Carotid artery disease: plaque features and vulnerability

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“Research is to see what everybody else has seen, and to think what nobody else has thought”

Albert Szent-Gyorgyi

To my family
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Abstract

**Background:** Atherosclerosis is an important cause of stroke. Ultrasound offers the convenience of real-time and detailed assessment of carotid plaque features as well as arterial wall thickening and composition. Evaluation of these features is important for determining patients’ risk of suffering vascular events and also contributes to selecting the best treatment strategy.

**Methods:** Using ultrasound data analysis we have determined plaque features in the bifurcation and internal carotid artery (ICA), including: surface plaque irregularities, calcification, echogenicity (grey scale median-GSM) and other textural plaque features (Juxtaluminal black area, entropy, coarseness). In addition, intima media thickens (IMT) and its grey scale median (IM-GSM) was measured in common carotid artery (CCA). Using Cone Beam CT (CBCT) we have quantified calcification volume of the carotid plaques extracted after carotid endarterectomy procedure. For the meta-analysis we have used comprehensive meta-analysis software version 3.

**Study I:** We have included 39 patients and we compared carotid plaque features of the contralateral arteries with those located ipsilateral to symptomatic side and arteries of asymptomatic patients.

**Study II:** The accuracy of US to detect atherosclerosis calcification was assessed against CBCT in 88 patients.

**Study III:** Based on the previous vascular events in coronary, carotid and lower extremity arterial system, 87 patients were divided into three groups: asymptomatic, symptoms in one vascular system and symptoms in more that one vascular system. IMT, IM-GSM and plaque features were compared between groups.

**Study IV:** We have meta-analyzed ten cohort prospective studies evaluating carotid plaque echogenicity for cerebrovascular symptoms prediction.

**Results:**

**Study I.** Plaques of the contralateral to symptomatic arteries had similar features to those in symptomatic and more vulnerable than asymptomatic arteries.

**Study II.** Carotid ultrasound was accurate in detecting calcification volumes of ≥8mm$^3$ with very high sensitivity but it was less accurate in detecting lower calcification volumes
(<8mm³). Carotid calcification was not different between symptomatic and asymptomatic patients.

Study III. Echogenicity of the intima-media complex (IM-GSM), but not its thickness (IMT), was significantly decreased with increasing number of arterial systems affected by atherosclerosis. IM-GSM was lower in patients with prior myocardial infarction and stroke.

Study IV. Carotid plaque echogenicity evaluated by US could predict future cerebrovascular events in patients with asymptomatic, relative risk RR 2.72 (95% CI, 1.86 to 3.96), and recurrent symptoms in symptomatic patients, RR 2.97 (95% CI, 1.85-4.78).

**Conclusion:** Plaques located in the contralateral to symptomatic arteries have similar features as symptomatic side and more vulnerable than asymptomatic arteries. Carotid ultrasound could accurately detect larger but not smaller carotid plaque calcification volumes (<8 mm³). Low IM-GSM could identify patients with multi-system atherosclerosis disease, suggesting a better marker for determining systemic atherosclerosis disease burden compared to conventional IMT. Finally, carotid plaque echogenicity predicts future cerebrovascular events in patients with symptomatic and asymptomatic carotid stenosis.

**Keywords:**
Carotid atherosclerosis, ultrasound, plaque features, echogenicity, calcification, surface plaque irregularities, subclinical atherosclerosis, cerebrovascular symptoms
List of papers


Abbreviations

ACAS  Asymptomatic Carotid Atherosclerosis Study
ACSRS Asymptomatic Carotid Stenosis and Risk of Stroke
ACST  Asymptomatic Carotid Surgery Trial
ANSYSCAP Additional Neurological Symptoms before Surgery of the Carotid Arteries
ARIC  Atherosclerosis Risk In Communities
BMT   Best Medical Treatment
CAS   Carotid Artery Stenting
CAV   Cardiovascular
CBCT  Cone Beam Computed Tomography
CCA   Common Carotid Artery
CEA   Carotid Endarterectomy
CEUS  Contrast Enhanced Ultrasound
CV    Cerebrovascular
CT    Computed Tomography
CTA   Computed Tomography Angiography
DSA   Digital subtraction angiography
ECST  European Carotid Surgery Trial
GSM   Grey Scale Median
HDL   High Density Lipoprotein
HR    Hazard Ratios
ICA   Internal Carotid Artery
IM    Intima-Media Thickness
IM-GSM Intima Media-Gray Scale Median
IPH   Intraplaque Hemorrhage
JBA   Juxtaluminal Black Area
LDL   Low Density Lipoprotein
MES   Microembolic Signal
MI    Myocardial Infarction
MRA   Magnetic Resonance Angiography
MRI   Magnetic Resonance Imaging
NASCET North American Symptomatic Carotid Endarterectomy Trial
NRI   Net Reclassification Improvement
OR    Odds Ratios
PET   Positron Emission Tomography
PT    Plaque Type
TCD   Transcranial Doppler
US    Ultrasound
VA    Veteran’s Affairs
INTRODUCTION

Atherosclerosis is a progressive inflammatory disease characterized by accumulation of lipids, fibrous elements and inflammatory cells in large and middle-sized arteries. It is the main cause cardiovascular (CAV) and important cause of cerebrovascular (CV) events (1). Epidemiologically, ischemic heart disease and cerebral ischemia represent the main cause of death and premature disability in developed countries (2). Moreover, due to the aging of the population, the global burden of atherosclerosis, and thereby its clinical consequences will continue to rise in the coming decades (3). The underlying pathogenesis of atherosclerosis involves an imbalance between lipid metabolism and maladaptive immune response and consequently chronic inflammation of the arterial wall (4). Although several risk factors associated with arterial wall disease have been identified, disease burden and ischemic events could not be predicted based on their identification alone, thus leaving space for other methods (e.g. arterial imaging and biomarkers) to be employed for better patients risk stratification. Advances in medical and interventional treatment strategies have emphasized the importance of identifying vulnerable patients, their risks and optimum treatment. Also, developments of different imaging techniques have aided considerably in this aspect by making non-invasive evaluation and quantification of atherosclerosis in-vivo possible, in particular ultrasound, which is an established imaging modality in atherosclerosis research.

Pathophysiology of atherosclerosis

The term atherosclerosis is derived from the Greek "athero," meaning gruel, or wax, and "sclerosis" for hardening (induration), with the former referring to the corresponding lipid pool (necrotic core) and the latter referring to the fibrous cap of the plaque's luminal edge. Arterial wall is composed of three layers: tunica intima, tunica media and adventitia. The intima is the inner lining of the vessel, directly adjacent to blood flow, a very dynamic layer which is composed of a monolayer of endothelial cells. The initial step of the atherosclerosis pathology is the dysfunction of these endothelial cells that allows the accumulation of low-density lipoprotein particles (LDL) into the intima. Subsequently LDL particles undergo oxidative modifications that may trigger a local
inflammatory response that signals the following steps in the lesion formation. This is followed by monocytes migration, accumulation into the intima and their conversion to macrophages, which characterizes the initiation of early atherosclerotic lesion formation. Upon occupying the intimal space, macrophages will uptake lipoprotein particles by receptor mediated endocytosis and become lipid-laden foam cells. This stage of the disease become macroscopically apparent as fatty streaks, that could be visible in the large arteries even in the first decade of life, as confirmed by autopsy studies. (4, 5)

Although formation of fatty streaks commonly precedes the development of a more advanced atherosclerotic plaque, not all of them will progress to atheroma formation. Also, not all mechanisms involved in atherosclerosis process are “pro-atherogenic”, in fact high-density lipoprotein (HDL) particles and M2-like phenotype macrophages have “anti-atherogenic” properties (4). Based on the above, atherosclerotic plaque could follow different ways: a) it could have stable progression (in most cases), b) it could have abrupt progression that leads to plaque rupture with superimposed thrombosis or c) it could regress, as shown in longitudinal follow-up imaging studies.

Lesion types

The pathogenesis of atherosclerotic lesions has been analyzed at different stages of disease and in different age groups. There are many histological classification of atherosclerotic lesions, however, we hereby present a simple one, which emphasizes the link between morphology and clinical manifestations (6). In addition, this classification could be properly related to plaque imaging during different stages of the disease. Figure 1, shows all plaque types presented on a graph together with corresponding ultrasound images.

A. Adaptive intimal thickening: Characterized by smooth muscle cells accumulation in a proteoglycan-rich matrix in the absence of lipids and inflammatory cells. This may provide a soil for lesion development, initially at the branching points but later could spread into adjacent parts.

B. Intimal xanthoma (“fatty streak”): Characterized by LDL accumulation into the intima. Subsequently, LDL particles will modify and undergo process of
oxidation and start to act as chronic stimulator for immune response. They induce endothelial cells to express adhesion molecules, which interact with receptors on the surface of the monocytes and stimulate their adhesion and migration into the intima. Macrophages could express several different phenotypes. Some of them attain pro-inflammatory (M1-like phenotype), and the others that have an M2-like phenotype may secret factors that favor resolution of the inflammation. These types of lesions could be fully reversible if the stimuli that caused them dissipate. They are present already in the aorta of some infants in the first 6 months, probably reflecting risk factors of the mothers, then their number decline in the subsequent years.

C. *Pathological intimal thickening*: Pathological intimal thickening is sometimes refereed to in the literature as an “intermediate lesion.” True necrosis is not apparent. The fibrous cap overlying the areas of lipid is rich in smooth muscle cells and proteoglycans. Macrophages and lymphocytes may also be present, but these are usually sparse.

D. *Fibroatheroma*: this corresponds to the formation of necrotic core. The foam cells, macrophages and smooth muscle cells accumulated in the intima over time could undergo apoptosis and secondary necrosis. The reason why necrosis happens in some but not the other lesions is not known. At this stage of the disease neovascularization and intraplaque hemorrhage are often present.

E. *Fibrocalcific plaque*: Calcification is common in progressive atherosclerosis and its content increases with age. Plaque components that undergo calcification are: apoptotic cells, extracellular matrix and necrotic core. Sometimes these structures undergo complete calcification; so calcification may constitute most of the plaque volume. The last two phases of the disease progression (D and E) can interchange between each other under different internal and external factors, e.g. statin therapy could increase fibrotic composition within the soft plaques and in high doses can aggravate plaque calcification.
Figure 1. Stages of atherosclerosis development and ultrasound imaging samples. In each box; top left- a cross sectional view of the carotid artery animation, top right- more detailed animation focused at the more active disease process, bottom right- ultrasound plaque features attempt to correlate with the plaque features represented in the animations.

A- adaptive intimal thickening, that can be accurately measured by ultrasound; B- Intimal xanthoma, there is an assumption, but it is not yet confirmed by direct histological studies if an echolucent intima media complex (low IM-GSM) represents the intimal xanthoma, characterized by accumulation of LDL and inflammatory cell particles; C- Pathological intimal thickening (intermediate lesion), early non-stenotic lesion on ultrasound; D- Fibroatheroma with typical features vulnerable plaques, echolucent plaque with juxtaluminal black area on US; E- Fibrocalcific plaque.
Pathomechanism of ischemic events

The most dramatic complication of atherosclerotic plaque progression is thrombosis, which may be caused by three different mechanisms: a) plaque rupture, b) plaque erosion or c) calcified nodule (7). The first and most common one is plaque rupture, which is defined as a fibrous cap disruption when the overlying blood contents are in continuity with the underlying necrotic core. Plaque erosion is identified when the thrombus is superimposed on a plaque substrate primarily composed of smooth muscle cells and proteoglycans, and without a communication between blood contents and the necrotic core. Calcified nodules are characterized by protrusion of the eruptive dense calcified bodies into the luminal space and represent the least frequent morphology associated with luminal thrombosis (8). Following plaque rupture, the pathomechanisms by which the disease is caused differs in different arterial systems. While in coronary arteries myocardial infarction (MI) is attributed to in-situ thrombosis, in carotid artery embolization is the most common mechanism causing ischemic stroke (1) (figure 2).

Figure 2. Stable and unstable plaque features. Pathomechanisms of ischemic events in coronary and carotid artery
Risk factors

There are multiple factors that contribute to atherosclerotic plaque progression. As described above, the processes involved in atherosclerosis include: endothelial cell injury, lipid metabolism, inflammation, and smooth muscle cell proliferation. Factors affecting these processes may inhibit or accelerate atherosclerosis. In general atherosclerosis risk factors are divided into two groups: non-modifiable (family history, male gender) and modifiable (hyperlipidemia, diabetes mellitus, cigarette smoking, hypertension, and dietary deficiencies of antioxidants) (1).

Epidemiology

Atherosclerosis-related cardiovascular (CAV) and cerebrovascular (CV) events are the most common causes of deaths in the West (2). The prevalence of atherosclerosis in carotid and coronary arteries increases exponentially with age, and is more prevalent in men than in women. According to the World Health Organization, 15 million people suffer stroke annually worldwide and of them 5 million die. In Europe alone nearly 650,000 stroke deaths occur per year (9). Approximately 85% of strokes are ischemic and 15-20% of them are attributed to carotid artery disease (10).

CAROTID ARTERY STENOSIS

Carotid artery stenosis is an important cause of ischemic stroke (11). Atherosclerosis could affect any segment of the carotid artery with a predilection at the point when it bifurcates into internal and external artery. The arterial wall is normally highly dynamic because its pulsations follow those caused by the cyclic heart contraction and relaxation. Arterial expansion alters artery radius, shape and curvature, which together interact to make the blood flow in the arteries (e.g. common carotid artery) laminar. Laminar blood flow is characterized by concentric layers of blood moving in parallel down the length of a blood vessel with the highest velocity in the center of the lumen. When blood separates at an arterial bifurcation, a complex pattern of fluid velocities is created. The highest
velocities at the center of flow come in contact with the flow divider, and this flow separation continues until some distance into the subsequent branches (figure 3). These regions have a low wall shear stress that contributes to alterations of endothelial cells, by inducing expression of endothelial leukocyte adhesion molecules that contribute to leukocyte migration into the intima and hence the development of atherosclerosis (12).

