Patterns of Non-invasive Imaging of Carotid Atherosclerosis

Pranvera Ibrahimi
To my grandfather, Idriz,

who emphasized the importance of education, and who instilled in me the inspiration to set high goals and the confidence to achieve them.
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Abstract

Atherosclerosis is an inflammatory disease that can be generalized, affecting more than one arterial bed simultaneously, or localized, manifested in one system. Ultrasound based measurements of plaque textural features, such as low grey scale median (GSM), echolucent (hypoechoic) plaque types and juxtaluminal black (hypoechoic) area (JBA) are manifestation of potentially unstable lesions. Conventional carotid IMT (intima media thickness) and the recently introduced IM-GSM (echogenicity of the intima media complex) are important measures of subclinical atherosclerosis and are used to predict future ischemic events.

The aims of this thesis were to study, in detail, the systemic nature of atherosclerosis by evaluating the carotid disease burden contralateral to symptomatic arteries, determining the relationship between proximal (subclinical atherosclerosis) and distal segments (well established disease) of the same artery and comparing local plaque features with systemic burden of atherosclerosis disease. In addition, the effect of statins on carotid plaque echogenicity was evaluated in a systematic review and meta-analysis.

Methods:
We have measured ultrasound-based textural carotid plaque features (GSM, JBA, entropy, coarseness), surface morphology, as well as IMT and IM-GSM. An in-house custom developed research software package was used for plaque feature extraction. For the meta-analysis we used Comprehensive Meta-Analysis version 3 software.

Results:
Study 1. In 39 patients, the carotid plaques contralateral to symptomatic arteries had similar morphological and textural features to those in the symptomatic arteries and are more vulnerable than those in asymptomatic arteries; more often mildly or markedly irregular with more vulnerable textural plaque features (lower GSM and larger JBA).

Study 2. In 87 asymptomatic patients, an increased IMT in CCA correlated with plaque irregularities in the bifurcation and ICA while IM-GSM was closely related to plaque echogenicity (GSM), and other textural plaque features.

Study 3. In the same cohort in study 2, patients with previous disease in the coronary arteries had higher IMT and lower IM-GSM and those with prior stroke had lower IM-
GSM. Neither IMT nor IM-GSM was different between patients with and without previous lower extremity disease. IM-GSM decreases significantly with increasing number of arterial territories p<0.001 (asymptomatic vs symptoms in one vs multiple arterial systems) but conventional IMT was not different between groups p=0.49.

**Study 4.** In a meta-analysis of 9/580 identified studies including 566 patients with 7.2 months follow-up, a consistent increase in the carotid plaques echogenicity after statin therapy, was reported. The perpetual (over 12 months) effects of which were shown in a meta-regression analysis to be related to changes in hsCRP.

**Conclusion:**
Symptomatic patients have similar plaque morphology and textural features of vulnerability in the contralateral carotid system, compared with asymptomatic ones. In the latter, measurements of proximal disease reflect distal pathology and the number of affected arteries. Finally, statin therapy and the drop of LDL cholesterol result in better plaque stability and optimum control of arterial inflammation, shown by arterial wall echogenicity and hsCRP changes, respectively.

**Key words:** atherosclerosis, carotid ultrasound, plaque features, grey scale median, juxtaluminal black area, surface plaque morphology, statins
Abbreviations

ACAS - Asymptomatic Carotid Atherosclerosis Study
ACRS - 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
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
ICA - internal carotid artery
IMT - intima-media thickness
IM-GSM - intima-media grey scale median
IPH - intraplaque hemorrhage
IVUS - intravascular ultrasound
JBA - Juxtaluminal black area
LDL - low density lipoprotein
MDCT – multidetector computed tomography
MI - myocardial infarction
MRA - magnetic resonance angiography
MRI - magnetic resonance imaging
NASCET - North American Symptomatic Carotid Endarterectomy Trial
OCT – optical coherence tomography
OR - odds ratio
PET - positron emission tomography
PT - plaque type
RF-IVUS – radiofrequency intravascular ultrasound
RR - relative risk
TCFA – thin cap fibroatheroma
US - ultrasound
List of Papers


INTRODUCTION

Atherosclerosis is a chronic inflammatory disease, which can lead to life-threatening cardiovascular and cerebrovascular events such as myocardial infarction (MI) or ischemic stroke (IS). It could simultaneously affect different segments of the same artery and also different arterial systems. The majority of ischemic events derive from the rupture or erosion of atherosclerotic plaque, with a superimposed thrombus. This may completely occlude the lumen (the commonest pathomechanism in MI) or may embolize and occlude a distal arterial branch (as in IS) (1). The risk of atherosclerotic plaque rupture is not necessarily related to stenosis severity, a dissociation that was shown in many studies (2, 3) and clinical trials. Furthermore, it has been shown that statins markedly reduce acute ischemic events (4) with only modest reduction in the degree of stenosis (5). There is a growing awareness that plaque features, such as: thin cap fibroatheroma, large necrotic core, composition, intra-plaque hemorrhage and intimal microcalcifications, could have additional value over and above the severity of stenosis. Thanks to advances of invasive and non-invasive imaging modalities these plaque features could now be visualized and quantified, and this is important in identifying patients at increased risk from future ischemic events. Furthermore, these features could be good markers for monitoring patients’ response to medical therapy.

Epidemiology

Despite recent advances in medical diagnosis and intervention, atherosclerosis remains the most important cause of death in the developed countries (6, 7). In the United States, coronary artery disease causes more than 400,000 deaths annually. Cerebrovascular diseases are the second leading cause of death worldwide. Although the data vary among countries, based on WHO data, the global incidence of stroke was estimated to be around 200 cases per 100,000 population. In a review of population based studies, the incidence varied from 130 to 410 cases per 100,000 person-years, with the highest rate in Japan and lowest in UK, Germany and New Zealand. In United States, approximately 795,000 people experience a new or recurrent stroke annually, with 610,000 cases reported as first attacks, and 185,000 as recurrent attacks. When gender difference was analyzed, a higher incidence for males
was confirmed in all studies. Even though atherosclerosis disease with its consequences are currently more prevalent in developed countries, contemporary predictions estimate that by 2030 more than 23.3 million persons will die annually from related cardiovascular disease (8).

**Risk factors**

The most accepted risk factors for atherosclerosis are: diabetes mellitus, hypertension, smoking, older age, obesity, raised low-density lipoprotein and low high-density lipoprotein cholesterol levels (9). Studies suggested that conventional CV risk factors have different impact in different arterial systems, with hypertension being particularly important in ischemic stroke, cholesterol in CAD, whereas smoking and diabetes in intermittent claudication (10, 11). Better understanding of these artery-specific risk factors should assist in optimum disease prevention. In addition, other risk factors have been recently recognized such as obesity, chronic infection, high sensitivity-C-reactive protein (hs-CRP) and insulin resistance (HbA1c), which remain to be determined. Many studies have shown that the prevalence of carotid and coronary atherosclerosis increase exponentially with age, and is more prevalent in men than women (12). Also, the median age of MI and stroke is lower in males, however, younger females have worse survival after MI compared with males (13, 14). In addition, after the age of 64 years no gender difference for ischemic heart disease seems to exist, whereas females have poorer short and long-term outcomes after stroke, independent of age and other covariates (15).

**Pathogenesis of atherosclerosis**

The term, atherosclerosis consists of two parts; atherosis (accumulation of fat accompanied by several macrophages) and sclerosis (fibrosis layer comprising smooth muscle cells [SMC] and connective tissue). Atherosclerosis starts early in life in the form of endothelial dysfunction, which allows lipid and inflammatory cells accumulation within the arterial wall. The disease evolution could be gradual and stable (as in the case of stable angina) or more aggressive, causing abrupt occlusion of
the arterial lumen in situ or artery-to-artery embolization (as in the case of MI and stroke) (16).

**Disease location**

Atherosclerosis of the arteries supplying the central nervous system is a frequent cause of ischemic strokes and transient ischemic attack (TIA), those supplying the heart cause MI and angina and in the peripheral circulation, atherosclerosis causes intermittent claudication and gangrene. Even within a particular arterial system, stenosis due to atherosclerosis tends to affect certain predisposed regions, usually the branching parts, e.g. carotid artery bifurcation. In some patients, atherosclerosis pathology could affect many arterial systems simultaneously, and this is considered as a sign of aggressive disease and patient’s vulnerability (17).

**Pathophysiology**

The evolution of atherosclerosis disease involves a combination of endothelial cell dysfunction, extensive lipid accumulation in the intima followed by accumulation of inflammatory cells, proliferation of vascular smooth muscle cells and remodeling of the extracellular matrix, resulting in the formation of an atherosclerotic plaque. The predilection of the disease at the branching points of the arteries could be explained by the disturbed blood flow at these regions. In a non-branching artery, pulsatile laminar shear of blood flow augments the production of nitric oxide by endothelial cells. This molecule has vasodilatory and anti-inflammatory properties by limiting local adhesion molecule expression (18). Dysfunction of the endothelial cells allows accumulation of LDL particles and inflammatory cells inside the arterial wall. Upon accumulation into the intima, monocytes mature to macrophages. These cells then start to uptake LDL particles and become lipid-laden foam cells. Foam cells formation corresponds with the initial lesions visible on the luminal wall as fatty streaks. The release of the cytokines by the macrophages will augment more inflammatory cells and also initiate the migration of smooth muscle cells from tunica media to the intima. This process is followed by degradation of the extracellular matrix and apoptosis of the foam cells, which results in the formation of necrotic core.
Apoptotic cells and necrotic core material together with extracellular matrix may act as nidus for microscopic calcium granules, which can subsequently expand to form larger nodules and plates of calcium deposits (19).

