studies addressing this issue should be implemented using more practically available noninvasive angiographic methods like CTA or MRA in order to generalize their results.

V. A4. Plaque morphology:

In a large systemic review, echolucent plaques were found to be more predominant among sICAD compared to aICAD (OR = 3.99, 95% CI = 3.06-5.19) (40). Gronholdt et al. examined 246 patients with carotid stenosis >50% of whom 111 patients were asymptomatic and 135 symptomatic. The patients were followed for 4.4 years, whereby the primary end point was the development of ipsilateral ischemic stroke. Echolucent plaques causing >50% carotid stenosis were found to be associated with a high risk for further ipsilateral strokes among sICAD but not among aICAD (***P*<0.001**) (87). Echolucent plaques were shown to be more predominant in relatively younger patients suggesting a maturation process (88). Another study found an association between echolucent plaques and the development of ipsilateral symptoms, before adjustment for the degree of stenosis (crude OR: 4.7, 95% CI: 1.0-22.9) (89). However, after adjustment, this association was no longer observed (Mantel-Haenszel OR: 4.2, 95% CI: 0.94-22.7) (89). An independent association was found between echolucent plaques and the development of acute stroke, irrespective of the degree of stenosis (90). Similarly, we found that hypoechoic plaques were more likely associated with the development of ipsilateral symptoms than other types of plaque, irrespective of the degree of stenosis. Previous studies found that hypoechoic plaques were frequently associated with the presence of hemorrhage (91). Intraplaque hemorrhage is thought to arise from rupture of fragile newly formed vessels, which sprout in and serve as substrates for plaque growth (92). In the plaque at risk study (PARSIK), intraplaque hemorrhage affected symptomatic rather than asymptomatic plaques (38% versus 11%, respectively, $P<0.001$) (93). The risk for cerebrovascular events was six times higher in relation to the plaques revealing intraplaque hemorrhage, showing an annual incidence

of 17.71% versus 2.43% in relation to plaques without hemorrhage (94). High intensity signal (HIS), detected by routine three-dimensional time-of-flight MRA (3D-TOF MRA) in patients with low grade stenosis (30-49%) was associated with prior ipsilateral ischemic strokes in 21% of the cases compared to only 1% in HIS negative cases (39). In patients with 50-69% HIS-positive carotid artery stenosis, prior ipsilateral ischemic stroke occurred in 67% compared to only 9% in HIS-negative cases ($p<0.001$) (44). Another study recruited 29 consecutive patients with low grade sICAD <50% treated with a single antiplatelet agent and a statin along with controlling the medical risk factors (45). Over a follow-up period of 31.3 ± 16.4 months, the presence of HIS in T1-weighted images as well as expansive arterial remodeling were associated with a higher rate of stroke recurrence warranting in some cases CEA.

V. A5. Relation between the risk factors and the white matter lesions:

In the current study, advancing age was the most consistent risk factor correlating with TWMLL. Diabetes**,** serum levels of HbA1c, hypertension and female sex were also statistically significantly related to TWMLL. Increased TWMLL was associated with more severe stroke and a longer duration of hospital stay. No relation was found between TWMLL and LDL-cholesterol serum levels. Interestingly, we found an inverse relationship between smoking and TWMLL. This observation is probably due to the fact that younger patients were more often smokers. Matching with our findings, several studies found that advancing age was strongly related to WML (58, 95-101). Hypertension was frequently found to be associated with increased WMLL (65, 66, 97, 101, 102). Large cohort studies found that women were more prone to develop WML (100-102). Moreover, TWMLL was more often seen among women (102). Other smaller cohorts found that males are more prone to develop WML (61) and TWMLL was more severe in males (98). In our study, TWMLL was more often found among females. Other studies identified diabetes mellitus, dyslipidemia and smoking as risk factors for WML (61, 100). Other cohorts found neither a statistically significant relation between various levels of lipid parameters nor diabetes and TWMLL (101). In our study, diabetes and serum levels of HbA1c were statistically significantly related to TWMLL but LDL-cholesterol serum levels were not related. Other authors found no statistically significant relation between smoking or diabetes mellitus and the presence

of WML (97). In the Rotterdam study, hypertension, serum levels of total cholesterol and body mass index were not statistically significantly related to the presence or absence of WML (58). The authors of the Atherosclerosis Risk in Communities Study (ARIC)-MRI study reported that cross sectional studies regarding the association between smoking and white matter lesions are less likely informative because of multiple confounding factors (103). For example in the Rotterdam study, the authors found more current smokers among patients without WML than those with WML (30% vs 10%, ***P*=0.04**) (58). However, they also found that smoking is a risk factor for WML progression.