![Hemodynamics at the carotid artery bifurcation](image)

*Figure 3. Hemodynamics at the carotid artery bifurcation*

**Who is at risk?**

Moderate to severe asymptomatic carotid stenosis is found in 2-5% of women and 2-8% of men over the age of 60 years. However the risk is much higher in high-risk groups. The prevalence of asymptomatic 50-90% carotid stenosis in patients over 60 years with three CAV risk factors is estimated at 16%, and in those with concomitant coronary artery disease and contralateral carotid disease is 15% and 6%, respectively. The Framingham study showed that predictors of carotid stenosis over 12 years are (13):
- Age (odds ratio (OR) 1.6-1.7/10 year increase in age)
- Smoking (OR 1.3-1.5 per 10 cigarettes smoked/day)
- Systolic blood pressure (OR 1.2 per 10 mmHg increase)
- Total cholesterol (OR 1.1-1.2 per 0.5 mmol/L increase).

Clinical identification of carotid artery stenosis used to be based on auscultation skills and the confirmation of a bruit. This clinical sign has recently been proven to be of low accuracy in depicting 70-99% stenosis with a sensitivity of 57% and specificity of 70% (14). This low accuracy of auscultation highlighted a need for other accurate imaging screening modalities such as ultrasound. In addition, detection of calcification on panorama radiograph or cone beam CT (CBCT), during patients’ evaluation for dental procedures, has been found to be associated with significant carotid stenosis (15). Recommending these patients for carotid US examination has made possible the identification of those with significant stenosis and implementation of different treatment strategies for stroke prevention.

*Degree of Carotid stenosis assessment*

The degree of carotid artery stenosis could be evaluated by different imaging techniques, however the gold standard against which other methods accuracy is evaluated is conventional angiography (16). Using angiography, the degree of stenosis could be quantified by two methods: one suggested by North American Symptomatic Carotid Endarterectomy Trial (NASCET) (17) and the other by European Carotid Surgery Trial (ECST) (18). Both methods evaluate the degree of stenosis as the percentage reduction in the linear diameter of the carotid artery. Because these two trials have used different strategies to quantify the degree of stenosis, applying both of them in the same carotid artery will demonstrate different values (e.g. 82% ECST usually corresponds to 70% NASCET stenosis) (19). This difference between the trials degree of estimated stenosis can be corrected using the formula: NASCET=(ESCET-40)/0.6 (20). Because angiography is an invasive investigation and is also associated with 0.3-1% risk of peri-procedural neurological events, a growing interest for other non-invasive modalities that could accurately evaluate the degree of stenosis and identify other plaque features that
could be of importance for patients risk stratification has been developed (21). A meta-
analysis conducted by Koelemay et al. (22) reported a sensitivity of 97% and specificity
of 99% for CTA in characterizing the degree of carotid artery stenosis. In particular, CTA
was accurate for assessing 70-99% stenosis and for excluding total artery occlusions.
Carotid duplex ultrasound (US) has been shown to be accurately comparable with digital
subtraction angiography (DSA) and today it is the most widely used method for
evaluating carotid stenosis. Carotid US has the benefits of speed, safety, bedside and its
radiation free properties. Stenosis is measured using Doppler principles of blood velocity
at the tightest stenosis point, which could be translated to equivalent DSA measurements.
One of the methods recommended for grading stenosis is to use peak systolic velocity. A
velocity more than 125 cm/s corresponds to >50% stenosis and a velocity >230 mm/s
corresponds to greater than 70% stenosis (23).
A meta-analysis of individual patients data showed an inter-observer variability
agreement, for ultrasound, for 70-99% degree of stenosis of 86% (79%-91%) (24). In
clinical practice, an additional confirmatory investigation to ultrasound has been
recommended before carotid endarterectomy (CEA); including another imaging modality
(CTA) or a repeat ultrasound examination (10). However, there are data that suggests that
ultrasound-only strategy is as effective as adding one more modality in patients with 70-
99% stenosis selected for surgical intervention within 14 days. Assessing moderate
symptomatic carotid stenosis by ultrasound has been shown to be less accurate (25). Also
the accuracy is limited in cases with moderate contralateral stenosis or occlusion as well
as in arteries with extensive calcification. In borderline cases, adding another modality,
CTA or contrast enhanced magnetic resonance angiography (MRA) can reduce
unnecessary CEA performed (26). It has been shown that symptomatic patients with
>70% stenosis benefit from CEA and is therefore very important to measure accurately
the degree of stenosis. However, it was later demonstrated that the risk of embolism and
thrombosis is not related only to the stenosis severity but also with its composition and
other plaque features.
Carotid artery plaque features: the concept of plaque vulnerability

Based on several morphological and histological characteristics, carotid plaques could be defined as stable and unstable. In general, plaques that have been associated with increased risk of ischemic events are defined as unstable or vulnerable. Vulnerable plaques are characterized by the presence of a thin fibroatheromatous cap that overlies a large lipid pool (>40% of the plaque), large necrotic core, intimal spotty calcification, high inflammatory cell concentration, neovascularization and intraplaque hemorrhage. While historically plaque features are evaluated histologically in autopsy studies or in the arterial segments removed after surgical procedures, advances in imaging techniques have made in vivo plaque visualization and identification of different components inside the plaque possible.

Imaging plaque features

A number of non-invasive imaging modalities exist for reliably determining carotid artery plaque features, including: ultrasound, magnetic resonance, computed tomography, and positron emission tomography (PET) (16). Because studies included in this thesis are mainly based on ultrasound technology, this section will be mostly focused on ultrasound characterization of carotid plaque features, and also its strength and weakness will be weighted against other non-invasive imaging modalities.

Surface plaque morphology

The surface morphology of the carotid plaques classified as smooth, mildly/markedly irregular and ulcerated, is an important determinant of vascular events. A smooth surface configuration is characterized by a regular luminal morphology which indicates a stable plaque. Irregular surface morphology, especially marked irregularity has been shown to be associated with increased risk of ischemic events (27). The third type of surface morphology –ulceration, is significantly associated with increased risk of cerebral events, and this was demonstrated in NASCET study (28). Of the different imaging techniques that have been used to assess surface plaque morphology, computed tomography
angiography (CTA) has the greatest accuracy. Compared to ultrasound, CTA sensitivity in detecting plaque ulceration has been shown the highest (93% vs. 37.5%) (29). Such sensitivity of US for identification of ulceration is higher (77%) in plaques causing >50% stenosis (27). Recently introduced ultrasound based techniques such as: contrast enhanced US (CEUS) and ultrasound 3D reconstructions have been shown to have very high accuracy for detecting ulceration (30, 31).

Plaque composition

As mentioned before, carotid plaques are composed of different components and the relative proportion of these components can vary from one plaque to another, which could determine its stability. Histologically, different plaque components have been described and their association with ischemic events was determined. Carotid plaque components most commonly found in patients with stroke and transient ischemic attack (TIA) are: lipid pool, necrotic core and hemorrhage. The presence of calcium seems to have a protective role, since it is found mostly in asymptomatic plaques. Because of the clinical importance identifying such plaque features in vivo, many imaging modalities have been employed (32, 33). Ultrasound as well as computed tomography (CT) and magnetic resonance imaging (MRI) offer important information about carotid plaque composition. On CT carotid plaques are classified based on the Hounsfield Units (HU) density as fatty (<60 HU), mixed (60-130 HU) and calcified (>130 HU) (34). Very low values (<0 HU) have been found to be associated with hemorrhage. Also MRI can depict carotid plaque composition, especially plaque hemorrhage (35, 36).

On ultrasound, plaque echogenicity is the main parameter that reflects composition. Plaque echogenicity has traditionally been evaluated by visual (subjective) grading plaques echo reflection (e.g. echogenicity and echolucency), and its echo pattern (heterogenous and homogenous). However, visual evaluation of plaque echogenicity has some limitations because it is subjective and also plaque echogenicity could be affected by ultrasound machine settings during the patients’ examination. Nevertheless, this limitation could be overcome by computer assisted plaque normalization using image processing and 2 references (blood and adventitia). The blood is scaled to zero, while the brightest area of the adventitia is normalized to a grey scale of 190. Following
normalization, the plaque is outlined and its overall brightness evaluated by means of grey scale median-GSM (grey scale range: 0-to-255; 0=black and 255=white). The lower the GSM the more echolucent is the plaque. Conversely, echogenic plaques will have higher GSM. However, because different studies have used different cut-off values to define plaque echolucency, its use in clinical practice could not be suggested yet. In addition to GSM evaluation, plaques could be objectively classified into four groups based on Gray-Weal classification modified by Geroulakos (37):

Type 1: uniformly echolucent with <15% of the pixels in the plaque area being occupied by pixel values >25;
Type 2: mainly echolucent where pixels with grey scale values >25 that occupy 15-50% of the plaque;
Type 3: mainly echogenic with pixels >25 occupying 50-85% of the plaque;
Type 4: uniformly echogenic with grey scale >25, occupying >85% of the plaque.

Studies correlating US findings with plaque histology have revealed that echolucent plaques are associated with lipids, necrotic core or intraplaque hemorrhage, while echogenic plaques are associated with fibrous and calcified tissue. In addition to GSM and plaque types there were other parameters developed such as: Juxtaluminal black area, plaque coarseness and entropy.

**Juxtaluminal black area (JBA)** of the plaque is defined as the area of the plaque components having grey-scale <25 without a visible echogenic cap. After imaging normalization this area is outlined and expressed as mm² (38). JBA <8 mm² has been shown to be associated with a 0.6 annual risk of stroke compared to 4.6% in patients with JBA >8 mm² (39).

**Thin cap fibroatheroma**

Histologically, fibrous cap is formed of smooth muscle cells relied within a collagen-proteoglycan matrix, associated with macrophages and T lymphocytes (40). A thin fibrous cap plays a critical role in determining plaque vulnerability. Following fibrous cap rupture, the exposure of thrombogenic plaque components to the luminal blood flow
represents a critical step that eventually leads to thromboembolic complications. The best non-invasive imaging modality to assess the status of the fibrous cap in carotid arteries is MRI, which could define the fibrous cap as normal, thin or fissured/ruptured (41). Currently, there are no imaging studies that have used ultrasonography to assess fibrous cap, and CT proved not accurate for this purpose.

*Neovascularization and intraplaque hemorrhage*

Adventitia of the normal middle and large size arteries has a vasculature network (vasa vasorum) that supplies the outermost part of the artery with oxygen and nutrition, while the intima part is supplied with oxygen from the lumen through diffusion. In pathological conditions (atherosclerosis), because the oxygen cannot achieve the deeper parts of the plaque, some small vessels will proliferate from the adventitia towards the intima as neovessels. The wall of these vessels lack pericytes and is very fragile and hence is prone to rupture. It has been suggested that rupture of the neovessels is the cause of production and expansion of intraplaque hemorrhage (IPH) (42). The presence of neovascularization and/or intraplaque hemorrhage is considered a feature of vulnerability (43). The best modality for visualizing IPH in the carotid arteries is magnetic resonance, which can recognize the age of the hemorrhage as well (35). Using ultrasound, neovascularization can be evaluated using microbubbles based contrast, known as contrast enhanced ultrasound (CEUS) (42). A recent study by Staub et al. concluded that the presence of neovascularization inside the carotid plaque is associated with morphological features of plaque instability (44).

*Arterial Calcification*

Calcium formation may be found in any arterial bed as well as in the microvessels. It may be present in the intima and/or media, the latter being common in chronic kidney disease or type II diabetes (16). The initial plaque calcification is associated with apoptotic smooth muscle cell and appears as micro-calcifications bound to membrane vesicles. Following plaque progression, the micro-calcifications combine together to form larger calcium deposits. In patients with carotid atherosclerosis calcification is detected in 50-
60% of cases (45) and even often in significantly stenotic lesions (>50%) (15) but its association with CV events is uncertain. Controversies exist as to the effect of calcification on plaque nature with some evidence suggesting more stability (45) and another indicating vulnerability, irrespective of the degree of stenosis (46). A systematic review has shown that symptomatic plaques have less calcification than asymptomatic ones (47). However, studies included in this review used wide range of methods to quantify carotid calcification, highlighting the need for a well-validated, accurate and reproducible technique for calcium quantification in patients with carotid atherosclerosis.

Plaque calcification in carotid arteries can be assessed by a number of imaging modalities including: CT, ultrasound and MRI. While ultrasound may detect the presence of calcification, it does not have the accurate means for quantifying its extent. Computed tomography on the other hand has the ability to identify and quantify calcification. Even calcification volumes of 1 mm$^3$ can be detected and quantified by cone beam CT (CBCT). Furthermore, it has been shown that the amount of calcification quantified by carotid CT correlated with respective histology sections (48). Despite, such uncertainty the extent of carotid calcification adds more accurate assessment to the plaque features. Carotid US is an easy and economically favorable method to study different plaque features and their potential association with symptoms. It has been reported that extensive calcified carotid plaques are associated with adverse outcome 30 days after carotid artery stenting (CAS), hence CEA should be recommended in such cases (49). Because many centers today rely mainly on carotid US information before intervention, identification of calcification and its severity seems to be important.