In addition to macrophages, other inflammatory cells, such as T-cells, are recruited in the atherosclerosis process. In the later phases of the disease, inflammatory cells could also be recruited after the rupture of the new vessels developed following a process of neovascularization. Activated T-cells secrete cytokines that inhibit the production of interstitial collagen, which is mandatory for maintaining the plaques protective fibrous cap. T cells also cause overproduction of interstitial collagenases (matrix metalloproteinases) that catalyze the collagen breakdown, which makes the fibrous cap more fragile and could lead to plaque rupture. Following plaque rupture, blood elements (platelets and coagulation proteins) get in direct contact with the lipid core and other constituents of the atherosclerotic plaque (collagen and tissue factor) promoting thrombosis (Figure 1) (20). Of note, during the initial steps of the atherogenesis, the atheroma grows outwardly, towards the adventitia, preserving the caliber of the lumen, this phenomenon is often prescribed as outward (positive) remodeling, and is responsible for the tendency of carotid conventional angiography to underestimate disease burden (21, 22).
Figure 1. The pathophysiology of atherosclerosis. 1 - Endothelial dysfunction and accumulation of LDL inside arterial wall. 2 - Monocyte migration into intimal layer. 3 - Monocyte conversion to mature macrophages. 4 - Foam cell formation. 5 - Migration of SMC from media to intima. 6 - Neovascularization and intraplaque hemorrhage. 7 - Apoptosis and necrotic core formation. 8 - Plaque rupture with superimposed thrombosis.
Plaque vulnerability

The vulnerable plaque is a concept used to determine plaques at high risk of thrombosis by any mechanism (rupture, erosion) and sometimes, to describe a set of histological features that by association are assumed to increase the risk of rupture and thrombosis. The most accepted features of the vulnerable plaque include: thin cap fibroatheroma (<64nm), large lipid core (>40% of plaque volume), intimal spotty calcification (as opposed to a calcified plaque cap), positive remodeling, intraplaque neovascularizations and intraplaque hemorrhage (23). The prototypical vulnerable (rupture-prone) plaque is a thin cap fibroatheroma (TCFA) infiltrated with macrophages covering a large necrotic core (24). However, not each ruptured plaque causes symptoms, there is evidence of multiple plaques that underwent rupture in a particular arterial system, but only one lesion was found to be a culprit lesion causing an ischemic event (25). Moreover, fibrous caps may show evidence of multiple ruptures and subsequent healing, despite lack of symptoms (25, 26). These findings support the recent appreciation of atherosclerosis as a dynamic pathology, with silent and stable plaques suddenly acquiring vulnerable characteristics followed by rupture. To better understand this unstable atherosclerotic pathophysiology, accurate longitudinal non-invasive arterial imaging able to detect vulnerable plaques and assess their response to various medications is of immense importance.

Imaging atherosclerosis

For many years conventional angiography had been the main imaging modality of atherosclerosis that revolutionized the diagnosis and management of significant high-grade arterial stenotic lesions (21). However, the technique allows only the silhouette of the arterial lumen to be visualized, leaving the structures beyond the stenosis unrecognized. With the introduction of non-invasive technologies such as: ultrasound (US) (27, 28), computed tomography (CT) (29), magnetic resonance imaging (MRI) (30), positron emission tomography (PET) (31), it has become possible to visualize plaque composition, positive remodeling and other features in addition to the severity of stenosis (Table 1) (32).
Table 1. Improvement of plaque features visualization using different imaging modalities and their modified imaging techniques

<table>
<thead>
<tr>
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<td>Extent of plaque behind calcium nodule (RF-IVUS)</td>
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<tr>
<td>OCT</td>
<td>TCFA</td>
<td>Inflammation</td>
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<tr>
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<td>Intimal spotty calcifications</td>
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**Ultrasound**

Ultrasonography (US) is now a well-established imaging modality for first-line diagnosis of carotid artery disease. This non-invasive technique is readily available, rapidly applicable, radiation free, and can be performed at relatively low cost. It has been successfully adopted for identifying characteristics of high-risk plaques in patients with atherosclerosis. High frequency linear transducers (>7 MHz) are ideal to assess the disease location and to evaluate plaque morphology, whereas lower frequency linear transducers (<7MHz) are preferred for Doppler examinations. In addition, US can be used to assess initial, subtle wall alteration in the early stages of the disease progression, the thickness of carotid wall known as intima-media thickness as well as its composition (33, 34).

**Evaluation of degree of stenosis**

In previous decades, the severity of carotid artery disease was determined by angiographic measurement of stenosis and luminal diameter. The degree of stenosis was used as the main parameter to select patients eligible for carotid endarterectomy (CEA) in the three large multi-center randomized studies: NASCET (North American Symptomatic Carotid Endarterectomy Trial), ECST (European Carotid Surgery Trial) and ACAS (Asymptomatic Carotid AtheroSclerosis Group) (35-38). In both NASCET and ECST trials, stenosis severity was determined by conventional angiography, since CTA and MRA were not available at that time. Subsequently, studies intended to standardize the degree of stenosis measured by angiography by using velocities evaluated by duplex ultrasound (39). However, as the angiographic measurement of the carotid stenosis differed between ECST and NASCET, mostly due to different arterial reference (distal ICA for NASCET and ICA lumen for ECST), there has been some uncertainty regarding the relationship between the flow velocity measured by ultrasound and the angiographically measured degree of stenosis (40). Two approaches were adopted to quantify the degree of carotid artery stenosis using duplex ultrasound (DUS): 1) morphological and 2) Peak-Systolic-Velocity (PSV) values. The morphological analysis is based on the ratio-percent method for the
quantification of stenosis degree. On the other hand, PSV evaluates the maximum peak of systolic velocity at the plaque site, in the affected vessel (41). It is widely accepted that average Doppler velocity rises proportionally with the degree of stenosis; hence flow velocity is commonly used to evaluate the severity of carotid stenosis and to plan the consequent diagnostic and therapeutic approach. In general, the diagnostic accuracy of DUS has been shown to be more than 90% when performed by experienced operators (39). However there are some controversial data regarding the value of PSV that corresponds to a 70% NASCET stenosis degree. Saba et al. (42) found that the PSV threshold for a NASCET stenosis ≥70% was 283 cm/s, which was higher compared to 220 cm/s defined by Heijenbrok-Kal et al. (43) and 230 cm/s as defined by Grant et al. (44).

Even though the diagnostic accuracy of DUS is high when compared to angiography it has some drawbacks because the increased resistance and turbulence can affect the flow velocity. Other situations that may reduce the flow velocity include: impaired cardiac function and good intracranial collateral compensation (45). On the other hand, when there is more severe contralateral stenosis or occlusion, an increased volume flow and flow velocity can overestimate the exact stenosis severity (46). Overall, flow velocity measurements with ultrasound should be combined with assessment of plaque burden on 2D picture. Currently, the severity of carotid stenosis is merely used as the only imaging marker for stroke risk stratification (47). Other imaging parameters such as plaque morphology and echogenicity are probably of increasing importance concerning the vulnerability of the atherosclerotic plaque.

**Plaque morphology and vulnerability**

Apart from the degree of stenosis, plaque morphology has emerged as an important contributing factor for stroke risk stratification. Ultrasound studies found that echolucent plaques and those with surface irregularities are more often associated with the occurrence of neurological symptoms than echogenic and smooth morphology ones (48, 49).
Surface plaque irregularities and plaque ulceration

Based on their surface morphology, carotid plaques are classified as smooth and regular, mildly irregular, markedly irregular and ulcerated plaques. The presence of plaque irregularities can represent clinically silent but potentially hazardous lesions, independent of the degree of stenosis. Plaque irregularities can be detected by invasive (angiography) or noninvasive (DUS, CTA or MRA) imaging modalities. Most studies used a cut-off of 0.4 mm for height variations along the contour of the lesion to discriminate moderate from severe irregularities (Figure 2). Using ultrasound, ulcerations are defined as those meeting three criteria: the recess must be 2 mm deep and 2 mm long at least, have a well defined wall at its base and exhibits an area of reversed flow within the recess or a zone of low flow signal at the level of the recess.

**Figure 2.** Different categories of surface plaque irregularities.
The sensitivity and specificity of DUS in detecting plaque ulceration has been shown to be 92.9% and 81.9%, respectively. MRA shows a slightly better specificity and sensitivity (50), however, its high cost is a serious drawback. In addition, recent results revealed that 3D ultrasound has higher accuracy in identifying plaque irregularities compared to Doppler US (51). Several studies have shown the association between carotid surface plaque irregularities and the development of cerebrovascular events (48, 52). Patients with plaque ulceration and or markedly irregular plaques detected by US have a 4.4-fold increase in the risk of stroke development, on multivariate analysis. (48). In addition, two other studies have demonstrated that, independent of the degree of lumen stenosis, carotid plaque surface irregularities and ulceration constitute a stronger predictor of the development of cerebrovascular symptoms compared to a smooth carotid plaque producing significant stenosis (53, 54).