V. A6. Relation between the degree and length of ICAD and the white matter lesions:

We did not find any statistically significant correlation between the length of ICAD as assessed by duplex ultrasound, MRA or even DSA and the TWMLL, periventricular or deep WML, whether ipsilateral or contralateral to the ICAD. Moreover, no statistically significant correlation was found between the degree of ICAD as assessed by duplex ultrasound and the above mentioned white matter lesions. However, in viewing all the correlations we can conclude that a statistically insignificant correlation was found between the length but not the degree of sICAD and the ipsilateral WML. We speculate that the length but not the degree of ICAD might be, at least to some extent, associated with the occurrence (or development) of WML. In our study the sample size was probably too small to unmask a possibly significant relation between the length of ICAD and WML. Previous authors observed a statistically significant correlation between the total carotid plaque volume but not the degree of ICAD and ipsilateral WML ($\rho=0.393$, $P=0.005$) (67) which is in line with our findings. The Rotterdam study examined the association between carotid atherosclerosis, assessed by ultrasound, and the WML of 111 people older than 55 years living in Rotterdam (58). Among those with WML, the intima-media thickness of the common carotid artery was statistically significantly larger than among those without WML. The presence of plaques in the carotid bifurcation but not in the common carotid artery was observed to be statistically significantly related to the presence of WML. Similar to our results, the authors found no relation between the degree of ICAD and the presence or absence of

WML. In a large population based study, the periventricular but not the deep white matter lesions were significantly associated with an increased number of plaques in the carotid artery (104). In another population based longitudinal study involving 640 individuals aged 59-71 years, the subjects with carotid plaques were found to have an odds ratio (OR) of 1.70 (95% CI: 1.05-2.74) to develop severe WML on a 4-year follow-up (105). On the other side, several studies found no relation between carotid atherosclerosis and WML (70, 71). For example, Potter et al. found no association between ipsilateral or contralateral carotid stenosis and WML, before and after adjusting risk factors (70). Other authors found no association between lipid rich necrotic core volume in the carotid plaques and ipsilateral WML (67).

In our patients with potentially symptomatic ICAD, a statistically insignificant trend was found between the length of ICAD and the ipsilateral WML. These findings may support, that at least some WML may have a microembolic origin. Similarly, previous authors observed that unstable type VI plaques, defined according to the American Heart Association (AHA) histological classification, were more likely than stable type V plaques to be associated with at least double ipsilateral WMLL (68). Type VI plaque is declined by its surface ulceration, rupture and hemorrhage (68).

VI. SUMMARY AND RECOMMENDATIONS

We found the following main findings (all according to the ultrasound findings):

1. A significant inverse correlation between the length and degree of sICAD ≥70% but neither for sICAD <70% nor for aICAD.
2. sICAD <70% were "borderline" longer than sICAD ≥70%.
3. sICAD was more likely associated with echolucent plaques than aICAD.

In conclusion, ICAD <70% with a length ≥ 16 mm and echolucent plaques might have a higher risk than ICAD<70% with a length <16 mm. However, the replication of our findings in larger prospective studies is mandatory before any meaningful conclusions can be drawn.

Regarding the relation with the white matter lesion, we did not find any statistically significant correlation between the length of ICAD assessed by duplex ultrasound, MRA or even by DSA and the TWMLL, periventricular or deep WML whether ipsilateral or contralateral to the ICAD. Moreover, no statistically significant correlation was found between the degree of ICAD as assessed by duplex ultrasound and the above mentioned white matter lesions. However, to a little extent, we found a tendency for a relation between the length but not the degree of sICAD and the ipsilateral WML, either periventricular or deep. This relation seems not to be strong enough to be proved in our small cohort. If these findings can be demonstrated in larger studies, this would suggest that the length but not the degree of ICAD might be, at least to some extent, related to the WMLL.

We found no specific risk factor which was related to the degree or length of ICAD. The absence of any statistically significant association between any risk factor and the length or degree of ICAD may be, to some extent, explained by the good control of risk factors among our cohort abolishing any potential deteriorating role on the degree or length of ICAD.