**ASYMPTOMATIC CAROTID STENOSIS**

Asymptomatic carotid stenosis refers to narrowing of the carotid artery caused by atherosclerosis in patients who have not experienced a stroke or transient ischemic attack in the brain related to cerebral circulation insufficiency. With the introduction of vascular ultrasound in 1980s, investigation of carotid artery disease became easy, and on the following years a high number of patients were diagnosed with a so-called asymptomatic
carotid disease. However, the prognosis and management of such patients remains uncertain. Both natural history studies, as well as several large randomized trials showed a low incidence of stroke in asymptomatic patients with carotid disease, only 1-2% per year and a rather high incidence of cardiovascular morbidity and mortality (5-10% per year) (50, 51).

**Management of asymptomatic patients with carotid stenosis**

The Asymptomatic Carotid Atherosclerosis Study (ACAS) (52) and the Asymptomatic Carotid Surgery Trial (ACST) (53) provided evidence that CEA offers a 50% risk reduction for ipsilateral stroke in patients with moderate to severe asymptomatic carotid artery stenosis compared to medical treatment alone (annual stroke risk: 1% vs. 2%). However, at the time when these trials were performed, patients had received a treatment that today would be considered as suboptimal medical treatment, e.g. in the ACST statins were used in only <10% of patients, while 20% of population was not taking antiplatelet therapy. Furthermore, dietary modification, blood pressure control and statin dosage were not close to those that are currently used in practice.

It has been suggested that up to 94% of CEA performed on the basis of these trials recommendations are unnecessary. As compared to CEA for symptomatic stenosis, with a number needed to treat (NNT) of 6 (or 3 for patients above 75 years old), to prevent a stroke in asymptomatic patients the NNT were 83 (54). The advancement of the best medical treatment (BMT) has questioned the invasive intervention (CEA or CAS) for asymptomatic patients. Recently, a low risk of ipsilateral stroke in patients with asymptomatic carotid stenosis has shown range from 0.4 to 1.0% per year (55). Nevertheless, for a subgroup of patients at higher risk of stroke, invasive intervention may still be justified. Identification of such high-risk patients who could benefit from invasive intervention in addition to BMT is thus of crucial importance.

**Identifying high-risk patients with asymptomatic carotid disease**

In the past three years, it has become increasingly clear that BMT is the best choice for the majority of patients with asymptomatic carotid disease, and there has been an
increasing effort in identifying factors that could stratify patients who may benefit of intervention therapy (56). Many clinical and imaging features have been reported that are associated with increased risk of stroke, and some of them will be described in the following sections.

*Bilateral carotid disease*

Patients with bilateral carotid disease are at higher risk from suffering CV symptoms. In asymptomatic carotid stenosis, it has been reported that patients with a history of contralateral TIA/stroke were associated with a 3.4% annual risk of stroke compared to 1.2% in those with no previous symptoms (57). It has also been concluded that medically treated patients with a history of contralateral symptoms prior to randomization were significantly more likely to develop CV symptoms within 5 years follow-up in the territory ipsilateral to asymptomatic stenosis compared to those without a history of contralateral symptoms, 29% vs. 22%, respectively (58). In addition, patients with bilateral carotid disease “vulnerable” textural features were confirmed in carotid plaques of both sides compared to more stable features in patients with unilateral disease (59).

*“Silent” brain infarction*

About 20% of asymptomatic patients with carotid stenosis will have the evidence of silent infarction on CT/MRI scans. Patients with ipsilateral “silent” infarction have been shown to have a significantly higher annual risk of ipsilateral stroke compared to those without, 3.6% vs. 1.0%, respectively (60). The same was confirmed in an ACST subgroup analysis. Compared to patients with no evidence of brain infarction or prior symptoms, those with ipsilateral infarction or a history of prior CV symptoms had a 5.8% absolute risk increase for stroke in the subsequent 10 years (61).

*Multisite atherosclerosis disease*

Reduction of Atherosclerosis for Continued Health (REACH) (62) study analyzing prospectively a cohort of 68,236 patients showed that after one year follow-up the rate of
vascular events is increased with the number of symptomatic diseased locations, ranging from 5.31% in asymptomatic patients to 12.58%, 21.41% and 26.27% in patients with 1, 2 and 3 symptomatic arterial disease locations, respectively. Based on these results it seems that the presence of multi-focal atherosclerosis disease has major clinical and prognostic impact. However, the pathomechanism behind widespread atherosclerosis at the level of distinct territories is complex; multiple risk factors, genetic, inflammatory markers and loco-regional hemodynamics, all may play an important role (63, 64). The impact of different risk factors on the disease prevalence in various locations is important in predicting the affected arterial system: smoking is three times more likely to cause lower extremity artery disease than coronary artery disease, compared to diabetes in coronary artery disease and hypertension in carotid atherosclerosis (1, 65).

*Ultrasound markers of plaque vulnerability*

**Degree of stenosis:** There is conflicting evidence with regards to any relationship between stenosis severity and future ischemic events. Subgroup analyses from the ECST and NASCET showed that stenosis severity (but not near occlusion) was associated with increased risk of stroke in medically treated patients. Also, the Asymptomatic Carotid Stenosis and Risk of Stroke (ACSRS) study (57) has reported that the risk of stroke is proportionally increased with the increase in the degree of stenosis from 0.8% to 1.4% and 2.4%, for 50-69% to 70-89% and 90-99% stenosis, respectively. On the other hand, neither ACAS (52) nor ACST (61) found any evidence that increasing stenosis severity is a predictive of increased risk of stroke in the medically treated asymptomatic patients.

**Stenosis progression:** It has been assumed that progression of the stenosis severity as shown with US could be associated with increased risk of stroke. Sabeti et al. (66) showed that stenosis progression was associated with two-fold increase of stroke risk in the 3-year follow-up period (OR 2.0; 95% CI 1.02-4.11). The ACSRS study (57) in a subgroup analysis on this issue showed that at follow up stenosis severity remained unchanged in 76%, regression was observed in 4% and stenosis progression in 20% of patients. The 8-year cumulative risk of ipsilateral stroke was 0% for patients with US evidence of stenosis regression, 9% in the group of patients with unchanged stenosis and
16% in patients with stenosis progression. Despite suggestions that stenosis progression was associated with doubling rate of stroke per annum, 68% of the ischemic strokes occurring during the follow-up affected patients with no evidence of stenosis progression, thus indicating a low accuracy of the method for identifying high-risk patients.

Textural plaque features: As stated above, there are many ultrasound-derived textural features that could be considered as a sign of plaque vulnerability. Soft (echolucent) carotid plaques today could be evaluated using different cut-offs of GSM values (low GSM=echolucent plaque) or by classifying plaques into plaque types (PT) as recommended by Gray-Weale and Geroulakos (PT 1 and 2=echolucent plaque). In the ACSRS study, asymptomatic patients with a GSM >30 had a very low annual rate of stroke (0.6%), the annual stroke rate increased to 1.6% for those with a GSM of 15-30 and to 3.6% for those with GSM <15. The annual rate of stroke was progressively increased when comparing plaque Types from 4 to 1, with Type 4 having a 0.4% annual risk of stroke, compared to 0.8% and 3.0% for Type 3 and Type 1 or 2. Most studies that used this classification to determine patients’ symptoms have defined plaques Type 1 and 2 as echolucent and plaque Types 3 and 4 as echogenic.

Microembolic signals detection by transcranial Doppler

Microembolic signal (MES) detection by transcranial Doppler (TCD) is another important imaging modality that was used to identify patients at increased risk of stroke; accordingly, consensus criteria have been established for interpreting these high-intensity transient signals (67). Several studies evaluated the predictive role of MES detected by TCD ultrasonography in patients with asymptomatic stenosis. Asymptomatic Carotid Emboli Study (ACES) showed that the presence of spontaneous embolization was associated with a five-fold increase in the risk of ipsilateral stroke (68). In 2005, Spence et al. (69) showed that asymptomatic carotid stenosis patients who had two or more microemboli during an hour of monitoring had a 1-year stroke risk of 15.6% compared to only 1% risk of stroke for patients with no microemboli. Furthermore, it was demonstrated that an aggressive approach to medical therapy has significantly reduced MES on TCD ultrasonography (70).
Figure 4. Proposed algorithm for the management of patients with asymptomatic carotid stenosis, relied on clinical data and data acquired by ultrasound imaging. BMT - Best Medical Treatment, CEA - Carotid Endarterectomy, CAS-Carotid Artery Stenting. Note: To decide between CAS and CEA other clinical and imaging features acquired by modalities other than US should be taken into consideration, e.g. intracranial extension of carotid plaque, patients with previous irradiation of the cervical region, carotid artery tortuosity, etc.

SYMPOTOMATIC CAROTID STENOSIS

First descriptions of carotid stenosis related to cerebral ischemic events date back to T. Willis (1621-1675) and J. Wepfer (1620-1695) (71). Symptomatic carotid stenosis is defined as carotid artery disease associated with ischemic symptoms (Stroke, TIA or amaurosis fugax) in the anterior brain circulation. After ischemic events, the presence of carotid artery stenosis is confirmed during an US, CTA, MRA or conventional
angiography examination. During the US examination the degree of stenosis is measured together with the description of exact plaque location and plaque morphology. After confirmation of the carotid disease in most cases, unless contraindicated, patients will be recommended carotid endarterectomy (CEA) intervention. CEA consists removal of the carotid atheroma together with intima and media layers, leaving the adventitia unattached.

**Management of symptomatic patients with carotid disease**

In patients with acute CV accidents associated with carotid stenosis the initial therapy comprises antiplatelet, statins and risk factors management (72). In patients with carotid stenosis an intervention should be performed within <2 weeks. Benefit of revascularization is greater in men, in older patients, and in severe stenosis (70-99%) (72). In addition, a subgroup of patients with moderate carotid stenosis (50-69%) might also benefit of revascularization, particularly those with moderate carotid plaque irregularities, ulceration and echolucency that are confirmed to be at higher risk for stroke recurrence (73). In addition, it was recently shown that the incidence of stroke recurrence is very high in the first 72 hours after initial symptoms and an emergency CEA intervention would be of benefit in this subgroup of patients.

*Medical therapy*

Medical therapy of stroke or TIA related to a carotid stenosis consists of immediate administration of secondary prevention with antiplatelet therapy. Either dipyridamole plus aspirin (74), clopidogrel alone (75), or aspirin alone is recommended (76, 77). In symptomatic patients with >50% carotid stenosis associated with microembolic signals administration of dual antiplatelet therapy (aspirin + clopidogrel) resulted in decreased risk of stroke recurrence (78). The Clopidogrel in High-risk patients with Acute Non-disabling Cerebrovascular Events (CHANCE) trial showed that the combined treatment of clopidogrel and aspirin for the first 21 days after TIA or stroke, followed by clopidogrel alone decreases the 90-day risk of stroke [hazard ratio, 0.68, 95% (CI, 0.57–
0.81), p<0.001], without increasing the risk of hemorrhage in comparison with aspirin alone (79).

In addition to antiplatelet therapy, statins have been found to be highly effective in stroke prevention among medically managed patients with CAV disease (71). In a meta-analysis the stabilizing effect of statin therapy on carotid plaque has been confirmed, and has been proposed to be related to the non-cholesterol-lowering, so-called pleiotropic effects of statins (80). Several studies have also demonstrated an independent benefit from statins on outcomes before carotid endarterectomy (81). A three-fold reduction in the rate of perioperative stroke, and five-fold reduction of perioperative mortality among 1,566 statin-treated patients undergoing carotid endarterectomy, has been shown (82). Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial has confirmed that in patients with recent stroke or TIA, 80 mg of atorvastatin daily reduced the overall incidence of strokes and of CAV events, despite a small increase in the incidence of hemorrhagic stroke (83).

**Interventional therapy**

It was not before 1950s when DeBakey and Estcot for the first time tried surgical intervention in the carotid stenosis for secondary stroke prevention. Later on, several large randomized studies demonstrated that CEA is associated with better clinical outcome after ischemic stroke. Indeed, the results of ECST and NASCET trials showed primarily that compared to medical treatment, CEA reduced the risk of stroke at 5 years of 15% among patients with a 70–99% and of 7.8% among those with a 50–69% stenosis. No benefit could be demonstrated in patients with 0-49% stenosis (17, 18). Also, surgery conferred no benefit in patients with “near-occlusion”, defined as a 90-95% stenosis when the distal internal carotid artery (ICA) lumen is collapsed, as shown on angiography (84). In a pooled analysis including ECST and NASCET trials data, a higher benefit from surgery in men has been reported (85). While CEA was clearly beneficial in women with >70% symptomatic carotid stenosis, this was not the case for those with 50–69% stenosis (85). Furthermore, women had a lower risk of ipsilateral ischemic stroke on medical treatment than men. This observation is possibly a reflection of the differences in the pathology of the atherosclerotic plaques, with men having unstable carotid plaques with
more pronounced features of inflammation compared to women (86). Because most of the recurrences occur early after stroke, it was confirmed that earlier interventions are associated with lower rate of adverse outcomes (87). In addition to CEA, carotid artery stenting (CAS) is another optional treatment for patients with carotid artery disease. Carotid Revascularization Endarterectomy Versus Stenting Trial (CREST) (88), showed that CEA and CAS can be performed with a procedural risk of <3% by carefully credentialed surgeons and interventionalists. However, it was demonstrated that CAS is associated with inferior outcomes compared with CEA for patients with symptomatic carotid disease (89). CAS was associated with higher rate of stroke, recurrent carotid stenosis and considerably higher cost compared with CEA (89). For the present, CAS is generally indicated for small proportion of symptomatic patients, those with: distal lesions where CEA is more difficult to perform; scarred or infected neck; recurrent stenosis following CEA; possibly contralateral internal carotid occlusion (90). However, improvements in CAS technical skill and technology (membrane covered stents, newer protection strategies) and better patient’s selection may alter this situation in the future.