**Plaque composition**

Plaques may histologically be described as fibrosed, fibro-fatty, fatty, hemorrhagic, necrotic or calcified (55). The different biochemical structure, internal architectural arrangement or physiological state of normal vs. diseased tissue can affect the physical properties of the tissue, thus enabling defining tissue characterization by different imaging modalities. Although different imaging modalities have been used to determine carotid plaque composition, e.g. MDCT and MRI, carotid ultrasound remains the one most widely used that allows characterization of carotid plaques by its echogenicity, defined as reflection of the ultrasound signal. Using ultrasound, tissue characterization of the carotid plaques can be performed using: visual assessment, software-assisted videodensitometry after imaging normalization, and backscatter analysis of native radiofrequency signal (56).

Early on, there was an attempt to differentiate plaque features visually based on their echogenicity. Various classifications were proposed to characterize carotid plaques using ultrasonography into; homogenous, having uniform high- or medium-level echoes, and heterogenous, having high-, medium-, and low-level echoes and containing areas with echogenicity similar to that of blood, as introduced by Reilly et al. (57). Furthermore, Johnson et al (58) classified plaques into calcified, dense or soft, and the Gray-Weale classification described 4 plaque types, from dominantly
echolucent with a thin, echogenic cap to dominantly echogenic with small areas of echolucency, through 2 types of mixed echogenicity (59).

The plaque type classification, widely used today, is that introduced by Geroulakos et al, which divides plaques into 5 types:

Type I: uniformly echolucent plaques with or without a thin echogenic cap.
Type II: predominantly echolucent plaques with less than 50% echogenic areas.
Type III: predominantly echogenic plaques with less than 50% echolucent areas.
Type IV: uniformly echogenic plaques.
Type V: plaques which cannot be classified due to heavy calcification producing acoustic shadows (60).

However, this method of plaque characterization had many limitations in view of its subjective way of assessment rather than objective measurement. Therefore, el-Barghouty et al in 1995 introduced the new approach of computer assisted grading of the echogenicity of the carotid plaque (61).

**Computer assisted videodensitometric carotid plaque tissue characterization**

The operator-dependent, subjective method of visual plaque characterization has been a major concern in the recent years. Therefore, a new method has been developed using computer-assisted analysis and providing a more quantitative and operator-independent assessment of plaque echogenicity. The best B mode images of carotid plaques captured through the examination are transferred from the ultrasound machine to a computer for further image standardization and analysis, performed using Adobe PhotoshopTM Version 3.0 or MATLAB based algorithms.

The protocol of image normalization and analysis follows 5 different steps:
1. The color information in the image has to be omitted so that all the processing and analysis performed on images to be in grey mode.
2. An area in the blood (free of noise) to be selected. Using the histogram facility in the program, the median value of the grey levels of all the pixels (grey scale median; GSM) is obtained.

3. Similarly part of the adventitia is selected and measurements of GSM are made at the brightest part of the adventitia on the same arterial wall as the plaque.

4. Image normalization and algebraic scaling of the whole image is performed using the "curves" facility of the software. This is linear and based on the two reference points: blood and adventitia. The scale is adjusted so that the grey value of the blood would be in the region of 0-5 and that of the adventitia in the region of 185-195. Thus the grey values of all pixels would change as defined by this new linear scale.

5. Analysis of echoic features in plaques. In normalized images, the plaque is outlined and the following measurements are obtained: (62)

**Grey Scale Median (GSM):** defined as the median of overall grey shades of the pixels in the plaque.

**Entropy:** defined as the degree of disorder or dissimilarity of grey values; a largest value is obtained when the plaque is non-homogenous and has a non-recognizable pattern (63).

**Coarseness:** defined as a measure of the variability of grey scale difference and hence coarseness of texture. A large value of contrast indicates large variation in grey values of the pixels (64).

**Plaque type:** Plaques are classified automatically by the software into the following types according to the modified Geroulakos classification: (60)

   a) Type 1, uniformly echolucent (black) and < 15% of pixels in the plaque area with values > 25;
   b) Type 2, mainly echolucent and pixels with grey-scale values > 25 occupying 15–50% of the plaque area;
   c) Type 3, mainly echogenic and pixels with grey-scale values >25 occupying 50–85% of the plaque area; and
d) Type 4, uniformly echogenic and pixels with grey-scale values >25 occupying >85% of the plaque area.

Besides, there are other measurements/parameters recently developed assessing plaque tissue characterizations, such as:

**Discrete white areas (DWA):** The presence of discrete white areas was defined as areas with pixels having grey scale values >124 (colored red by the software) that does not produce acoustic shadowing in plaque types 1 to 3. An association between DWA and Stroke/TIA risk has already been shown (54).

**Juxtaluminal black area:** JBA is defined as black area when it is close to the lumen without visible echogenic cap (65). This feature has been shown to correlate with the necrotic core in studies that correlated US imaging with histology (66).

**Reproducibility and validity**

Several studies have shown the good reproducibility for GSM measurements (67-69) indicating that it is a robust method for the assessment of plaque characteristics. However, when comparing two software packages “Adobe Photoshop” and “Artery measurement Software” there is a significant difference in absolute GSM values, even though the results are highly correlated and the agreement between the two methods is good (67).

**Radiofrequency analysis**

Radiofrequency analysis or integrated backscatter (IBS) is a more technologically demanding “third generation” approach, commercially developed over the last 15 years and is theoretically considered the most accurate, since the native ultrasound signal is sampled upstream to the video display, and is not distorted by the post-processing function of the imaging chain (56, 70).

The system is calibrated with blood equals to 0 decibels (dB) and the optimal artificial reflector to 50 dB. Along with this setting the lipid-hemorrhagic plaques remain below 14, fibrous and fibro-fatty plaques between 14 and 26, and calcific plaques
above 27. Again, the radiofrequency approach is considered the best quantitative however, the other approaches are also able to provide clinically valuable information for in vivo characterization of the atherosclerotic plaques using ultrasound technology (56, 70).

**Ultrasound carotid plaque tissue characterization vs. histology**

Vulnerable, high-risk plaques are characterized not only by having a larger plaque burden but also for having higher content of lipids, with necrotic cores due to invasion of lipid pools by macrophages and other inflammatory cells covered by a thin fibrous cap; with speckled micro-calciﬁcation and the luminal contours may be irregular rather than smooth. These histological features have various patterns of backscatter which are affected by the degree of acoustic attenuation, angular variability, spatial texture, neo-vascularization and homogeneity of the spatial grey-level distribution.

While, histological characterization can identify features associated with plaque instability, there has been inconsistent correlation between preoperative imaging visual plaque feature characterization and post-operative (histological) plaque analysis. Kardoulas et al (1996) presented inconsistency of ultrasound plaque features such as, plaque surface regularity, irregularity, ulceration and plaque haemorrhage with plaque pathology (71). However, these US features were of clinical importance in identifying an asymptomatic high-risk subgroup of patients that might benefit from surgical treatment.

The grey scale median (GSM) measurement combined with color mapping of the carotid plaques have been shown to adequately correlate with the different histopathological components and thus allow relatively accurate identiﬁcation of determinants of plaque instability (27). El-Barghouty et al. reported that a high ﬁbrous content of plaques was associated with a high GSM and high hemorrhage and lipid content with a low GSM on histology, and the computerized measurement of carotid plaque echogenicity could be used to predict plaque composition (72). Szajtel et al. (2005) showed that a quantitative analysis of plaque echogenicity using GSM measurement provided a good correlation with histological findings and also allowed
identification of some characteristics suggesting plaque instability such as the thickness of the fibrous cap or the juxtaluminal position of the necrotic core (66). Salem et al. demonstrated that an echolucent plaque (GSM <25), a large juxtaluminal black area (>6 mm²), and a large plaque area (>95 mm²) significantly predicted an unstable carotid plaque by histology (73). Furthermore, carotid plaque echogenicity, as quantitatively assessed by IBS analysis, have been shown to correlate well with the histologic contents of the plaques (74).

Why it is important to objectively perform tissue characterization of the carotid plaque?

Observational studies have shown a clear relationship between low GSM and ischemic events and increased brain microembolisations (75) and it tends to increase with statins (76). Furthermore, carotid plaque echolucency measured by GSM has been shown important in planning the treatment procedure. Moreover, carotid plaque echolucency with a GSM <25 increased the risk of stroke in carotid artery stenting (77). Despite that, controversies remain regarding the accuracy of such value in differentiating symptomatic from asymptomatic plaques with GSM values ranging between 15 up to 75 (78-80). A sound explanation of such controversies is because of nature of the GSM measurement of the median brightness of the whole atherosclerotic plaque and therefore may not necessarily reflect the presence of particular regional components. It has recently been demonstrated that a stratified GSM assessment, analyzing each millimeter from the surface to the bottom of the plaque combined with color mapping could predict plaque histology better than usual overall GSM measurement (81). Using this method, a profile of the regional GSM as a function of the distance from the plaque surface could be generated.

Plaque neovascularization and intraplaque hemorrhage

The vasa vasorum consists of small blood vessels that supply the arterial wall with nutrients and oxygen. They are predominantly located in the adventitial layer, but factors such as hypoxia and inflammation may stimulate the extent and distribution of the vascular network within the arterial wall (82). Histological studies have confirmed
a network of microvessels within atherosclerotic lesions, known as intraplaque neovascularization (IPN), which originate from the adventitia and extend to the media and intima (83). IPN is regarded as a marker of the vulnerability of carotid plaque as it increases the risk of rupture (84) causing intraplaque hemorrhage or creating immature microvessels responsible for trafficking inflammatory cells inside the plaque (85). DUS is not able to identify these small microvessels inside the plaque on its own, however, using a microbubble based contrast, contrast enhanced ultrasound (CEUS) has made its visualization possible. The hypothesis was based on the ground that microbubbles remain only in the vascular lumen, thus when seen inside the plaque, they enable identification of intraplaque neovessel (86). The CEUS identification of intraplaque neovessels has been confirmed against histology in many studies (28) (Figure 3). On the other hand, intraplaque hemorrhage could be associated with low echogenic areas within the plaque or they can be suspected during follow-up imaging in case the plaque enlarges abruptly.