There are several limitations of this study. The non-randomized design of this single-center cross-sectional retrospective study with a convenient sample might have influenced the comparative analysis between aICAD and sICAD. We recommend the

conduction of prospective studies addressing this issue. Another limitation of our study concerns the predominance of sICAD in comparison to aICAD since only patients with stroke or TIA were included. Furthermore, the reliability of the above-mentioned method, which we used to measure the length of ICAD has to be assessed in further studies. In part 1 of this study, 38/121 had bilateral stenosis. That is why the presented model of logistic regression using a generalized estimation equation method including only 38 subjects with bilateral stenoses, was not stable and the results showed are to be viewed only with caution.

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Appendix

Table S1: National institute of health stroke scale (NIHSS) (106, 107):

1a. Level of consciousness	0=Alert 1=Not alert, arousable 2=Not alert obtunded 3=Unresponsive
1b. Questions	0=Answers both correctly 1=Answers one correctly 2=Answers neither correctly
1c. Commands	0=Performs both tasks correctly 1=Performs one task correctly 2=Performs neither task
2. Gaze	0=Normal 1=Partial gaze palsy 2=Total gaze palsy
3. Visual fields	0=No visual loss 1=Partial hemianopsia 2=Complete hemianopsia 3=Bilateral hemianopsia
4. Facial palsy	0=Normal 1=Minor paralysis 2=Partial paralysis 3=Complete paralysis
5a. Left motor arm	0=No drift 1=Drift before 10 s 2=Falls before 10 s 3=No effort against gravity 4=No movement
5b. Right motor arm	0=No drift 1=Drift before 10 s 2=Falls before 10 s

	3=No effort against gravity 4=No movement
6a. Left motor leg	0=No drift 1=Drift before 5 s 2=Falls before 5 s 3=No effort against gravity 4=No movement
6b.Right motor leg	0=No drift 1=Drift before 5 s 2=Falls before 5 s 3=No effort against gravity 4=No movement
7. Ataxia	0=Absent 1=One limb 2=Two limbs
8. Sensory	0=Normal 1=Mild loss 2=Severe loss
9. Language	0=Normal 1=Mild aphasia 2=Severe aphasia 3=Mute or global aphasia
10. Dysarthria	0=Normal 1=Mild 2=Severe
11. Extinction/inattention	0=Normal 1=Mild 2=Severe

Fazekas scale (59):

Periventricular hyperintensity (PVH) is graded as:

0 = absence

1 = “caps” or pencil-thin lining

2 = smooth “halo,”

3 = irregular PVH extending into the deep white matter.

Separate deep white matter hyperintense signals (DWMH) are rated as:

0 = absence

1 = punctate foci

2 = beginning confluence of foci

3 = large confluent areas.

Table S2: Grading of the internal carotid artery stenosis (29):

NASCET definition	10	20-40	50	60	70	80	90	Occlusion
ESCT definition	45	50-60	70	75	80	90	95	Occlusion
Main criteria								
1. B-mode	+++	+						
2. Color coded images	+	+++	+	+	+	+	+	+++
3. PSV in the maximal stenotic area (cm/s)			200	250	300	350-400	100-500	
4. Poststenotic PSV (cm/s)					>50	<50	<30	
5.Collaterals (periorbital/ACA)					(+)	++	+++	+++
Additional criteria								
6. Prestenotic diastolic flow restraint (CCA)					(+)	++	+++	+++
7. Poststenotic turbulence of flow			+	+	++	+++	(+)	
8. End diastolic velocity in the maximal stenotic area (cm/s)			Up to 100	Up to 100	>100	>100		
9. Konfetti sign				(+)	++	++		
10. Stenosis index ICA/CCA			≥2	≥2	≥4	≥4		

PSV: peak systolic velocity, ACA: anterior cerebral artery, CCA: common carotid artery, ICA: internal carotid artery

Table S3: Maximal systolic flow velocity as well as post stenotic flow in relation to different stenotic grades among all available patients:

Degree of stenosis	Length of stenosis (mm)	PSV in the maximal stenotic area (cm/s)	Post stenotic flow velocity (cm/s)
20-40%	17 (12-20)	130 (104-156)	75 (67-84)
50%	14 (12-20)	206 (200-221)	67 (59-94)
60%	18 (15-24)	260 (250-290)	60 (54-68)
70%	18 (15-21)	300 (310-331)	58 (53-85)
80%	18 (14-24)	360 (318-383)	41 (33-47)
90%	13 (10-17)	400 (400-464)	23 (17-29)

Descriptive results are expressed as median (interquartile range)
PSV: peak systolic velocity

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