One example is the use of the flow-reversal or flow-cessation embolic protection. A meta-analysis of 4747 patients in 24 CAS studies found a significant benefit from protected distal procedures with a relative risk reduction of 0.59 (95% CI, 0.47–0.73) (91). Another important strategy is the avoidance of CAS in patients with echolucent carotid disease or extensive calcification and in patients aged >70 who were shown to have worse results with CAS (92). Several studies have confirmed that echolucent plaques are associated with higher rates of emboli signals and in-stent restenosis compared to echogenic plaques. There are controversial data according to CAS interventions on echolucent plaques and recurrent symptoms. On the basis of this, we undertook a systematic search on PubMed for studies evaluating associations between carotid plaque echolucency and adverse outcomes (stroke, microembolization and in stent restenosis). Out of 412 studies found after the first medical databases search, we identified five studies appropriate to be qualitatively and quantitatively analyzed, which evaluated different adverse outcomes in patients with echolucent plaques after CAS. Pooled analysis showed that CAS in echolucent carotid plaques is associated with higher risk of stroke (OR 2.33; 95% CI 1.73-4.65, p=0.015), microembolization (OR 2.77; 95% CI 1.40-5.45, p=0.003), and in stent restenosis (OR 3.8; 95% CI 1.93-7.44, p<0.001).
In general, pooled OR of adverse outcomes for CAS performed in echolucent compared to echogenic plaques was 2.92 (95% CI 1.97-4.32), p<0.001 (92-96).

Identifying patients at high risk for stroke recurrence

An analysis of pooled data of randomized clinical trials showed that the risk of stroke recurrence is 21% at five years after symptoms (20). However, the risk of stroke in the early period after onset of symptoms seems to be much higher than it was previously thought, with contemporary natural history studies suggesting that within 72 hours of the presenting symptom 8-15% of patients with a 50-99% stenosis may suffer a recurrent stroke (97, 98). Accordingly, it would be of clinical benefit to identify this subgroup of patients at increased risk and to deliver interventions as soon as possible (emergency CEA) after onset of symptoms, accepting that benefit must always be balanced against the potential for perioperative stroke.

ABCD2 (99) and ABCD2-I scoring system (100) that integrate clinical and brain imaging data has been shown to have predictive value for symptom recurrences after TIA. In addition, there are several imaging carotid plaque features that could also assist in identifying high risk patients, more frequently associated with stroke recurrence including: stenosis severity, plaque irregularity and ulceration and plaque echolucency and the presence of contralateral occlusion. Even though, significant benefit of CEA procedures was determined for 50-99% stenosis degree, a meta-analysis including more than 6000 patients randomized in ECST, NASCET, and the Veteran’s Affairs (VA) Study, revealed that for 1000 CEAs intervention only 78 strokes would be prevented at 5 years in patients with 50-69% stenosis, compared with 156 strokes prevented at 5 years in patients with 70-99% stenosis (20). Plaque irregularity was associated with significantly higher rates of ipsilateral stroke in patients randomized to BMT, compared with smooth stenosis. In patients with smooth carotid plaques, CEA prevented about 100 strokes/1000 interventions at 5 years compared to 200 strokes/1000 CEAs at 5 years in patients with an irregular plaque producing 75% stenosis (20, 28). Salem et al. reported that the presence of plaques with GSM <25 were predictive of a significantly increased risk of recurrent neurological events in the early period, prior to CEA (101). Furthermore, the presence of
contralateral occlusion is associated with a significant increase in the risk of late stroke ipsilateral to the treated ICA stenosis in medically treated patients (85). In a recent systematic review, it has been found that MES were highly predictive of stroke recurrence in patients with carotid stenosis having an OR of 9.57; (95% CI, 1.54–59.38) (102).

**Figure 5. Proposed algorithm for the management of symptomatic patients with carotid stenosis; BMT - best medical treatment, DUS - Duplex ultrasound, CEA - carotid endarterectomy, CAS - carotid artery stenting.**

**Ultrasound imaging markers of vascular risk: subclinical atherosclerosis**

Many large studies have confirmed the predictive value of carotid intima-media thickness (IMT) for cardiovascular risk. On 2010 American Heart Association/American College of Cardiology guidelines designated carotid IMT a class IIa recommendation for
cardiovascular risk assessment in asymptomatic adults with intermediate risk of cardiovascular disease (103). However, a careful evaluation of carotid IMT studies recently together with quantitative assessment of their results revealed discrepancies in methods with which IMT was assessed and suggested that IMT measurement in common carotid artery (CCA) have only a minimal predictive power beyond traditional risk factors, with no clinical importance (104, 105). Carotid plaque assessment appears to be a better predictor of CAV and CV risk compared to IMT alone (106). In addition, quantitative assessment of plaques such as plaque thickness, echogenicity, area, and volume appears to be progressively more sensitive in predicting CV risk than assessment of plaque presence alone (106). Recently, a new measure of carotid artery wall - intima-media grey scale median (IM-GSM), that attempt to quantify the echogenicity of the intima-media complex seems to have a better predictive value compared to IMT (107).

*Carotid artery wall measurements: intima-media thickness and intima-media GSM*

Carotid IMT is measured between intima-lumen interfaces and media-adventitia interfaces of the arterial wall represented as a double-line density on carotid ultrasound image (figure 6). Its accuracy for the measurement of far wall IMT in common carotid artery was validated against histological specimens representing the true biological thickness of the vessel wall (108). Otherwise, near-all assessment of IMT was associated with systematic measurement error, because of the echogenicity of the adventitia layer, the boundary between media and adventitia in the near wall, is not easy to be defined (109). The differences of the IMT values between 25th and 75th percentile are very small (<1mm) and therefore, for the measurement of IMT a high precision is required, including, proper training of the sonographers and implementation of protocols for the measurement of IMT. In general, IMT measurement has been shown to have a good intra and interobserver correlation coefficient of >0.90. Furthermore, advances of US technology have made possible automatic measurement of IMT, obviating the need to perform manual measurements, and this have improved the reproducibility of the measurements (110). These systems for IMT measurements incorporate IMT datasets from large clinical studies that allow generation of carotid IMT percentile values for individual patients (111).
One of the reasons behind the limitation of IMT measurements was the different
definition between studies. Some have used a cut-off of ≥0.9 mm or ≥1 mm (112, 113),
others defined an abnormal IMT as >75\textsuperscript{th} percentile for age, ethnicity and sex, which is
also recommended by American Society of Echocardiography (114).

Salonen et al. were the first to demonstrate the association of carotid IMT with future
coronary events on 1991 (115). They showed that each 0.1 mm increment of IMT was
associated with 11\% increased risk of MI on follow up. Subsequently, there were several
other large clinical studies, including: ARIC (Atherosclerosis Risk in Communities)
study (113), the CAPS (Carotid Atherosclerosis Progression Study) (116) the CHS
(Cardiovascular Health Study) (117), the MDCS (Malmo Diet and Cancer Study) (118)
and the Rotterdam Study (119), all showed that carotid IMT can be used to assess
incident CV disease risk. However, studies that evaluated the predictability of the IMT
over and above traditional risk factors (Framingham risk score) have been in most cases
negative. In the Multi-Ethnic Study of Atherosclerosis (MESA) (120), IMT measurement
in the CCA did not predict either MI or ischemic stroke after adjusting for Framingham
risk score. Lau et al. showed that the IMT measurements have an area under the curve of 0.69 for predicting CV events compared to 0.66 for Framingham risk score (121). These data showed that IMT is not a highly predictive marker as it was thought, highlighting the need for finding another measure (marker) on the carotid artery wall that could be a better predictor of future risk. Measurement of echogenicity of the intima-media complex (IM-GSM) was recently introduced as a possible marker of subclinical atherosclerosis. This could probably differentiate between: adaptive intimal thickening (representing a hyper reactivity of the smooth muscle cells in the tunica media) from intimal xanthoma or pathological intimal thickening of the arterial wall (representing the accumulation of LDL particles and inflammatory cells into intima) (figure 1). An echolucent IM-complex (low IM-GSM) was associated with different risk factors of atherosclerosis and increased risk of vascular events and mortality rate (122).

It has been shown that adding plaque and IMT data improved risk prediction in intermediate risk patients but plaque presence in carotid artery is more effective than IMT in predicting future ischemic events (106). A meta-analysis showed that compared to IMT, carotid plaque had a 35% better predictive value of future ischemic events, after adjusting for Framingham risk factors. In the ARIC study the model that performed best for prediction of ischemic events, death and revascularization, included traditional risk factors plus IMT plus carotid plaque (113). For intermediate risk patients NRI was 21% and it was higher for women than man, 25.4% vs. 16.4% respectively. It was suggested that carotid plaques grow faster in their length than thickness, and based on this it seems that assessment of carotid plaque area is potentially a better indicator of disease burden. In multivariable linear regression models, traditional risk factors explained only 15-17% of the IMT compared to 52% of the carotid total plaque area. In addition, advancement of US techniques has made possible assessment of carotid plaque volume by 3D images. Early results showed plaque volume assessment is a more reliable and sensitive to changes than cross-sectional area, and could be a better marker to evaluate the effects of different treatments on the carotid plaque (123).
OBJECTIVES

Study I
To evaluate the status of the plaques located in the contralateral to symptomatic carotid artery and to compare those plaques with plaques located in the same patients ipsilateral side and also plaques located in asymptomatic carotid arteries.

Study II
To evaluate the accuracy of ultrasound in detecting carotid artery calcification as compared to cone beam CT and in addition to compares calcification presence and volume between different subgroup of patients and different risk factors.

Study III
To evaluate the ability of two different carotid arterial wall measures, IMT and IM-GSM in differentiation between patients with and without prior vascular events in different arterial systems and also between numbers of arterial systems affected by symptomatic atherosclerosis disease.

Study IV
To evaluate the association between carotid plaque echogenicity and future cerebrovascular ischemic events in asymptomatic and symptomatic patients, in a systematic review and meta-analysis study.
METHODS

Preoperative patients evaluation

In all new cases presented with ischemic stroke a brain CTA, MRA or rarely conventional angiography is performed and analyzed by experienced neuro-radiologists, either at the Radiology department in Umeå University hospital or it would have been performed at the referring hospital. Ultrasound examination is performed at the department of Clinical Physiology at the Umeå University Hospital by experienced vascular sonographers. All carotid scans are reported and also the images are illustrated as a graph describing the location and the degree of stenosis and plaque calcification. Ultrasound carotid imaging follows the NASCET-type protocol for assessing carotid stenosis. All patients undergo a detailed neurological examination by a neurologist, who determines if the symptoms are related to the diseased circulatory area in the brain. Also, an echocardiogram is performed in these patients. Finally, the decision for CEA is undertaken after discussion among the neurologist, internal medicine specialist and vascular surgeon. For more complicated cases, there are meetings regularly organized, “carotid rounds”, where cases are presented and discussed in detail by a group of multi-disciplinary specialists (internal medicine, radiologists and vascular surgeons), and after careful evaluation of risk and benefit the decision for BMT or invasive strategy (CEA vs. CAS) is made.

Definitions and clinical data of patients included in Study I, II and III

Symptomatic patients with carotid stenosis were defined as those who had an ipsilateral ischemic CV event (stroke, TIA or retinal artery embolization) within the 6 months prior to the carotid scan, whereas asymptomatic patients were those with carotid atherosclerosis but with no CV event within the same period (6 months).

Asymptomatic patients were identified because of a) objective or subjective bruit during auscultation, b) a suspicion of CV symptoms which was later confirmed to be not of CV
origin, c) follow-up of a known carotid stenosis, d) previous known CV symptoms > 6 months before, e) detection of calcification in the territory of carotid artery by panorama imaging, f) CV symptoms related to posterior circulation, or g) carotid disease detection during evaluation of the thyroid gland.

Patients clinical data were prospectively analyzed to establish; a) the number and duration of ischemic events; b) time of last symptom; c) risk factors (smoking, diabetes, hypertension and dyslipidemia) and d) the surgical information. Data for coronary artery disease, ongoing condition or prior events, were also collected. Routine biochemical data were collected from the patient's clinical notes including lipid profile and glycated hemoglobin (HbA1c).

**Carotid ultrasound examinations**

All patients included in Study I, II and III underwent preoperative carotid Doppler ultrasound examinations, using a Siemens Acuson Sequoia 512® system with an 8L5 linear transducer. Using conventional Doppler ultrasound criteria the degree of carotid stenosis was assessed (124).

**Ultrasound data retrieval**

Studies included in this thesis are retrospective analysis of the patients who were engaged into two prospective studies:

1) Panorama arm of the SPACE study (15),

2) Additional Neurological SYmptoms before Surgery of the Carotid Arteries — a Prospective study (ANSYSCAP) (125).

In the study I and II we used data of the Panorama arm of the SPACE study and in study III we used only a subgroup of asymptomatic patients of the ANSYSCAP study. Over the course of these studies, the ultrasound imaging storage process was upgraded from analog to digital.
In study I and III, we aimed to obtain a detailed carotid plaque features analysis, we decided not to combine in the same study data stored in digital and those stored in analog. So in study I, we included only carotid ultrasound images stored in analog and for study III we used only images of asymptomatic patients stored in digital. Because in study II our intention was to only determine carotid plaque calcification (presence/absence), and not undergoing detailed plaque analysis we decided to use both, data stored in analog as well as those in digital.

**Extraction of the carotid ultrasound examinations**

The ultrasound images stored in analog or digital system were exported to the EchoPac software (General Electric, EchoPac version 8.0.1, Waukesha, WI) and the IMT was measured and the morphological features (plaque irregularities and calcification) were assessed. We performed imaging normalization and measurements of the plaque and intima-media complex features by using an in-house custom developed research software package (Department of Biomedical Engineering — R&D, Umeå University Hospital, Umeå, Sweden).