Figure 3. Usefulness of the different imaging modalities for differentiating vulnerable and stable plaques in carotid and coronary arteries
Imaging subclinical atherosclerosis in carotid arteries

Carotid intima-media measurements

Intima media thickness of the carotid artery (c-IMT) is associated with several cardiovascular risk factors and manifest cardiovascular and cerebrovascular disease. C-IMT measured by B-mode US of the carotid artery is defined as the distance between the lumen-intima interface and the media-adventitia interface (87). C-IMT is best visible in the far wall of the distal segment of the common carotid artery with high reproducibility. There are several large prospective studies, each including more than 1000 participants, which evaluated the c-IMT in predicting coronary artery disease (Table 2) and stroke (Table 3). The ARIC (Atherosclerosis Risk in Communities) study (88) indicated that in middle-aged patients for every 0.19 mm increment of c-IMT, the risk for MI or sudden cardiac death increases by 36%. C-IMT measurements also improved traditional risk factors for prediction of CV events. In particular, among intermediate-risk patients, the addition of c-IMT and plaque information led to clinical net reclassification improvement of approximately 9.9% (89). Likewise, statins trials have consistently shown that a regression in c-IMT is associated with a reduction in CV events (90), particularly in patients with intensive therapy (91). However, different studies have used different cut-offs for defining the c-IMT (Table 2 and 3). There are some suggestions to define abnormal c-IMT as an absolute value of ≥1mm, or c-IMT at the upper quartile or upper tertile, but the most accepted definition is the IMT higher than 75th percentile for age, gender and ethnicity (87). Recently, a meta-analysis showed that c-IMT does not add clinically important incremental prediction over traditional cardiovascular risk factors (92). Even, adding c-IMT to the Framingham risk score did not improve risk prediction in diabetic patients (93). Furthermore there are assumptions indicating that increased IMT represents intimal hyperplasia and fibro-muscular hypertrophy rather than lipids accumulation in the intimal layer. The presence of carotid artery plaques has been shown to be a better predictor than c-IMT (89) (Table 2 and 3). However, there was a need to find other imaging markers for patients’ vulnerability, measurement of which could be implemented to all patients, because plaque presence is not very frequent, even in intermediate risk patients, or it may rely on distal assessment where US
imaging is limited. Nevertheless, recently new carotid artery wall measurement that quantifies the echogenicity of the intima media complex (IM-GSM) has been introduced. This provides a description of tissue composition of the intima and media layers of the arterial wall and seems to be a sensitive marker of arterial wall disease that could discriminate between adaptive and pathological intimal thickening. While the GSM of plaques was studied against histology, the IM-GSM has to be evaluated and the prognostic impact of this new variable has to be investigated. Indeed, there are studies that confirmed the association of IM-GSM with several risk factors not related to IMT, such as dyslipidemia, oxidative stress and inflammation, the results of which need further investigation in prospective clinical setting. The Prospective Investigation of the Vasculature in Uppsala Seniors (the PIVUS study) (94), a population-based cohort study demonstrated a close relationship between IM-GSM and plaque GSM being independent of small plaque size. However, this relationship was not examined in patients with large and highly stenotic plaques. Moreover, PIVUS group, in a community-based cohort study of elderly men, has found that IM-GSM was a significant predictor of both all-cause and cardiovascular mortality (95). According to these findings, IM-GSM could be an important and easily measurable characteristic of the carotid artery wall that could be obtained in most subjects. The predictive information offered by an echolucent IM might add important information above and beyond c-IMT.
Table 2. Prospective studies evaluating carotid intima-media thickness and plaque presence for stroke prediction

<table>
<thead>
<tr>
<th>Study/year</th>
<th>Subjects (n)</th>
<th>Age (y)</th>
<th>Female %</th>
<th>Follow-up (y)</th>
<th>Subclinical marker</th>
<th>Cut-off point for IMT</th>
<th>RR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>O’Leary et al., 1999 (96)</td>
<td>4476</td>
<td>&gt;65</td>
<td>39</td>
<td>6.2</td>
<td>Maximum IMT</td>
<td>Highest quintile</td>
<td>2.13 (1.38-3.28)</td>
</tr>
<tr>
<td>Lorenz et al., 2006 (97)</td>
<td>5056</td>
<td>19-90</td>
<td>50</td>
<td>4.2</td>
<td>Mean IMT</td>
<td>0.16 mm increase</td>
<td>1.11 (0.97-1.28)</td>
</tr>
<tr>
<td>Chambless et al. 2000 (98)</td>
<td>14,214</td>
<td>45-64</td>
<td>55</td>
<td>7.2</td>
<td>Mean IMT</td>
<td>0.18 mm increase F: 1.32 (1.10-1.58) M: 1.38 (1.16-1.65)</td>
<td></td>
</tr>
<tr>
<td>Kitamura et al., 2004 (48)</td>
<td>1289</td>
<td>60-74</td>
<td>0</td>
<td>4.5</td>
<td>Maximum IMT</td>
<td>Highest quartile</td>
<td>4.9 (1.9-12.0)</td>
</tr>
<tr>
<td>Kitamura et al., 2004 (48)</td>
<td>1289</td>
<td>60-74</td>
<td>0</td>
<td>4.5</td>
<td>Plaque presence</td>
<td>N/A</td>
<td>3.2 (1.4-7.1)</td>
</tr>
<tr>
<td>Prabhakaran et al., 2007 (99)</td>
<td>1938</td>
<td>&gt;40</td>
<td>59</td>
<td>6.2</td>
<td>Plaque presence</td>
<td>N/A</td>
<td>3.1 (1.1-8.5)#</td>
</tr>
<tr>
<td>Chien et al., 2008 (100)</td>
<td>2190</td>
<td>&gt;35</td>
<td>55</td>
<td>10.5</td>
<td>Maximum IMT</td>
<td>1SD</td>
<td>1.47 (1.28-1.69)</td>
</tr>
</tbody>
</table>

*RR* - adjusted relative risk
**Table 3. Prospective studies evaluating carotid intima-media thickness and plaque presence for myocardial infarction prediction**

<table>
<thead>
<tr>
<th>Author/year</th>
<th>Subjects (n)</th>
<th>Age (y)</th>
<th>Female %</th>
<th>Follow-up (y)</th>
<th>Subclinical marker</th>
<th>Cut-off point for IMT</th>
<th>RR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salonen et al., 1991 (101)</td>
<td>1288</td>
<td>42-60</td>
<td>0</td>
<td>~2</td>
<td>Plaque presence</td>
<td>N/A</td>
<td>4.15 (1.50-11.47)</td>
</tr>
<tr>
<td>Salonen et al., 1991 (101)</td>
<td>1257</td>
<td>42-60</td>
<td>0</td>
<td>3</td>
<td>Maximum IMT</td>
<td>0.11mm increase</td>
<td>1.11 (1.06-1.16)</td>
</tr>
<tr>
<td>Chambless et al., 1997 (88)</td>
<td>12,841</td>
<td>45-65</td>
<td>57</td>
<td>5.2</td>
<td>Mean IMT</td>
<td>0.19mm increase</td>
<td>F: 1.46 (1.22-1.74)</td>
</tr>
<tr>
<td>O’Leary et al., 1999 (96)</td>
<td>4476</td>
<td>&gt;65</td>
<td>39</td>
<td>6.2</td>
<td>Maximum IMT</td>
<td>Highest quintile</td>
<td>2.46 (1.51-4.01)</td>
</tr>
<tr>
<td>Hunt et al., 2001 (102)</td>
<td>12,375</td>
<td>45-64</td>
<td>54</td>
<td>7</td>
<td>Plaque presence</td>
<td>N/A</td>
<td>2.02 (1.42-2.41)</td>
</tr>
<tr>
<td>van der Meer et al., 2004 (103)</td>
<td>6389</td>
<td>&gt;55</td>
<td>62</td>
<td>7 to 10</td>
<td>Maximum IMT</td>
<td>Highest quartile</td>
<td>1.95 (1.19-3.19)</td>
</tr>
<tr>
<td>Johnsen et al. 2007 (104)</td>
<td>6226</td>
<td>25-84</td>
<td>44</td>
<td>6</td>
<td>Mean IMT</td>
<td>Highest quartile</td>
<td>2.86 (1.07-7.65)</td>
</tr>
<tr>
<td>Chien et al. 2008 (100)</td>
<td>2190</td>
<td>&gt;35</td>
<td>55</td>
<td>10.5</td>
<td>Max IMT</td>
<td>1SD</td>
<td>1.38 (1.12-1.70)</td>
</tr>
<tr>
<td>Cournot et al. 2009 (105)</td>
<td>2561</td>
<td>51.6=1 0.5</td>
<td>38.2</td>
<td>2 to 10</td>
<td>Mean IMT</td>
<td>&gt;0.63</td>
<td>2.26 (1.35-3.79)</td>
</tr>
<tr>
<td>Cournot et al. 2009 (105)</td>
<td>2561</td>
<td>51.6=1 0.5</td>
<td>38.2</td>
<td>2 to 10</td>
<td>Plaque presence</td>
<td>N/A</td>
<td>2.81 (1.84-4.29)</td>
</tr>
<tr>
<td>Plichart et al. 2011 (106)</td>
<td>5895</td>
<td>65-85</td>
<td>62.9</td>
<td>5.4</td>
<td>Plaques &gt;2 sites</td>
<td>N/A</td>
<td>2.2 (1.6-3.1)</td>
</tr>
<tr>
<td>Plichart et al. 2011(106)#</td>
<td>5895</td>
<td>65-85</td>
<td>62.9</td>
<td>5.4</td>
<td>Mean IMT</td>
<td>Fifth quintile</td>
<td>0.8 (0.5-1.2)</td>
</tr>
<tr>
<td>Jeboah et al. 2012(107) #</td>
<td>6814</td>
<td>45-84</td>
<td>33.3</td>
<td>7.6</td>
<td>Mean IMT</td>
<td>N/A</td>
<td>1.17 (0.95-1.45)</td>
</tr>
<tr>
<td>Polak et al. 2013 (108)</td>
<td>6562</td>
<td>61.1=1 0.2</td>
<td>52.6</td>
<td>7.8</td>
<td>Plaque presence</td>
<td>N/A</td>
<td>F: 1.67 (1.33-2.10)</td>
</tr>
</tbody>
</table>