**Definitions of ultrasound derived plaque and intima-media complex features**

Plaque definition was taken as a focal protrusion that proved to be more than 50% the thickness of the adjacent wall thickness (126). Each plaque was analyzed for plaque type, calcification, irregularity, GSM, JBA, GSM of the JBA, DWAs, coarseness and entropy. In the arteries with only one plaque, the feature of the latter was taken to represent the artery. Arteries with more than one plaque were treated in a way that the congregated information/measurements from all plaques represented that artery. When it came to irregularity measurements, the worst abnormality of irregularity in all plaques was taken to represent the artery. The presence of either calcification, JBA and DWA was marked in all studied arteries too. An area-weighted average was taken for continuous features (GSM, area of JBA and entropy) and used in relation to the size of the artery. The overall plaque feature was assessed as an averaged sum of individual features multiplied by plaque area and divided by the total plaque area.
**Plaque irregularity (Study I and III):** The surface plaque irregularities were categorized into three groups: (1) smooth, (2) mildly irregular (height variations 0.4 mm along the contour of the lesion), or (3) markedly irregular (height variations >0.4 mm) (127).

**Grey scale median (GSM) (Study I and III):** It was measured as the median of the grey values of all pixels within cropped plaque image after image normalization (figure 7).

**Juxtaluminal black-area (JBA) Study (III):** These black areas close to the lumen without visible echogenic cap represent the JBA (38). The area was manually detected and outlined, which together with the corresponding GSM were calculated by the software. The larger value was used in cases with several JBAs within a plaque.

**Plaque type (Study I and III):** According to the modified Geroulakos classification, after image normalization and plaque delineation, plaques were classified by the software into the four types (128) (Fig. 3):
Type 1, uniformly echolucent (black), <15% of pixels in the plaque area with values >25;
Type 2, mainly echolucent, pixels with grey scale values >25 occupying 15-50% of the plaque area;
Type 3, mainly echogenic, pixels with grey scale values >25 which occupy 50-85% of the plaque area; and
Type 4, uniformly echogenic, pixels with grey scale values >25 which occupy >85% of the plaque area.

Calcifications (Study I and II): Were defined as hyperechogenic spots that produced posterior shadowing.

**Discrete white areas (DWAs):** Discrete white areas, when present, were defined as those without posterior shadowing but with pixels of grey scale values >124, colored red in the grey scale-based stratified color mapping of the plaque types 1 to 3 (129).
Entropy (Study I and III): Entropy was taken as a measure of the random nature of the grey-tone values within the plaque (130), with low values for homogenous tissue and high values for heterogeneous composition.

Coarseness (Study III): Coarseness quantifies the granularity of the plaque texture, which is consistent with high degree of local uniformity in intensity for large areas. Coarseness was calculated using the neighborhood grey-tone difference matrix method (131). It has already been demonstrated that coarseness discriminates symptomatic from asymptomatic plaques with low values in symptomatic plaques consistent with heterogenic, more granular texture and less uniform composition (132).

![Figure 7. Image normalization and plaque features assessment](image)

Intima–media complex measurements (Study III)

Both IMT and IM-GSM were measured in the distal segment of the common carotid artery within 1 cm (± 2 mm) distance starting from the carotid bifurcation at the right and left carotid systems. When a plaque was present in this region, the IM-complex free-of-
plaque was selected, and if it was <8 mm long the artery was excluded from the study (n = 8).

**Carotid-intima media thickness (IMT)**

IMT was conventionally defined as the distance between the media–adventitia interface and the lumen–intima interface. One-centimeter long segments of the far wall were measured and an average of three cycles was taken (133).

**Intima–media grey scale median (IM-GSM)**

The median of the grey values of all pixels within a one-centimeter length cropped intima media complex image was measured to reflect IM-GSM. The distal intima-media complex in the normalized and standardized image was manually outlined, prior to feature calculation.

**Cone Beam CT (CBCT) analysis**

Excised plaques after CEA were examined by CBCT (Cone Beam Computed Tomography, 3D Accuitomo 170, J Morita MFG Corporation, Kyoto, Japan; 60 kV, 1 mA, 360°). Volumes of 4 × 4 or 6 × 6 cm were used and they had a resolution of 0.08–0.125 mm voxel size. The reconstructions were made with 0.5 mm slice thickness and 0.5 mm increment. A software program (General Electric Company, Barrington, IL, USA, advantage workstation 4.3, Volume Viewer 2) for volume measurements was also used. All calcification spots were measured in mm³, with resolution of the volume reconstructions of 1 mm³. Plaques with multiple dispersed calcifications had the largest calcification nodule volume used to represent the intra-plaque calcification.

**Carotid endarterectomy (CEA) procedure**

All patients were operated under general anesthesia. Common, internal and external carotid arteries were exposed and a longitudinal incision was made in the common carotid artery and then extended into the internal branch until a healthy area distal to the plaque. The intimal thickening of the common carotid artery was divided and the plaque was gently dissected from the artery, leaving the adventitial layer intact with careful
handling of the plaque. After excision, the plaque was immediately placed in a plastic tube and stored in −20 °C, then transferred to a −80 °C freezer until later taken out for CBCT analysis.

**Source of data**

Study I and II

This is a secondary analysis of the Ultrasound of the Panorama arm of the SPACE study [15]. In the main study, 100 consecutive patients with symptomatic or asymptomatic carotid stenosis (≥50%) who were eligible for CEA were included. All patients included agreed to extensive preoperative evaluation and underwent CEA.

Study III

This is a secondary analysis of the ANSYSCAP study (125). In the main study consecutive symptomatic and asymptomatic patients with 50-99% stenosis were included between August 2007 and December 2009. In ANSYSCAP, consecutive patients with a 50-99% carotid stenosis eligible for CEA were prospectively included between August 2007 and December 2009.

Study IV

For the systematic review and meta-analysis we have systematically searched electronic databases (PubMed, MEDLINE, EMBASE and Cochrane Center Register) up to January 2015 for studies evaluating the effect of carotid plaque echogenicity on CV symptoms. The search terms used were: “carotid atherosclerosis”, “carotid plaque”, “echogenicity” “ultrasound” “symptoms”, “Stroke”, “TIA” “amaurosis fugax” in various combinations. Two researchers (FJ and PI) independently performed the literature search, study selection and data extraction. There was no time or publication limit in the literature search. The selected reports were manually searched, and relevant publications, obtained from the reference lists, were retrieved.
Inclusion/exclusion criteria

Study I
Out of 47 cases with a preoperative ultrasound examination stored in the analog format we analyzed 39. Eight patients were excluded because either the examinations could not be retrieved and analyzed (n = 7) or the patient had an asymptomatic carotid stenosis but a recent (<6 months) CV event on the contralateral side. Of the 39 patients included, 33 had symptomatic carotid stenosis (50-99%) and 6 had unilateral or bilateral asymptomatic stenosis (50-99%). All patients had at least one atherosclerotic plaque on both sides. We further excluded 12 arteries because of total occlusion (n=2) or because of >50% of the plaque covered by shadow derived from extensive plaque calcification (n=10). So, in total we analyzed 66 arteries (25 contralateral to symptomatic, 30 symptomatic and 11 asymptomatic). The contralateral arteries were compared with the symptomatic and asymptomatic arteries.

Study II
Out of 101 carotid plaques (of 94 patients) we finally analyzed 94 plaques (from 88 patients). Seven patients were excluded, five because of suboptimal image quality and two because there was no calcification in the internal carotid artery (ICA) plaque but a distinct calcification in the external carotid artery (ECA). Since it was unclear whether the ECA would be included in the extirpated plaque, it was impossible to make a reasonable assessment without breaking the blinding.

Study III
Out of 87 asymptomatic patients stored in the digital system we finally analyzed 166 arteries. Seven arteries were excluded because the common carotid artery segment free of plaque was <0.8mm.
Figure 8. Flowchart of the patients selections for study I, II and III

Study IV
Studies reporting results on US assessment of plaque echogenicity and their association with CV symptoms (Stroke, TIA, amaurosis fugax) were included in our analysis. Specific inclusion criteria for the systematic review were: (1) Cohort prospective studies; (2) English language articles; (3) studies with ≥30 subjects, and (4) ultrasound based characterization of carotid artery plaque, visually based on Gray-Weale, or using computer-assisted analysis (Geroulakos classification or GSM). Studies that visually categorized plaques in only two groups echogenic/echolucent, that evaluated plaque heterogeneity, that used imaging techniques other than US (e.g. MRI, CT, IVUS, PET), and those that analyzed plaque features other than echogenicity (volume, degree of
stenosis, plaque irregularities, ulceration, neovascularization) as predictors of CV symptoms were excluded (figure 9).

Figure 9. Flowchart of the studies selection for the study IV

Statistical analysis

**Study I, II and III.** All statistical analyses were performed using IBM SPSS Statistics 22. Categorical variables were expressed as percentages and continuous variables as mean ± SD (median). Mean values of the plaque texture features were compared between groups, using independent samples t-test and Man-Whitney test. Baseline differences between groups for each plaque texture feature were tested using one-way ANOVA, for more than two groups in analysis. Post hoc analysis (Bonferroni) was also performed for
continuous variables. If variables did not have normal distribution, the non-parametric Kruskal-Wallis test was used. Fisher's exact probability test or the Chi-2-test was used when comparing two sets of binary or categorical values, with a pre-selected significance level of p < 0.05.

Quartile analyses were used for categorizing calcification volume into four groups. Kappa values were calculated to determine the accuracy of US in detecting calcification. Logistic regression analysis was used to determine the association of an echolucent intima-media complex with multi-system atherosclerosis disease.

Study IV.

We have separately analyzed studies that evaluated asymptomatic and symptomatic patients. $I^2$ statistic was used to measure the heterogeneity. When there was a significant variation between studies size, length and follow-up, as well as patient’s characteristics, we used random-effect. Publication bias was examined using Begg–Mazumdar test. All analyses were conducted using Comprehensive Meta Analysis version 3 software (Biostat inc., Englewood, NJ, USA).
RESULTS

Study I

Clinical data

The severity of carotid artery stenosis and other risk factors are presented in figure 10 and 11. There was no difference between asymptomatic and symptomatic patients with carotid stenosis.

Figure 10. Clinical data in symptomatic and asymptomatic patients. BP-blood pressure, SD-standard deviation
Figure 11. Distribution of the different risk factors, previous or ongoing ischemic events and therapy in symptomatic and asymptomatic patients

Degree of stenosis

Based on the carotid Doppler velocities there was no difference in the degree of stenosis between contralateral and asymptomatic arteries. In symptomatic arteries the degree of stenosis was higher compared to contralateral (p<0.001) and asymptomatic arteries (p=0.006).

Contralateral vs. Symptomatic

The GSM and JBA in the plaques located in the contralateral to symptomatic side had similar features to those in the symptomatic side (26.2 ± 7.3 vs. 24.9 ± 7.8, p = 0.536) (figure 12) and (5.0 ± 3.9 vs. 4.6 ± 3.0, p = 0.80), respectively. Also, the plaque irregularities and PT were not different between these two groups. The only plaque feature that differentiated between symptomatic and contralateral arteries was the prevalence of JBA, which was higher in symptomatic arteries (p=0.001).
Contralateral vs. Asymptomatic

Contralateral arteries had more vulnerable morphological and textural plaque features compared to those in the asymptomatic arteries, with lower GSM (26.2 ± 7.3 vs. 49.4 ± 14.6, p < 0.001), lower GSM of the JBA (5.0 ± 3.9 vs. 11.4 ± 2.1, p = 0.001), higher prevalence of plaque type 1 and 2 and lower prevalence of type 3 and 4 (p = 0.001) and higher prevalence of mild (60% vs. 36%) and marked irregularities (28% vs. 9%), p = 0.03, (Figure 12).

![Figure 12. Echogenicity (left) and surface plaque morphology (right) between asymptomatic, contralateral to symptomatic and symptomatic arteries](image)

Reproducibility analyses

Interobserver variability was expressed using intra-class correlation coefficient. For GSM it was 0.928 (95% CI, 0.899-0.950) and for JBA it was 0.972 (95% CI, 0.848-0.965). The interobserver agreement for JBA was (K=0.948, p<0.0001) and for PT was (0.843, p<0.001).
Study II

Clinical data

Mean age of the patients included in this study was 70 ± 7 years and 33% were females. Symptomatic carotid artery stenosis was observed in 73 patients and asymptomatic carotid stenosis in the remaining 15 patients. Six of the symptomatic patients underwent bilateral carotid endarterectomy (CEA). A stenosis severity of 50%–69% and 70-99% was found in 6 and 85 cases, respectively, and near-occlusion in three arteries. Patient’s baseline characteristics are presented in Table 1.

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Study population (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean±SD</td>
<td>70±7</td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>29 (33)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg), mean±SD</td>
<td>147±22.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg), mean±SD</td>
<td>78±12</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l), mean±SD</td>
<td>4.61±1.03</td>
</tr>
<tr>
<td>LDL (mmol/l), mean±SD</td>
<td>2.60±0.92</td>
</tr>
<tr>
<td>HDL (mmol/l), mean±SD</td>
<td>1.27±0.48</td>
</tr>
<tr>
<td>Creatinine (μmol/l), mean±SD</td>
<td>84±25</td>
</tr>
<tr>
<td>HBA1c (mmol/mol), mean±SD</td>
<td>52.5±12.7</td>
</tr>
<tr>
<td>Symptomatic carotid stenosis, n (%)</td>
<td>73 (83)</td>
</tr>
<tr>
<td>Prior myocardial infarction, n (%)</td>
<td>14 (16)</td>
</tr>
<tr>
<td>Current angina pectoris, n (%)</td>
<td>7 (8)</td>
</tr>
<tr>
<td>Heart failure, n (%)</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Previous stroke (&gt;6 months to the present evaluation), n (%)</td>
<td>14 (16)</td>
</tr>
<tr>
<td>Claudication (lower extremity artery disease), n (%)</td>
<td>10 (11.4)</td>
</tr>
<tr>
<td>Any previous revascularization for ischemia, n (%)</td>
<td>24 (27.3)</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>9 (10.2)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>29 (33)</td>
</tr>
<tr>
<td>Lipid lowering medicine, n (%)</td>
<td>82 (93.2)</td>
</tr>
<tr>
<td>Platelet inhibiting or anticoagulation medicine, n (%)</td>
<td>88 (100)</td>
</tr>
<tr>
<td>Blood pressure reducing medicine, n (%)</td>
<td>84 (95.5)</td>
</tr>
</tbody>
</table>

Table 1. Patients’ data
Ultrasound vs. CBCT

Carotid artery calcification was detected in 87.2% of cases in the preoperative ultrasound examinations and in 98.9% in CBCT examinations after CEA. There was no difference between the 50%–69% and the 70%–99% degree of stenosis groups (Table 2). Furthermore, there was no difference in carotid calcification between different risk factors (Table 2). In general, the sensitivity of carotid ultrasound in detecting the presence of calcification was 88.2%. The calcification volumes acquired by CBCT were divided into 4 groups using quartile analysis; <8, 8–35, 36–70 and >70 mm$^3$ (Figure 13). Calcification volumes $\geq$8 mm$^3$ were accurately detectable by ultrasound with a higher sensitivity of 96% compared to the sensitivity of 62% for the calcification volume of <8 mm$^3$ (Table 2, Figure 13).

<table>
<thead>
<tr>
<th>CBCT/Ultrasound</th>
<th>Total arteries, n</th>
<th>Calcification on CBCT</th>
<th>Calcification on US</th>
<th>US Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcification</td>
<td>94</td>
<td>93</td>
<td>82</td>
<td>88.20%</td>
</tr>
<tr>
<td>Calcification $\geq$8 mm$^3$</td>
<td>72</td>
<td>72</td>
<td>69</td>
<td>96%</td>
</tr>
<tr>
<td>Calcification $&lt;$8 mm$^3$</td>
<td>22</td>
<td>21</td>
<td>13</td>
<td>62%</td>
</tr>
</tbody>
</table>

Table 2. Accuracy of Doppler ultrasound in detecting carotid calcification
Calcification vs. Symptoms

The presence of calcification, evaluated by US was not different between symptomatic and asymptomatic patients. In addition, we explored whether dispersed calcification within the plaque could be associated with patient’s symptoms, but we didn’t find any difference between groups. Even using quartiles of calcification volumes evaluated by CBCT couldn’t discriminate between symptomatic and asymptomatic patients. In table 3 we have compared calcification volume (< and ≥ than 8 mm) between different risk factors.

Reproducibility analyses

The inter-observer agreement for the presence of calcification in the carotid artery plaques evaluated by US was good (K=0.905, p<0.001).
<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Arteries n</th>
<th>&lt;8mm³ calcification volumes on CBCT, n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;75</td>
<td>68</td>
<td>18 (26.5)</td>
<td>0.21</td>
</tr>
<tr>
<td>≥75</td>
<td>26</td>
<td>4 (15.3)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
<td>7 (23.3)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>64</td>
<td>15 (23.4)</td>
<td>0.99</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>79</td>
<td>20 (25.3)</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>15</td>
<td>2 (13.3)</td>
<td>0.31</td>
</tr>
<tr>
<td>Current smoker</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10</td>
<td>4 (40.0)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>84</td>
<td>18 (21.4)</td>
<td>0.19</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>31</td>
<td>8 (25.8)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>63</td>
<td>14 (22.2)</td>
<td>0.60</td>
</tr>
<tr>
<td>Previous stroke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>2 (11.8)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>77</td>
<td>20 (26.0)</td>
<td>0.21</td>
</tr>
<tr>
<td>Previous MI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15</td>
<td>3 (20.0)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>79</td>
<td>19 (24.0)</td>
<td>0.73</td>
</tr>
<tr>
<td>Statin therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>87</td>
<td>21 (24.1)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>7</td>
<td>1 (14.3)</td>
<td>0.55</td>
</tr>
<tr>
<td>Degree of stenosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-69%</td>
<td>6</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>70-99%</td>
<td>85</td>
<td>20 (23.5)</td>
<td></td>
</tr>
<tr>
<td>near-occlusion</td>
<td>3</td>
<td>2 (66.7)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Table 3. Subgroup analysis
**Study III**

*Clinical data*

In this study we included 87 asymptomatic patients (166 carotid arteries). Mean age of the patients was 69±6 year and 34.5% were females. 23% of patients had previous MI, 16% had angina, 21% had previous ischemic stroke, and 22% (n=19) had lower limb atherosclerosis in the form of intermittent claudication (Table 4). 50% (n=43) of the patients were asymptomatic, 34% (n=30) had previous disease in one arterial system and 16% (n=14) had previous disease in multi-arterial systems.

<table>
<thead>
<tr>
<th>Age, years, mean±SD</th>
<th>69±6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>30 (34.5)</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>18 (20.7)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>25 (28.7)</td>
</tr>
<tr>
<td>Previous MI, n (%)</td>
<td>20 (23)</td>
</tr>
<tr>
<td>Current angina, n (%)</td>
<td>14 (16.1)</td>
</tr>
<tr>
<td>Previous stroke, n (%)</td>
<td>18 (20.7)</td>
</tr>
<tr>
<td>Intermittent claudication, n (%)</td>
<td>19 (21.8)</td>
</tr>
<tr>
<td>SBP (mmHg), mean±SD</td>
<td>145.7±20</td>
</tr>
<tr>
<td>DBP (mmHg), mean±SD</td>
<td>77±12</td>
</tr>
<tr>
<td>Anti-platelet or anti-coagulation therapy, n (%)</td>
<td>84 (96.6)</td>
</tr>
<tr>
<td>Blood pressure lowering therapy, n (%)</td>
<td>80 (92)</td>
</tr>
<tr>
<td>Lipid lowering therapy, n (%)</td>
<td>78 (89.7)</td>
</tr>
<tr>
<td>HbA1c (%), mean±SD</td>
<td>5.2±1.1</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l), mean±SD</td>
<td>4.67±0.9</td>
</tr>
<tr>
<td>LDL (mmol/l), mean±SD</td>
<td>2.57±0.9</td>
</tr>
<tr>
<td>HDL (mmol/l), mean±SD</td>
<td>1.34±0.4</td>
</tr>
</tbody>
</table>

*Table 4. Patients’ data*

*Intima-media measurements vs. previous vascular symptoms*

In patients with previous MI the IMT was higher (1.06±0.2 vs. 0.95±0.2 mm, p=0.034) and IM-GSM was lower (21±15 vs. 33±16, p=<0.001). Also, patients with previous stroke had lower IM-GSM (24±12 vs. 33±16, p=0.007) but IMT was not different between those with and without stroke (1.03±02 vs. 0.96±0.2 mm, p=0.195). IMT and
IM-GSM were not different in patients with and without previous atherosclerosis disease in the lower extremity (Figure 14).

Figure 14. IM-GSM in patients with previous stroke, MI angina and claudication intermittent

**IMT and IM-GSM vs. multi-system atherosclerosis disease**

IM-GSM was significantly different between groups, it decreased significantly with increasing number of arterial systems affected by symptomatic atherosclerosis disease, 37.7±15.4 vs. 29.3±16.4 vs. 20.7±12.9, p<0.001, for asymptomatic, symptoms in one and in multi-arterial system disease, respectively. When analyzing IM-GSM measured in the artery-side with higher IMT the results were similar (Table 5). IMT was not significantly different between groups, 0.95±0.2mm vs. 0.98±0.2mm vs. 1.02±0.02mm, p=0.49 (Table 5, Figure 15).
<table>
<thead>
<tr>
<th></th>
<th>Asymptomatic (n=83)</th>
<th>Previous symptoms in one arterial system (n=58)</th>
<th>Previous symptoms in &gt; one arterial system (n=25)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IM-GSM, mean±SD*</td>
<td>37.7±15.4</td>
<td>29.3±16.4</td>
<td>20.7±12.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IM-GSM, mean±SD</td>
<td>35.0±15.7</td>
<td>29.5±18.6</td>
<td>17.4±13.9</td>
<td>0.001</td>
</tr>
<tr>
<td>IMT, mean±SD</td>
<td>0.95±0.2</td>
<td>0.98±0.2</td>
<td>1.02±0.2</td>
<td>0.49</td>
</tr>
<tr>
<td>Plaque GSM, mean±SD</td>
<td>41.4±17.0</td>
<td>33.0±16.0</td>
<td>20.9±10.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plaque Coarseness, mean±SD</td>
<td>13.7±4.4</td>
<td>11.4±5.1</td>
<td>7.6±3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plaque Entropy, mean±SD</td>
<td>3.5±0.2</td>
<td>3.6±0.2</td>
<td>3.6±0.2</td>
<td>0.13</td>
</tr>
<tr>
<td>Plaque JBA, n (%)</td>
<td>14 (19.4)</td>
<td>14 (28.6)</td>
<td>13 (72.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plaque GSM of JBA</td>
<td>6.8±5.0</td>
<td>12.1±9.0</td>
<td>3.8±3.3</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Table 5.** Common carotid wall and bifurcation plaque measurements in different groups.

(*IM-GSM mean of both sides. (†) IM-GSM measured on the side with higher IMT

**Figure 15.** IM-GSM, plaque GSM and plaque coarseness were significantly decreased with increasing number of arterial systems affected by atherosclerosis
Using ROC curve analysis we determined IM-GSM of 25 as the best cut-off value to differentiate between numbers of arterial systems affected by the atherosclerosis disease. Out of 25 arteries in the multi-system group, 16 (64%) had an IM-GSM <25, having an OR of 3.23 (95%CI 1.33-7.85), p<0.01. Even after adjusting for IMT this association remained significant with OR 2.65 (95%CI 1.56-6.67), p=0.03.

Reproducibility analyses

For intima-media complex and plaque measurements there was a good agreement between the two observers. The interobserver variability for IMT measurements expressed by intra-class correlation coefficient was 0.977 (95% CI, 0.963-0.988) and for IM-GSM it was 0.934 (95% CI, 0.783-980). For plaque measurements intraclass correlation was 0.921 (95% CI, 0.890-0.942) and for JBA it was 0.910 (95% CI, 0.871-0.960). In addition we performed Bland-Altman test for the intima-media complex measurements and there was not any proportional bias for IMT and IM-GSM measurements, mean difference between the two observers measurements was not significantly different from zero.

Study IV (Systematic review and meta-analysis)

Study selection

After the first searching of online medical databases we identified 1385 studies. In addition ten studies were identified through related citations. After excluding duplicates, 1387 studies were screened (table 6). By reading the abstracts of the selected papers, we first depicted 30 potentially eligible articles for further review, of these only 10 (101, 134-142) met the inclusion criteria for qualitative analysis. One of the studies (137) had analyzed separately symptomatic and asymptomatic patients and we included both groups in the meta-analysis. The remaining 20 studies were excluded (figure 9).
Qualitative assessment and study characteristics

There were considerable differences between reports in the degree of stenosis between asymptomatic studies included in this analysis with 3 studies that have included patients with mild to severe carotid stenosis (30-99%), 3 studies with moderate to severe stenosis (50-99%) and 2 studies with severe carotid stenosis (≥70%) stenosis.

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Number of subjects</th>
<th>No. of arteries analyzed</th>
<th>Degree of carotid stenosis</th>
<th>Echolucency definition</th>
<th>Mean follow-up (months)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Polak et al. 1998 (134)</td>
<td>4886</td>
<td>N/A</td>
<td>30-99%</td>
<td>PT 1 and 2</td>
<td>39.6</td>
<td>Stroke, TIA</td>
</tr>
<tr>
<td>2. Mathiesen et al. 2001 (135)</td>
<td>223</td>
<td>N/A</td>
<td>35-99%</td>
<td>PT 1 and 2</td>
<td>36</td>
<td>Stroke, TIA, AF</td>
</tr>
<tr>
<td>3. Liapis et al. 2001 (136)</td>
<td>332</td>
<td>442</td>
<td>30-95%</td>
<td>PT 1 and 2</td>
<td>44</td>
<td>Stroke, TIA, AF</td>
</tr>
<tr>
<td>4a. Grønholdt et al. 2001 (137)</td>
<td>111</td>
<td>111</td>
<td>50-99%</td>
<td>GSM&lt;75</td>
<td>52.8</td>
<td>Stroke</td>
</tr>
<tr>
<td>4b. Grønholdt et al. 2001</td>
<td>135</td>
<td>135</td>
<td>50-99%</td>
<td>GSM&lt;75</td>
<td>52.8</td>
<td>Recurrent symptoms</td>
</tr>
<tr>
<td>5. Nicolaides et al. 2005 (138)</td>
<td>1115</td>
<td>N/A</td>
<td>50-99%</td>
<td>PT 1, 2, 3</td>
<td>37.1</td>
<td>Stroke, TIA, AF</td>
</tr>
<tr>
<td>7. Topakian et al. 2011 (140)</td>
<td>435</td>
<td>N/A</td>
<td>70-99%</td>
<td>PT 1 and 2</td>
<td>24</td>
<td>Stroke, TIA</td>
</tr>
<tr>
<td>8. Salem et al. 2012 (101)</td>
<td>158</td>
<td>N/A</td>
<td>50-99%</td>
<td>PT 1 and 2</td>
<td>2 weeks</td>
<td>Recurrent symptoms</td>
</tr>
<tr>
<td>9. Silvestrini et al. 2013 (141)</td>
<td>621</td>
<td>N/A</td>
<td>70-99%</td>
<td>PT 1 and 2</td>
<td>22</td>
<td>Stroke TIA</td>
</tr>
<tr>
<td>10. Singh et al. 2013 (142)</td>
<td>206</td>
<td>N/A</td>
<td>30-99%</td>
<td>PT 1 and 2</td>
<td>6</td>
<td>Recurrent symptoms</td>
</tr>
</tbody>
</table>

Table 6. Studies included in the qualitative and qualitative analysis. GSM - grey scale median, AF - amaurosis fugax, TIA - transient ischemic attack, PT - plaque type based on Gray-Weale or Geroulakos classification

Four studies have visually (qualitatively) evaluated plaque echogenicity and four others performed computer-assisted (quantitative) analysis of the plaque echogenicity. Two studies defined plaque echolucency based on a cut-off value of GSM (<69 and <75) and six studies defined plaque types (PT) 1 and 2 as echolucent and PT 3 and 4 as echogenic. All studies have used longitudinal section US images for carotid echogenicity evaluation. Patient’s clinical outcome was defined as stroke, TIA or amaurosis fugax (AF) in 8 studies and as recurrent CV accidents in 3 studies (Table 6).