RR* - adjusted relative risk. #studies that have excluded cases with carotid plaque presence in their analysis. Other studies have not excluded or even not reported if they have excluded cases with plaque presence or not. IMT was measured in CCA in all cases presented in this table.
In addition to c-IMT, carotid total plaque area and coronary artery calcium score (CACS) were proposed as subclinical measures of atherosclerosis that might be useful to predict vascular events.

**Quantifying carotid artery plaques: total plaque area and volume**

Measurement of the carotid plaque area has been proposed as a better predictor of future vascular events than merely their presence (109). In addition, carotid plaques have usually a twice higher rate of longitudinal enlargement than transverse growth, which makes measurement of plaque area much more accurate for assessing disease progression than measurement of its thickness (32). Measurement of carotid plaque area at baseline is a strong predictor of the combined outcome of MI, IS and vascular death. After adjusting for baseline patient’s characteristics, the combined 5-year risk increased by quartile of plaque area: 5.6%, 10.7%, 13.9%, and 19.5% (P < 0.001 for all) (109). Whereas c-IMT predicts IS more strongly than MI (110), carotid plaque area predicts both events; an area of 0.46–1.18 cm$^2$ has a 5 year risk of 12.3% for both events and a 3.9% risk for IS alone (109). Plaque area was also shown to be useful for accurate assessment of the effect of therapy. Measurement of total plaque volume (TPV), particularly its progression, has recently been shown as a better predictor for TIA, stroke, MI or death compared to c-IMT and plaque area (32).

**Coronary artery calcium score (CACS)**

The process of arterial wall calcification is an active process that involves osteoblast-like cells. Measurements of coronary arteries calcification (CACS) have been shown to provide incremental information over and above traditional risk factors for predicting coronary events (111), hence improving individual’s risk stratification (112). Although, a correlation between CACS in coronary and c-IMT in carotid arteries was determined (113), the former has higher predictive accuracy for CV events (114). Assessment of CACS by CT is easy, rapid, reproducible, and relatively cheap and requires only low-dose radiation. A CAC score (CACS) >300 has been shown to have a likelihood of coronary events almost 10 times higher compared to CACS of zero (112). Furthermore, CACS accurately reclassified patients at
intermediate cardiac risk according to Framingham Risk Score, with a net reclassification index (NRI) of 21.7% as low risk (CACS <100) and 30.6% as high risk (CACS >400) (115). However, there are some studies indicating that the absence of CAC does not completely eliminate the possibility of CAD in the future (116). A recent analysis of the Multi-Ethnic Study of Atherosclerosis (MESA) showed that 16% of cases with CACS of zero at baseline were found to have significant coronary artery stenosis (117). Furthermore, in almost 20% of intermediate risk asymptomatic patients with CACS of zero, there was significant stenosis confirmed with angiography and in one third of them myocardial ischemia was evident (118).

**Imaging atherosclerosis in multiple sites**

Some individuals with atherosclerotic disease are particularly prone to instability followed by plaque rupture and clinical complications. This instability could be influenced by systemic factors, such as infection, autoimmunity or genetics that affects simultaneously different arterial systems. Patients with unstable plaques in multiple arterial systems are at higher risk of suffering recurrent symptoms and complications. After three year follow up, the rates of MI, IS or vascular death were 25% for patients with symptomatic disease in one vascular system and >40% in those with multiple diseased arterial systems (1). Indeed, patients with detectable disease in the coronary and peripheral arteries carry twice the level of risk compared to those presenting with disease in coronary artery alone (119). Interestingly, the presence of bilateral carotid disease was found to be a better predictor of CAD than the extent or severity of disease in either bifurcation (120). Moreover, the presence and severity of peripheral artery disease have been shown to be associated with high prevalence of carotid artery stenosis in the large self-referred and Life Line cohort, regardless of lower extremity symptoms (121). Furthermore, coronary artery disease with three vessels affected has proven to be an independent predictor of severe internal carotid artery stenosis and even total occlusion (122).
Management of carotid atherosclerosis

Early means for managing carotid artery stenosis was solely surgical endarterectomy. This approach has now changed and evolved. Patients are first commenced on medical therapy to control risk factors and to stabilize the arterial plaques. Non-surgical management includes percutaneous intervention with stent implantation (123). Carotid endarterectomy continues to be the treatment of choice in symptomatic patients with severe (>70%) luminal stenosis (36). Strict modification of the risk factors, including optimized antihypertensive therapy, lipid management, smoking cessation, and antiplatelet therapy, have led to less-compelling indications for immediate surgery in asymptomatic populations (124).

The two trials, Asymptomatic Carotid Atherosclerosis Study (ACAS) (125) and the Asymptomatic Carotid Surgery Trial (ACST) (126) provided level I evidence that CEA offers a 50% risk reduction for ipsilateral stroke in patients with moderate to severe asymptomatic carotid artery stenosis when compared with medical treatment (annual stroke risk: 1% vs 2%, respectively). However, at the time these trials were performed, patients received medical treatment that today would be considered suboptimal. The most definitive evidence for disease management in asymptomatic patients exists for medical therapy, with particular emphasis on statins, more effective antihypertensive medications, and antiplatelet therapy. Specifically, the advent of statins has provided a means for not only delaying plaque progression, but also possibly regressing already formed lesions and change of the plaque composition (127).

Stabilizing effects of statins on carotid atherosclerosis disease

It is well established that the cerebrovascular (CV) system benefits from statins (3-hydroxy-3-methylglutaryl-coenzyme A inhibitors [HMG-CoA]) with reports as early as 1994 (4S trial) (4). However, the decreased risk of CV disease could not be explained by inhibition of cholesterol biosynthesis alone. Later on, it was determined that in addition to lipid profile improvement; statins also induce some cholesterol-independent “pleiotropic” effects that contribute to atherosclerotic plaque stabilization. Indeed, numerous experimental and clinical studies indicated that some
of these effects might involve: restoring endothelial function, decreasing oxidative stress and decreasing vascular inflammation (128). By blocking the synthesis of isoprenoid intermediates (Rho proteins), statins play crucial roles in cellular events. Including improvement of endothelial cell function or restoration, inhibition of secretion of several matrix metalloproteinases (MMPs) from smooth muscle cells (SMCs) (129) and macrophages (130), inhibition of proliferation and migration of SMC from media to intima (131), also decreasing vascular inflammation that consists to stability of atherosclerotic plaques (132).

Reduction of stroke risk with statin therapy

Statins are among the most effective drugs in reducing the risk of stroke. An early meta-analysis (133) showed a significant reduction of stroke risk in patients receiving statins compared to placebo with an overall risk reduction of 31% . Also, two other meta-analyses published recently confirmed the effect of statins on stroke reduction. The first one (134) determined that for each 10% decrease in LDL cholesterol the estimated risk reduction for any stroke was 15.6%. And the second study, using individual data from 90,000 individuals, came to the same conclusion (135) showing a significant reduction in fatal and nonfatal stroke with a good overall safety profile and no increased incidence of hemorrhagic stroke. The Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) study was a randomized clinical trial which showed that in patients with recent stroke or TIA, five-year treatment with atorvastatin 80 mg daily reduced the incidence of stroke and cardiovascular events compared to placebo (5-year absolute risk reduction 2.2%) (136). However, approximately 25% of patients in the placebo group were prescribed a commercially available statins outside the trial. In a post hoc analysis, LDL-C reduction was, therefore, used as the best marker for being adherent to the allocated treatment. The group with more than 50% LDL-C reduction from baseline was probably adherent to atorvastatin 80 mg/day who showed a 31% relative risk reduction in stroke without increase risk in brain hemorrhage (137).
Safety of statin therapy

Statins are usually well-tolerated drugs with few adverse effects, including myopathy and elevation of liver enzymes. Rhabdomyolysis occurs rarely (138). Recently, the FDA recommended removing routine monitoring of liver enzymes because serious liver injury is rare and unpredictable (139). There is conflicting data on the possible effects of statins in increasing risk of intracranial hemorrhage, cognitive impairment, diabetes mellitus and cancer, but this suggestion has been refuted by a recent meta-analysis which showed that statins were not associated with intracranial hemorrhage (140). In contrast to what was believed before, two recent studies found a decrease in newly diagnosed cases of dementia in elderly patients (141) and patients with diabetes who had received statins (142). As for diabetes mellitus, two comprehensive meta-analyses (143, 144) have shown a slightly increased risk of diabetes development in subjects on statins, however, the risk is low both in absolute terms and when compared with the reduction in cardiovascular events. The increased risk of cancer was also suspected since the finding that low levels LDL were associated with a higher risk of cancer incidence. Until now, two studies (145, 146) found an increased risk of cancer in patients on statins, but two meta-analyses (147, 148) failed to confirm this observation. In addition, a recent study showed a decreased incidence of hepatocellular carcinoma among patients treated with statins (149).
Based on the foregoing knowledge of carotid atherosclerotic plaque appearance on US imaging and the effect of statins on stabilizing plaques, several questions remain to be answered, among which 4 are addressed in this thesis.