Seven studies showed a higher risk of ipsilateral CV accidents in asymptomatic patients with echolucent carotid plaques. Two studies showed higher risk of recurrent CV
accidents and one study showed that echolucent plaques could predict future events in symptomatic but not asymptomatic patients.

Although, we observed a difference in the follow-up periods between different symptomatic studies included in the analysis, the association between plaque echolucency and recurrent ischemic events was determined even at only 2 weeks after symptoms initiation. The degree of stenosis among symptomatic patients studies ranged from 30-90%.

**Meta-analysis results**

We have meta-analyzed 8 studies with 7937 asymptomatic patients and 3 studies with 499 symptomatic patients. Among studies that met the set criteria for inclusion in the meta-analysis, one has reported separately data for symptomatic and asymptomatic patients and we included both of them in the analysis separately. Echolucent carotid plaques predicted future CV accidents in asymptomatic patients with pooled relative risk (RR) of 2.72 (95% CI, 1.86 to 3.96), p<0.001 (figure 16), and also recurrent symptoms in symptomatic patients with pooled RR of 2.97 (95% CI, 1.85-4.78), p<0.001 (figure 17).

<table>
<thead>
<tr>
<th>Study name</th>
<th>Risk ratio</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Z-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polak et al. 1998</td>
<td>2.780</td>
<td>1.359</td>
<td>5.686</td>
<td>2.800</td>
<td>0.005</td>
</tr>
<tr>
<td>Liapis et al. 2001</td>
<td>1.900</td>
<td>1.080</td>
<td>3.341</td>
<td>2.229</td>
<td>0.026</td>
</tr>
<tr>
<td>Mathiesen et al. 2001</td>
<td>2.380</td>
<td>1.181</td>
<td>4.795</td>
<td>2.426</td>
<td>0.015</td>
</tr>
<tr>
<td>Gronholdt et al 2001</td>
<td>1.010</td>
<td>0.430</td>
<td>2.371</td>
<td>0.023</td>
<td>0.982</td>
</tr>
<tr>
<td>Nicolaides et al. 2005</td>
<td>4.800</td>
<td>2.256</td>
<td>10.215</td>
<td>4.071</td>
<td>0.000</td>
</tr>
<tr>
<td>Hashimoto et al 2009</td>
<td>4.400</td>
<td>1.182</td>
<td>16.383</td>
<td>2.209</td>
<td>0.027</td>
</tr>
<tr>
<td>Topakian et al 2011</td>
<td>6.430</td>
<td>1.352</td>
<td>30.573</td>
<td>2.339</td>
<td>0.019</td>
</tr>
<tr>
<td>Silvestrini et al 2013</td>
<td>4.150</td>
<td>1.764</td>
<td>9.763</td>
<td>3.261</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>2.720</td>
<td>1.864</td>
<td>3.968</td>
<td>5.191</td>
<td>0.000</td>
</tr>
</tbody>
</table>

![Figure 16. Forest plot of the studies evaluating the association between echolucent plaques and future CV accidents in asymptomatic patients. \(I^2=39\)](image)
Figure 17. Forest plot of the studies evaluating the association between echolucent plaques and recurrent CV accidents in symptomatic patients. $I^2=0$, Kendall’s Tau=0.66, $p=0.29$

Subgroup analysis

Based on the degree of carotid artery stenosis (mild-to-severe vs. moderate-to-severe vs. severe), the time period during which patients were recruited in the studies and US imaging was performed (before 2000 vs. since 2000) definition of the echolucent plaque (GSM cut-off vs. PT 1 and 2), and the methods used for assessment of carotid plaque types (visually vs. computer assisted), we have performed some comparison analyses between subgroup (table 7).

There was clear association between echolucent plaques and future CV symptoms for all degrees of stenosis categories, however, the highest RR was observed among studies with severe stenosis 4.72 (95% CI, 1.86-12.00), $p<0.001$ (figure 18). Also, studies that included US data collected after 2000 showed higher RR 4.65 (95% CI, 2.84-7.61) than those before the year 2000, RR 1.97 (95% CI, 1.40-2.78) (figure 19). In addition, analyzing separately only studies that have used a GSM cut-off to define carotid plaque echolucency the prediction of future CV events was not significant ($p=0.23$), whereas RR of the studies that defined echolucency as PT 1 and 2 was 3.01 (95% CI, 2.02-4.50), $p<0.001$ and in cases when the PT was defined using computer-assisted Geroulakos classification RR was even higher 3.56 (95% CI, 2.08-8.08), $p<0.001$ (table 7)
Figure 18. Subgroup analysis between different carotid stenosis

<table>
<thead>
<tr>
<th>Study name</th>
<th>Stenosis</th>
<th>Risk ratio</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Z-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polak et al. 1998</td>
<td>Mild-severe</td>
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<td>1.359</td>
<td>5.686</td>
<td>2.800</td>
<td>0.005</td>
</tr>
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<td>Liapis et al. 2001</td>
<td>Mild-severe</td>
<td>1.900</td>
<td>1.080</td>
<td>3.341</td>
<td>2.229</td>
<td>0.026</td>
</tr>
<tr>
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<td>Mild-severe</td>
<td>2.380</td>
<td>1.181</td>
<td>4.795</td>
<td>2.426</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.292</td>
<td>1.311</td>
<td>4.006</td>
<td>2.911</td>
<td>0.004</td>
</tr>
<tr>
<td>Gronholdt et al 2001</td>
<td>Moderate-severe</td>
<td>1.010</td>
<td>0.430</td>
<td>2.371</td>
<td>0.023</td>
<td>0.982</td>
</tr>
<tr>
<td>Nicolaides et al. 2005</td>
<td>Moderate-severe</td>
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<td>2.256</td>
<td>10.215</td>
<td>4.071</td>
<td>0.000</td>
</tr>
<tr>
<td>Hashimoto et al. 2009</td>
<td>Moderate-severe</td>
<td>4.400</td>
<td>1.182</td>
<td>16.383</td>
<td>2.209</td>
<td>0.027</td>
</tr>
<tr>
<td>Topakian et al. 2011</td>
<td>Severe</td>
<td>6.430</td>
<td>1.352</td>
<td>30.573</td>
<td>2.339</td>
<td>0.019</td>
</tr>
<tr>
<td>Silvestrini et al. 2013</td>
<td>Severe</td>
<td>4.150</td>
<td>1.764</td>
<td>9.763</td>
<td>3.261</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.725</td>
<td>1.860</td>
<td>12.003</td>
<td>3.264</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.801</td>
<td>1.756</td>
<td>4.469</td>
<td>4.321</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Figure 19. Subgroup analysis based on the year of patients’ recruitment and US image analysis
### Table 7. Subgroup analysis of asymptomatic patients studies

<table>
<thead>
<tr>
<th>Degree of stenosis</th>
<th>Relative risk</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Z value</th>
<th>p value</th>
<th>Between subgroups (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild-to-severe</td>
<td>2.29</td>
<td>1.31</td>
<td>4</td>
<td>2.51</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Moderate-to-Severe</td>
<td>2.64</td>
<td>1.34</td>
<td>5.2</td>
<td>2.82</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>4.72</td>
<td>1.86</td>
<td>12</td>
<td>3.26</td>
<td>&lt;0.001</td>
<td>0.42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Period of ultrasound data collections</th>
<th>Relative risk</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Z value</th>
<th>p value</th>
<th>Between subgroups (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 2000</td>
<td>1.97</td>
<td>1.4</td>
<td>2.78</td>
<td>3.89</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Since 2000</td>
<td>4.65</td>
<td>2.84</td>
<td>7.61</td>
<td>6.1</td>
<td>&lt;0.001</td>
<td>0.005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Echolucency definition</th>
<th>Relative risk</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Z value</th>
<th>p value</th>
<th>Between subgroups (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSM cut-off</td>
<td>1.66</td>
<td>0.71</td>
<td>3.92</td>
<td>1.19</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>PT 1 and 2</td>
<td>3.01</td>
<td>2.02</td>
<td>4.5</td>
<td>5.4</td>
<td>&lt;0.001</td>
<td>0.21</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PT assessment</th>
<th>Relative risk</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Z value</th>
<th>p value</th>
<th>Between subgroups (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual</td>
<td>2.56</td>
<td>1.64</td>
<td>4</td>
<td>4.15</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Computer assisted</td>
<td>3.56</td>
<td>2.08</td>
<td>6.08</td>
<td>4.65</td>
<td>&lt;0.001</td>
<td>0.35</td>
</tr>
</tbody>
</table>

In addition, we performed meta-regression analysis of the echolucent plaques and different stenosis category on future ischemic events risk in asymptomatic patients. Although there was an increasing trend of future events with increased stenosis severity, the slope was not significant (p=0.19) (figure 20).

![Figure 20. Meta-regression analysis. Regression of Log risk ratio (RR) on carotid stenosis severity in patients with echolucent plaques](image)
Publication Bias

There was no publication bias reported for the studies included in the meta-analysis (Figure 17 and 21).

Figure 21. Asymptomatic patients, funnel plot of the standard error by log risk ratio. Kandall’s Tau=0.392, p=0.173
DISCUSION

Study I

Findings: This study shows that carotid plaques in the contralateral to symptomatic side have similar features compared with the symptomatic ones but more vulnerable features than the asymptomatic arteries. Contralateral plaques have lower GSM, lower GSM of the JBA, higher prevalence of plaque type 1 and 2 compared with asymptomatic arteries. In addition, they have more mild and marked irregular plaques, than the asymptomatic ones. JBA presence was the only plaque features that showed difference between contralateral and symptomatic arteries.

Data interpretation: It is generally believed that the pattern of carotid atherosclerosis is unpredictable. The findings in this study show that symptomatic patients had more aggressive disease, in both carotid sides, in the form of irregular contour and signs of inconsistent texture, suggesting vulnerable plaque features compared to those seen in asymptomatic patients. Even though the symptoms were unilateral, our results clearly show that contralateral arteries have very similar features. However, the JBA was more frequent in the ipsilateral carotid arteries and this could represent an additional feature for plaque instability which could also help in better patient risk stratification. Previous studies confirmed that vulnerable patients may have multiple unstable lesions, other than the culprit ones, either distally or even in another territory (1, 143). These claims support our results. However, despite our findings and the differences we identified between symptomatic and asymptomatic arteries, it must be mentioned that identification of vulnerable plaques remains a contentious issue in clinical practice. Quantitative assessment of carotid plaque echogenicity and textural features analysis can identify potentially unstable plaques, which are echolucent (low GSM) compared to echogenic (high GSM) asymptomatic plaques. Again using histology as the gold standard, it has been confirmed in imaging studies that echogenic plaques represent densely fibrotic and calcific composition (144), whereas, Low GSM was associated with large hemorrhagic areas and lower GSM values located in the juxtaluminal position that was associated with predominant necrotic cores. Morphological plaque features (e.g. surface plaque
irregularities), GSM and JBA have been used in the prospective studies as an attempt to predict future vascular events (145, 146). To our knowledge this is the first study that, used computer based morphological and textural plaque features in providing information on plaque vulnerability in contralateral carotid arteries.

**Clinical implications:** The findings of this study might have important clinical implications, as it: (a) confirms the generalized nature of atherosclerosis in patients with carotid disease, for not only involving more than one segment at the side of the symptoms but also the contralateral side, (b) confirms the assessment of carotid plaque features by computer assisted videodensitometric plaque features as a valuable analysis that could reliably differentiate symptomatic from asymptomatic carotid disease, and (c) confirm that the presence of JBA in carotid arteries could be of clinical relevance in addition to plaque echogenicity, because it was the only feature that showed difference between the two carotid sides of symptomatic patients. In addition, it may have therapeutic implication since; being at high risk of recurrent events, symptomatic patients with bilateral disease should receive aggressive secondary prevention treatment in addition to revascularization.

**Study II**

**Findings:** The results of this study show the sensitivity of US for calcification detection compared to CBCT images acquired in plaque specimens collected after CEA. US sensitivity was high in identifying calcification volume ≥8 mm³ by CBCT, but, calcification volumes <8 mm³ were inconsistently detected, with a sensitivity of only 62%. There was no difference for calcification between symptomatic and asymptomatic patients, neither between other risk factors and degree of stenosis.

Data interpretation: The most accurate method for detection and quantification of carotid artery calcification is computed tomography, which is highly correlated with the amount of calcification evaluated by histology sections (48, 147). Because CT involves radiation and is an expensive procedure, imaging plaque features, including calcification, by other
radiation free method that is more patient friendly such as US could have significant clinical relevance. But, why is the detection of calcification important. Firstly, it has been confirmed that the presence of calcification in carotid plaque is associated with lower risk of future brain ischemic events, suggesting that the presence of calcification is an indicator of plaque stability (47). Sometimes identifying vulnerability based on its composition is time consuming, and demands expertise, in such circumstances detection of calcium in the plaque could be a fast and important indicator of plaque stability. However, it should be mentioned that calcification is not always a sign of stability. In the contrary, intimal plaque microcalcification is an accepted sign of plaque vulnerability, however detection of its presence is not possible with non-invasive imaging modalities, but only with optical coherence tomography (OCT) (16). Secondly, detection of calcification could be important in decision making for interventional treatment of carotid atherosclerosis (CEA vs. CAS). It has been reported that implanting a stent in a calcified carotid plaque is associated with increased risk of ipsilateral stroke, within days after intervention (49). As many centers now rely mainly on the carotid US findings before intervention, identification of calcification may guide towards better patient risk stratification.

An attempt has been made to evaluate the accuracy of US in detecting plaque calcification by quantifying the total plaque echogenicity (148), however, because the carotid plaques are very frequently heterogenic, this method proved unreliable. We believe that our study is the first to demonstrate the US accuracy for calcification detection, defined as hyperechoic spots that produce posterior shadowing. Also, our findings show that non-detectable calcifications are of lower volumes and usually dispersed throughout the plaque. US was highly accurate in identifying carotid plaque calcification volume of at least 8mm³. However, this volume seems to be relatively significant when compared with normal intima-media thickness, which in most cases is <1mm thick but yet reliably quantifiable by US. The same imaging method failed in some cases to detect calcification volume of less than 8mm³ with reliable accuracy. In addition, we did not find any difference in calcification presence and volume between symptomatic and asymptomatic patients. It has been shown that statin therapy increases carotid plaque echogenicity (149) and also coronary artery calcification (150) and we
wished to assess this effect in our study. However, we have been limited to undergo such comparison since, 87.3% of our patients were already using statin therapy, limiting us in carrying out a reliable comparison between groups.

Clinical implication: Our finding showed that calcification volumes of at least 8mm$^3$ are accurately detectable by US, that could be taken as a validation of this method to decide for CAS intervention, since only extensive plaque calcification that was correlated with adverse events after carotid stenting. US seems not well suited for the detection of smaller calcium deposits, although they might play a role in plaque vulnerability. Although the method we used to determine US accuracy is subjective in nature, it establishes a potential foundation for the future development of quantitative models, which could guide towards improved identification of plaque characteristics as a step towards achieving plaque stabilization through optimum treatment. Determining a grey scale media (GSM) cut-off of the hyperechoic spots that do not produce shadow but otherwise represent calcification could enable detection of earlier stages of calcification and reduce the need for advanced CT examinations and the resultant radiation for patients.

Study III

**Findings:** This study demonstrates that common carotid artery measures, IMT and IM-GSM are associated with significant differences in the severity of the disease and distribution of other arterial systems. It was only IM-GSM, but not IMT that was able to differentiate between the numbers of arterial systems affected by atherosclerosis. It was proportionally decreased with increasing number of diseased arterial systems, starting from asymptomatic to more than one vascular system. The conventional measure of subclinical atherosclerosis, IMT, was less sensitive in showing similar differences between groups.

**Data interpretation:** In general, patients suffering from atherosclerosis in any system are at increased risk for recurrent events in the same arterial system or even symptomatic
disease in another territory. This is explained by the systemic inflammatory nature of atherosclerosis. Patients with previous stroke have been shown to be at high risk of suffering recurrent strokes but even higher risk of suffering a future MI (1). Patients with symptomatic disease in one vascular system have been reported to have a 25% risk for developing MI, stroke or even vascular death compared to >40% in those with multisystem arterial disease (1). Based on these data patients with multisite atherosclerosis can be described as vulnerable in the view of the high risk they carry for vascular events. It was in the light of our findings, that IMT showed differences only when comparing patients with and without previous MI. On the other hand IM-GSM proved much more sensitive in showing significantly progressive lower values as patients went from asymptomatic to symptomatic single arterial system and finally to symptomatic multisystem disease. In addition IM-GSM was significantly different in patients with previous stroke and MI, compared to those without.

Conventional carotid IMT has been widely used in many studies attempting to identify patients at increased risk for future vascular events. On the other hand, measurements of the composition of arterial wall free of plaque (IM-GSM) were only attempted in few cases. The association between IM-GSM and future vascular events has recently been demonstrated, as it was shown to be associated with a three-fold greater risk of all-cause mortality and an eight-fold greater risk of cardiovascular mortality (122). Recently it has been demonstrated that IMT represents an adaptive thickening of the arterial wall more than real atherosclerosis pathology (16). It was confirmed that age and hypertension are the dominant risk factors for increased IMT whereas low values of IM-GSM related to low levels of HDL-C and increased level of CRP (151). Furthermore, it has been suggested that IM-GSM could add incremental valued over and above IMT for patients risk stratification, since they both were related to different plaque features in the distal segments of the carotid artery (ICA and bifurcation) (59).

Clinical implication: The ability of the IM-GSM to differentiate between patients with and without previous ischemic events suggests that it could also identify asymptomatic patients at higher risk. Identifying such patients should warrant aggressive medical management of modifiable risk factors in an attempt to reduce/prevent vascular events.
However our findings could find clinical implication once they are reproduced prospectively and the reproducibility of the technique is confirmed in a number of studies.

**Study IV**

Findings: Our analysis shows that atherosclerotic plaque echogenicity evaluated by carotid ultrasound accurately predicts the development of CV symptoms in asymptomatic and symptomatic patients. This prediction was preserved for all degrees of stenosis.

Data interpretation:

*Asymptomatic patients*

Although a small benefit of CEA was observed in asymptomatic carotid stenosis, advances in medical therapies and the well-documented fall in stroke rates in such patients over the last decade questioned the need of surgical treatment in these group of patients (50, 52, 53, 152). However, there is a subgroup of high-risk asymptomatic patients, which could benefit from CEA, and identification of such patients could have an important clinical impact. The analysis we undertook in this study provides a strong evidence for the additional value of plaque echogenicity in identifying asymptomatic patients at higher risk of future CV events. To strengthen the argument further, such accuracy for predicting future events, based on plaque echogenicity, stood firm even after analyzing separately different categories of carotid artery stenosis, although asymptomatic patients with severe stenosis again seem to be at a higher risk.

A higher association between plaque echolucency and future CV events was found among studies that started patients’ recruitment and US data collection from 2000 and thereafter, studies that defined plaques as PT 1 and 2 and when computer assisted analysis was used to evaluate plaque echogenicity. Accordingly, more advanced US devices and computer-assisted analysis of plaque echogenicity could help better recognition of plaque echolucency and consequently, better prediction of future ischemic events. Even if the cost-benefit of these findings is ignored, the clinical benefit to patients cannot be over emphasized if our findings are implemented, particularly in asymptomatic patients with severe stenosis. However, carotid plaque echogenicity

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quantification is not real life analysis since it needs special software and expertise. It is also an operator dependent technique with some potential technical difficulties in some patients.

*Symptomatic patients*

Several large clinical randomized trials demonstrated a significant surgical benefit for symptomatic patients, over and above medical treatment, particularly in those with 70-99% stenosis (17, 153). However, there is a subgroup of patients with lower degree of stenosis who carry a high risk of stroke recurrence. Although, our analysis showed that plaque echogenicity analysis could predict recurrent ischemic events in symptomatic patients with moderate to severe stenosis, the small number of studies and differences in the follow-up periods between them limits us in providing a strong conclusion. A high rate of stroke recurrence even within the first 72 hours of symptoms and within the following two weeks has recently been reported (98). More studies are needed to confirm the association between plaque echolucency and recurrent events in the early hours/days after initial which could indicate a need for an emergency CEA in order to prevent another stroke, as has been suggested (101).

*Clinical implications:* Quantification of carotid plaque echogenicity is a technique with great potential in daily clinical service, which may result in better patient stratification according to potential development of disease related complications. Such approach may guide towards identifying high-risk patients with asymptomatic stenosis as well as symptomatics with moderate stenosis and implement treatment strategy. Based on our results plaque echolucency in asymptomatic patients, particularly in those with severe stenosis, should be taken as a sign for vulnerability that supports the need for surgical intervention.
LIMITATIONS

Study I

The small number of patients, particularly the asymptomatic and the retrospective nature of the study are the most important limitations. When comparing different groups, the small number of patients may carry the risk of false negative. In addition, lack of detailed information for atherosclerosis burden in other arterial systems and lack of body mass index data is another limitation. The images used for the analysis were slightly clouded, however, applying standard image despeckle-filtering prior to the image analysis minimized the noise (154). In general, the little noise that remained in the images for the final analysis might influence the value of entropy and weaken its potential to differentiate between groups.

Study II

The evaluation of US positive and negative predictive value was not possible; because almost all (98.9%) carotid plaques were calcified in CBCT. During CEA procedure there might be small-calcified material not excised, but we have been working on the assumption that the largest plaque with the largest calcium volume was removed and assessed by CBCT. Furthermore, we cannot be sure that the calcification detected and quantified by CBCT was the same hyperechogenic nodule seen by US, particularly in cases with multiple calcifications, however, we made a further assumption that the nodule with the largest volume was the same that caused the posterior shadowing. In addition, our subgroup comparisons were weak, since the number of asymptomatic patients was small compared with symptomatic patients.

Study III

This is a retrospective study with known limitations. We have compared the two carotid artery wall measures with previous symptoms in different arterial systems, an information
that was acquired from patients’ medical records. Another limitation is the lack of imaging plaque features in other vascular territories (coronary and lower extremity arteries). Assessing plaque/IMT and GSM in other arterial system, particularly the ones related to the developed events, would have been of great interest but were not possible with the retrospective nature of the study. In addition, information on the duration of individual risk factors that could have influenced the results was not available.

Study IV

A systematic review based on relevant key words might have missed important publications, but two investigators blinded to each other’s means of search checked the search. We used only publications in the English language; other relevant ones in different languages might have been missed. Studies included in the meta-analysis were of different designs and different definitions to determine plaque echolucency were used, however we tried to overcome this by using the random effect meta-analysis. Another limitation is the low number of studies included in the meta-analysis, particularly those with symptomatic patients.
CONCLUSIONS

Study I

Symptomatic patients with carotid atherosclerosis seem to have profound features of plaque vulnerability in the contralateral arteries as well. Carotid plaques of asymptomatic patients were more stable compared to symptomatic and contralateral to symptomatic side. These findings, support the concept of generalized atherosclerotic pathology rather than incidental unilateral disease, suggest increased relative vulnerability of the contralateral plaques in symptomatic carotid stenosis and hence emphasize the need for more aggressive treatment for secondary atherosclerosis prevention.

Study II

Carotid US accurately detected carotid artery calcification volumes of at least 8mm³, but it was less accurate in detecting smaller volume calcified plaques, with relatively high false negativity. We didn’t find any difference in calcification presence or volume between symptomatic and asymptomatic patients. Since calcification is a dynamic process, finding a method that can quantify atherosclerotic calcification by avoiding radiation could be of importance for longitudinal assessment of its progression and effects on plaque stability.

Study III

Our results showed that IM-GSM but not conventional IMT to differentiate between patients with multiple system atherosclerosis disease. Because it was confirmed that patients with multisite atherosclerosis are at higher risk of ischemic events in future, measurement of intima-media complex echogenicity (IM-GSM) seems to be a promising method to identify high-risk patients.
Study IV

There is a clear association between carotid plaque echolucency and future CV events in asymptomatic and symptomatic patients. These findings if implemented, should result in better management of vulnerable patients as well as early recruitment of those requiring surgical intervention, who else would have been considered wrongly stable. Before implementing such strategy for selecting asymptomatic patients for surgical intervention, current results need to be confirmed in randomized clinical trials testing isolated medical treatment in echogenic plaque against medical treatment plus CEA in asymptomatic patients with echolucent plaque.
Acknowledgement

Though only my name appears on the cover of this dissertation, a great many people have contributed to its production. I owe my gratitude to all those people who have made this dissertation possible.

First and foremost I express my sincerest gratitude to my supervisor Professor Michael Henein. I have been amazingly fortunate to have an advisor who gave me the freedom to explore on my own, and at the same time the guidance to recover when my steps faltered. Michael taught me how to question thoughts and express ideas. His patience and support helped me overcome many difficult situations and complete this dissertation. I hope that one day I would become as good an advisor to my students as Michael has been to me.

My co-supervisor Professor Per Wester deserve a special thanks for his help, very professional comments that always made important changes in my scientific work and for scholarly devotion to my work during realizing the thesis. So many things I have learned from him will be very helpful for my academic career and also will help me for proper diagnosis and treatment of stroke patients.

I would like to thank very much Elias Johansson who provided me the opportunity to use the clinical patients’ data he collected very carefully making my scientific work much easier, and also for his insightful discussions that helped me to do the best with the data analysis and also to widen my knowledge in the carotid stenosis field.

This thesis couldn’t be possible without the help of Professor Christer Grönlund, who made special software that I have used in my studies and also for his fruitful comments in all of my studies.

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