I. The morphology, echogenicity and texture of carotid plaques of the contralateral side of symptomatic arteries
II. The relationship of the morphology and texture of the carotid plaque at bifurcation and ICA, with the conventional measurement of c-IMT and its echogenicity
III. The relationship between CCA measurements (IMT and IM-GSM) and the systemic burden of atherosclerosis
IV. Systematic review analysis of the studies on the net effect of statin therapy on plaque echogenicity as measured by GSM.
OBJECTIVES

The objective of the thesis was to study, in detail, the systemic nature of atherosclerosis by evaluating the disease burden in contralateral to symptomatic arteries, determining the relationship between proximal (subclinical atherosclerosis) and distal segments (well established disease) of the same artery. Also, we aimed to determine the intima-media complex measurements relationship with the systemic burden of atherosclerosis and to evaluate, in a systematic review and meta-analysis design, the effect of statins on carotid plaque echogenicity.

Study I

The aim of this study was to evaluate in detail the status of the contralateral carotid system to symptomatic artery. To compare the plaque morphology and textural characteristics of the contralateral carotid arteries, with respect to the culprit side in a group of patients who received carotid endarterectomy as well as patients who did not have a prior cerebrovascular event.

Study II

To assess the potential associations between the proximal carotid artery markers of atherosclerosis and the more distal and clinically relevant lesions, which could be used for optimum disease risk assessment.

Study III

To compare carotid artery wall measurements (IMT and IM-GSM) between different patient groups, based on prior vascular symptoms in different arterial systems and the number of diseased arteries.

Study IV

To determine, in a systematic and meta-analysis model, the response of plaque features ‘echogenicity” to statin therapy in patients with carotid artery disease.
METHODS

The preoperative evaluation before CEA at Umeå Stroke Center

As a clinical routine, a carotid ultrasound is performed at the department of physiology at the University Hospital of Northern Sweden, by an experienced vascular sonographer. A CT, MRI, or conventional angiography is performed, either at the University Hospital of Northern Sweden or would have been performed at the referring hospital. Several experienced neuroradiologists review the results of the CTA, MRA or conventional angiography results. All carotid imaging is aimed at reproducing NASCET-type carotid stenoses. An echocardiogram is also routinely performed, either at the University Hospital of Northern Sweden or at the referring hospital. This is followed by a detailed neurological examination by a neurologist who evaluates the symptoms and whether they conform to the anterior circulation. Before the CEA, an internal medicine specialist evaluates the perioperative risk and adjusts the medical treatment if warranted. Then the decision for CEA is undertaken after discussion among the neurologist, internal medicine specialist and vascular surgeon.

Carotid ultrasound

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. The severity of carotid stenosis was assessed by conventional Doppler ultrasound criteria (150). All velocity cut-off values were related to the angiographic evaluation of the carotid stenosis according to the criteria of the NASCET (151).

Ultrasound data retrieve and imaging extraction

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

1) Ultrasound to panorama (UtP) arm of the SPACE study (152),
2) Additional Neurological SYmptoms before Surgery of the Carotid Arteries — a Prospective study (ANSYSCAP) (153)

Over the course of both studies, the ultrasound imaging storage process was upgraded from analogue to digital. In the first study, we used data of the UtP arm of the SPACE study stored as analog, and in the 2-nd and 3-rd study, we used only a subgroup of asymptomatic patients of the ANSYSCAP study, stored in digital.

The ultrasound images stored in the VHS or digital system were exported to the EchoPac software (General Electric, EchoPac version 8.0.1, Waukesha, WI) where morphological features (plaque irregularities and calcification) were assessed and IMT was measured. Following this and using an in-house custom developed research software package (Department of Biomedical Engineering — R&D, Umeå University Hospital, Umeå, Sweden) we performed imaging normalization and measurement of plaque IM-GSM, plaque GSM and other textural plaque features.

**Morphological plaque feature calculation**

Plaque irregularity: The surface irregularities of the plaque were categorized as:
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) (154)

Plaque calcification: was defined as hyperechogenic spots within the plaque with posterior shadowing.

**Textural plaque features calculation**

The image was normalized by selecting a ROI within the darkest spot of the vessel lumen avoiding areas of “noise”, and another ROI including the brightest part of the adventitia. Then the image pixels were normalized using the ROIs, such that the intensity of the blood was set at 0 and the brightest adventitia at 190. The pixel density was standardized to 20 pixel/millimeter. The plaque in the normalized and standardized image was then manually outlined and cropped prior to feature
calculation (Figure 4). The described technique was similar to that used by Nicolaides et al. (155).

**Figure 4.** Carotid ultrasound image normalization and textural plaque features evaluation

*Grey scale median (GSM):* This was the median of the grey values of all pixels within cropped plaque image.

*Juxtaluminal black-area (JBA):* These are black areas close to the lumen without visible echogenic cap (65). The area was manually detected and outlined. The area and corresponding GSM were calculated by the software. The larger value was used in cases with several JBAs within a plaque.

*Plaque type:* Plaques were classified automatically by the software into the following four types according to the modified Geroulakos classification (60):

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 which occupy 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.

Discrete white areas (DWAs): The presence of discrete white areas was defined as those with pixels having grey scale values >124 (colored red by the software) not producing acoustic shadowing in plaque types 1 to 3 (54).

Entropy: This is a measure of the random nature of the grey-tone values within the plaque (156), giving high values for heterogeneous composition, and low values for homogenous tissue.

Coarseness (Study II): This feature quantifies the granularity of the plaque texture. The texture is coarse when there is a high degree of local uniformity in intensity for large areas. Coarseness was calculated using the neighborhood grey-tone difference matrix method (64). Previous research has demonstrated that this feature discriminates symptomatic from asymptomatic plaques (157) where the former had lower coarseness consistent with more heterogenic, more granular, and less uniform composition.

Intima–media complex measurements (Study II and III): Both IMT and IM-GSM measurements were made in the distal segment of the common carotid artery within 1 cm (±2 mm) distance starting from the carotid bifurcation at both sides. 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.

Carotid intima-media thickness (IMT) (Study II and III): IMT was conventionally defined as the distance between the lumen–intima interface and the media–adventitia interface. One centimeter segments of the far wall carotid arteries were measured in triplicate on a single frame, and then averaged (158).

Intima–media grey scale median (IM-GSM) (Study II and III): This was the median of the grey values of all pixels within a one-centimeter length cropped intima media complex image (Figure 5). The distal c-IM complex in the normalized and
standardized image was manually outlined and cropped, prior to feature calculation (Figure 5).

**Figure 5.** IM-GSM measurement in CCA and plaque GSM measurement in carotid artery bifurcation

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

All patients with symptomatic carotid stenosis had an ipsilateral ischemic cerebrovascular event (stroke, TIA or retinal artery embolization) within the 6 months prior to the carotid scan, whereas none of the patients with asymptomatic carotid stenosis had any cerebrovascular event within the same period.

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

Patients clinical data were prospectively followed 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. All patients underwent a thorough clinical examination including neurological assessment. 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).

A plaque was defined as focal protrusion into the carotid lumen, 50% greater than the surrounding wall thickness \(^{(159)}\). Each individual plaque was analyzed for irregularity, calcification, GSM, JBA, GSM of the JBA, plaque type, DWAs and entropy. In the arteries with 1 plaque, the features of that plaque represented the artery. In the arteries with >1 plaque the findings from all plaques were congregated to represent that artery i.e. the area of JBA denotes the area of all JBAs. For the categorical features, the worst abnormality in all plaques was used to represent the artery, e.g. the most irregular plaque and lowest plaque type number. If a calcification, JBA and DWA were detected in any plaque, that artery was considered positive for calcification, JBA and DWA, respectively. For the continuous features (GSM, area of JBA and entropy) we used an area-weighted average by taking the relative size of the plaques into account. The overall plaque feature was assessed as the sum of the individual features multiplied by plaque area and divided by the total plaque area.
Study I
This is a secondary analysis of the Ultrasound of the Panorama arm of the SPACE study (152). In the main study, we included consecutive patients with symptomatic or asymptomatic carotid stenosis. We included patients who underwent carotid endarterectomy and agreed to undergo an extensive pre-operative evaluation.

Inclusion criteria
There were 47 cases with a preoperative ultrasound examination stored in the analog format. We excluded 8 patients 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) cerebrovascular event that was not ipsilateral to the stenosis. Thus, 39 patients were analyzed, 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 was covered by shadow derived from extensive plaque calcification (n=10). In total, we have analyzed 66 arteries (25 contralateral, 30 symptomatic and 11 asymptomatic). The contralateral arteries were compared with the symptomatic arteries and with the asymptomatic arteries.

Study II and III
These studies represent a secondary analysis of the ANSYSCAP study.

ANSYSCAP inclusion criteria
In ANSYSCAP (153), consecutive patients with a 50–100% carotid stenosis/occlusion were prospectively included between August 2007 and December 2009. In the main analysis, only those with a recently (within 6 months) symptomatic 50–99% carotid stenosis preliminarily eligible for carotid endarterectomy (CEA) were analyzed. However, an additional 133 patients with asymptomatic 50–99% carotid stenosis were also included, which were not analyzed in the main analysis.
Secondary analysis inclusion criteria

In this study we included only asymptomatic patients whose carotid ultrasound examinations were stored in digital format and could be retrieved and analyzed (n = 87). In addition to carotid plaque features we evaluated the intima-media complex of the common carotid artery. IMT and IM-GSM were measured in 137 arteries of 87 patients and the derived data were correlated with plaque GSM and other morphological and textural features (Figure 6).

![Flow-chart of patients' selection](image)

**Figure 6.** Flow-chart of patients' selection. Of note, in the third study we have excluded only cases when the CCA segment free of plaque was <8mm

Study IV

The methodology for this study was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (160).

*Information Search and Data Collection*

Up to April 2015, we systematically searched electronic databases (PubMed, MEDLINE, EMBASE and Cochrane Center Register) for studies evaluating the effect
of statins on carotid plaque echogenicity. The search terms used were: “carotid atherosclerosis”, “carotid plaque”, “ultrasound” “statins”, “HMG-CoA reductase inhibitors” and “lipid-lowering drugs”, in various combinations. Two researchers (Pranvera Ibrahimi and Fisnik Jashari) independently performed the literature search, study selection and data extraction. There was no time, language or publication limit in the literature search. The selected reports were manually searched, and relevant publications, obtained from the reference lists, were retrieved.

Study Eligibility Criteria

Clinical studies that reported results on the effect of statins therapy on the plaque echogenicity (GSM, IBS) evaluated by duplex ultrasound were eligible. Specific inclusion criteria were: (1) observational, non-randomized or randomized studies that explored the effect of statins treatment either as primary or secondary cardiovascular disease prevention; (2) ultrasound of the carotid arteries before and at least once at a follow-up of at least one month; (3) English language articles; (4) studies with ≥15 subjects; and (5) ultrasound-based characterization of carotid artery plaque composition. All other studies that used different imaging techniques (e.g., MRI, CT, IVUS, PET) and those that used plaque features other than echogenicity (volume, degree of stenosis, ulceration, neovascularization) as a target for monitoring statins therapy were excluded. We have performed a quality score of the retrieved studies utilizing the methodological index for the non-randomized studies (MINORS) (161). Studies that scored over 20 out of 24 (or 14 out of the 16 for those non-comparative, but rather solely observational) were considered of adequate quality. In the meta-analysis, we included studies that specified duration of the study and presented plaque echogenicity means and standard deviations prior to and during (or at the completion of) the intervention or the percent change in plaque echogenicity before and during intervention.
Statistical Analyses

Study I, II and III. Categorical variables were expressed as percentages and continuous variables were expressed as mean ± SD (median). Mean values of the plaque texture features were compared between groups, using independent samples t-test and Mann-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. Spearman's correlation coefficient was used to correlate carotid wall measurements between intima–media and plaques and Pearson's correlation coefficient was used to correlate measurements between continuous variables. Linear regression analyses were used to determine the relationship of the intima media measurements with plaque features, independently of IMT and other risk factors. Statistical significance was indicated by a p value <0.05. We used SPSS statistics 22.0 software.

Study IV. For the plaque echogenicity analysis, the treatment effects of interest were the differences in the extent of changes in echogenicity (GSM or IBS), low-density lipoprotein cholesterol (LDL), high-density lipoprotein (HDL) and high sensitivity C-reactive protein (hsCRP) before and after treatment. Because of the significant variation in study size, length and follow-up, as well as patient’s characteristics, we used random-effects. Heterogeneity was measured using I² statistics. We performed analyses within each imaging group stratified by pre- vs. post-treatment. All analyses were conducted using Comprehensive Meta Analysis Version 3 software (Biostat inc., Englewood, NJ, USA).
RESULTS

Study I

In this study we included 39 patients. Mean age of the patients was 70±7 years and 33% were females. There was no difference in the risk factors (e.g. hypertension, diabetes, dyslipidemia, smoking) between symptomatic and asymptomatic patients (Table 4). The degree of stenosis was higher in symptomatic arteries, but there was no difference in the degree of stenosis between contralateral to symptomatic and asymptomatic arteries.

Contralateral carotid plaques had similar features to symptomatic ones, with GSM (26.2 ± 7.3 vs. 24.9 ± 7.8, p = 0.536) and GSM of the JBA (5.0 ± 3.9 vs. 4.6 ± 3.0, p = 0.8). Plaque irregularities and plaque types were similar between the two groups with no statistical difference (p = 0.25) and (p = 0.75) respectively. The only difference observed between the contralateral and symptomatic arteries was the prevalence of JBA, which was lower in the contralateral artery plaques than the plaques in the symptomatic arteries (p = 0.001).

On the other hand, contralateral arteries had more vulnerable morphologic and textural plaque features compared to those in the asymptomatic arteries, with higher prevalence of mild (60% vs. 36%) and marked irregularities (28% vs. 9%), p = 0.03, 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), and higher prevalence of plaque type 1 and 2 and lower prevalence of type 4 (p = 0.001) (Figure 7, 8, 9 and Table 5). Other textural plaque features including: JBA, mean entropy, frequency of plaque calcification and DWA, were not statistically different between the groups (Table 5).
<table>
<thead>
<tr>
<th></th>
<th>Symptomatic (n = 33)</th>
<th>Asymptomatic (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>71.2 ± 7.3</td>
<td>66 ± 7.8</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>11 (33.3)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>2 (6.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>10 (30.3)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Previous myocardial infarction (%)</td>
<td>5 (15.2)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Current angina, n (%)</td>
<td>1 (3.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Stroke 6 months before, n (%)</td>
<td>8 (24.2)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Claudication, n (%)</td>
<td>6 (18.2)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Previous revascularization of any type, n (%)</td>
<td>9 (27.3)</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Any lipid lowering medication, n (%)</td>
<td>31 (93.9)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Any antiplatelet or anticoagulation medication, n (%)</td>
<td>33 (100)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Any blood pressure lowering therapy, n (%)</td>
<td>32 (97)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg), mean (SD)</td>
<td>152.91 ± 24</td>
<td>147 ± 16</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg), mean (SD)</td>
<td>79.7 ± 13.2</td>
<td>82.4 ± 8</td>
</tr>
<tr>
<td>Total cholesterol, mean (SD)</td>
<td>4.5 ± 1</td>
<td>4.57 ± 1.3</td>
</tr>
<tr>
<td>HDL cholesterol, mean (SD)</td>
<td>1.3 ± 0.7</td>
<td>1.14 ± 0.2</td>
</tr>
<tr>
<td>LDL cholesterol, mean (SD)</td>
<td>2.51 ± 0.9</td>
<td>2.52 ± 0.9</td>
</tr>
<tr>
<td>HbA1c, mean (SD)</td>
<td>5.19 ± 1.2</td>
<td>4.7 ± 0.5</td>
</tr>
</tbody>
</table>
Table 5. Morphological and textural features between groups

<table>
<thead>
<tr>
<th>Features</th>
<th>Asymptomatic ( (n = 11) )</th>
<th>Contralateral to symptomatic ( (n = 25) )</th>
<th>Symptomatic ( (n = 30) )</th>
<th>Between three groups ( (p) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSM, mean (SD)</td>
<td>49.38 ± 14.6</td>
<td>26.18 ± 7.3</td>
<td>24.9 ± 7.8</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Entropy, mean (SD)</td>
<td>3.58 ± 0.1</td>
<td>3.51 ± 0.2</td>
<td>3.51 ± 0.2</td>
<td>0.727&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>JBA, ( n ) (%)</td>
<td>5 (45)</td>
<td>7 (28)</td>
<td>24 (80)</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>JBA area (mm&lt;sup&gt;2&lt;/sup&gt;), median</td>
<td>0.5</td>
<td>5.2</td>
<td>5</td>
<td>&lt;0.001&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSM of the JBA, mean (SD)</td>
<td>11.4 ± 2.1</td>
<td>5.0 ± 3.9</td>
<td>4.6 ± 3.0</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Calcification, ( n ) (%)</td>
<td>6 (55)</td>
<td>19 (76)</td>
<td>21 (70)</td>
<td>0.456&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DWA, ( n ) (%)</td>
<td>9 (82)</td>
<td>13 (52)</td>
<td>16 (53)</td>
<td>0.216&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irregularities</td>
<td></td>
<td></td>
<td></td>
<td>0.006&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Smooth, ( n ) (%)</td>
<td>6 (55)</td>
<td>3 (12)</td>
<td>2 (7)</td>
<td></td>
</tr>
<tr>
<td>Mildly, ( n ) (%)</td>
<td>4 (36)</td>
<td>15 (60)</td>
<td>13 (43)</td>
<td></td>
</tr>
<tr>
<td>Markedly, ( n ) (%)</td>
<td>1 (9)</td>
<td>7 (28)</td>
<td>15 (50)</td>
<td></td>
</tr>
<tr>
<td>Plaque types (PT)</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PT 1, ( n ) (%)</td>
<td>0 (0)</td>
<td>3 (12)</td>
<td>5 (17)</td>
<td></td>
</tr>
<tr>
<td>PT 2, ( n ) (%)</td>
<td>0 (0)</td>
<td>11 (44)</td>
<td>15 (50)</td>
<td></td>
</tr>
<tr>
<td>PT 3, ( n ) (%)</td>
<td>6 (55)</td>
<td>10 (40)</td>
<td>10 (33)</td>
<td></td>
</tr>
<tr>
<td>PT 4, ( n ) (%)</td>
<td>5 (45)</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Degree of stenosis</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0-49%, ( n ) (%)</td>
<td>4 (36)</td>
<td>13 (52)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>50-69%, ( n ) (%)</td>
<td>1 (9)</td>
<td>6 (24)</td>
<td>5 (17)</td>
<td></td>
</tr>
<tr>
<td>70-99%, ( n ) (%)</td>
<td>6 (55)</td>
<td>6 (24)</td>
<td>25 (83)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> One way ANOVA; <sup>b</sup> Exact 2-sided chi-2-test; <sup>c</sup> Kruskal–Wallis test; <sup>d</sup> Student T-Test.
Figure 7. Surface plaque irregularities between groups

Figure 8. Differences of GSM between groups
Figure 9. A-asymptomatic echogenic plaque with high GSM, B-contralateral to symptomatic echolucent plaque without visible JBA, C-symptomatic echolucent plaque with low GSM and high JBA

Study II
In this study we have analyzed 87 asymptomatic patients with significant carotid artery stenosis; mean age 69 ± 6 years, 34.5% females. The severity of carotid stenosis and the risk factors for atherosclerosis (e.g. age, hypertension, dyslipidemia) and also concomitant therapy are presented in table 6. Carotid stenosis > 50% was found in 67.8% (n = 93) of the arteries included in the final analyses. Mildly irregular plaques were found in 24% (n = 34) and moderately irregular plaques in 13% (n = 18) arteries.

The mean value of the IMT was 0.97 ± 0.2 mm, and the mean value of IM-GSM was 31 ± 16. A mean value of GSM was 36±17 and the median value of JBA was 6.53 mm². Plaque type (PT) 1 was detected in 2%, PT 2 in 31%, PT 3 in 42% and PT 4 in 25% of the arteries. Higher IMT values in CCA correlated with plaque irregularities (p<0.001) and with the degree of stenosis (p=0.001) in the bifurcation and ICA (Figure 10, Table 7). IM-GSM was closely related to plaque echogenicity (GSM) (r =
0.76, p < 0.001), (Table 8, Figure 11), and modestly related to plaque coarseness (r = 0.65, p < 0.001), the JBA (r = −0.52, p < 0.001) and the GSM of the JBA (r = 0.48, p = 0.005). IM-GSM also correlated with plaque GSM of arteries with ≥ 50% and <50% stenosis, r = 0.72 and r = 0.75, respectively (Table 9 and 10).

Patients with bilateral carotid stenosis (≥ 50%) had more vulnerable arterial wall and plaque measures compared to those with unilateral carotid stenosis; higher IMT (p=0.006), lower IM-GSM (p<0.001) and lower plaque GSM (p=0.016) and higher JBA (p=0.006) (Figure 12).

**Table 6. Patients’ data**

<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, (%)</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 (mm Hg), mean ± SD</td>
<td>145.7 ± 20</td>
</tr>
<tr>
<td>DBP (mm Hg), 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 7. Correlation between IMT in the CCA and other textural and morphological features in the plaques located in the bifurcation and ICA

<table>
<thead>
<tr>
<th>IMT</th>
<th>Plaque (bifurcation/ICA)</th>
<th>Correlation coefficient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSM</td>
<td>-0.132*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Coarseness</td>
<td>-0.157*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>JBA area (mm²)</td>
<td>0.165*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>GSM of the JBA</td>
<td>0.258*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Entropy</td>
<td>0.049*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Calcification</td>
<td>0.013#</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Degree of stenosis</td>
<td>0.274#</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Irregularities</td>
<td>0.533#</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Bilateral stenosis</td>
<td>0.213#</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

*Pearson correlation, #Spearman rho

Table 8. Correlation between IM-GSM in the CCA and other textural and morphological features in the plaques located in the bifurcation and ICA

<table>
<thead>
<tr>
<th>IM-GSM</th>
<th>Plaque (bifurcation/ICA)</th>
<th>Correlation coefficient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSM</td>
<td>0.761*</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Coarseness</td>
<td>0.649*</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>JBA area (mm²)</td>
<td>-0.524*</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>GSM of the JBA</td>
<td>0.478*</td>
<td>&lt;0.01</td>
<td></td>
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<tr>
<td>Entropy</td>
<td>0.017*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Calcification</td>
<td>0.106#</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Degree of stenosis</td>
<td>-0.186#</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Irregularities</td>
<td>0.004#</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Bilateral stenosis</td>
<td>-0.269#</td>
<td>&lt;0.001</td>
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</tr>
</tbody>
</table>

*Pearson correlation, #Spearman rho
Table 9. Correlation between IM-GSM in the CCA and other textural and morphological features in the plaques located in the bifurcation and ICA, for arteries with ≥50% stenosis

<table>
<thead>
<tr>
<th>IM-GSM (CCA)</th>
<th>≥50% stenotic plaques (bifurcation/ICA)</th>
<th>Correlation coefficient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSM</td>
<td>0.722*</td>
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<td></td>
</tr>
<tr>
<td>Coarseness</td>
<td>0.642*</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>JBA area (mm²)</td>
<td>-0.535*</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>GSM of the JBA</td>
<td>0.573*</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Entropy</td>
<td>0.062*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Calcification</td>
<td>0.122#</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Degree of stenosis</td>
<td>-0.016#</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Irregularities</td>
<td>0.032#</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

*Pearson correlation, #Spearman rho

Table 10. Correlation between IM-GSM in the CCA and other textural and morphological features in the plaques located in the bifurcation and ICA, for arteries with <50% stenosis

<table>
<thead>
<tr>
<th>IM-GSM (CCA)</th>
<th>&lt;50% stenotic plaques (bifurcation/ICA)</th>
<th>Correlation coefficient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSM</td>
<td>0.753*</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Coarseness</td>
<td>0.645*</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>JBA area (mm²)</td>
<td>-0.488*</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>GSM of the JBA</td>
<td>0.306*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Entropy</td>
<td>0.178*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Calcification</td>
<td>0.107#</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Irregularities</td>
<td>0.250#</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

*Pearson correlation, #Spearman rho
Figure 10. IMT in the CCA and plaque irregularities in the bifurcation and ICA

Figure 11. Correlation between IM-GSM in the CCA and GSM of the plaque located at the bifurcation and ICA
Study III

In this study we included 87 asymptomatic carotid patients: mean age 69±6 year, 34.5% females. 23% of patients had previous MI, 16% had angina, 21% had ischemic stroke >6 months, and 22% had lower limb atherosclerosis in the form of intermittent claudication (Table 4).

Patients with previous MI had higher IMT (1.06±0.2 vs. 0.95±0.2 mm, p=0.034) and lower IM-GSM (21±15 vs. 33±16, p<0.001) than those without. 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). There was no difference in IMT or IM-GSM between patients with and without previous atherosclerosis disease in the lower extremity (Figure 13).

50% of the patients were asymptomatic, 34% had previous disease in one arterial system and 16% had previous disease in multi-arterial systems. IM-GSM showed significant difference between groups, it was significantly decreased 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 asymptomatics, symptoms in one and symptoms in multi-arterial system disease, respectively. When analyzing IM-
GSM measured in the arteries on the side with higher IMT, the results were similar (35.0±15.7 vs. 29.5±18.6 vs. 17.4±13.9), p=0.001. Using ROC curve analysis a cut-off of 25 was determined for IM-GSM association with multisite atherosclerosis. Unadjusted and adjusted odds ratios (OR) for IM-GSM <25 association with multisite atherosclerosis disease was [OR 3.23 (95%CI 1.33-7.85), p<0.01] and [OR 2.65 (95%CI 1.56-6.67), p=0.03], respectively.

In addition, plaque GSM was significantly decreased with increasing number of previously symptomatic arterial systems (Table 11). Conventional IMT showed an increasing trend (0.95±0.2mm vs. 0.98±0.2mm vs. 1.02±0.02mm) for asymptomatics, symptoms in one and symptoms in multi-system arterial disease, respectively, but it was not significantly different between groups p=0.49 (Table 11). The degree of carotid artery stenosis was not different between groups (p=0.32) (Figure 14).
Table 11. Common carotid wall and bifurcation plaque measurements in different groups

<table>
<thead>
<tr>
<th></th>
<th>Asymptomatic (n=43)</th>
<th>Previous symptoms in one arterial system (n=30)</th>
<th>Previous symptoms in &gt;one arterial system (n=14)</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>

(*: IM-GSM mean of both sides; #: IM-GSM measured on the side with higher IMT)

Figure 13. IM-GSM, plaque GSM and plaque coarseness were significantly decreased with increasing number of arterial systems affected by atherosclerosis
Figure 14. IM-GSM in patients with previous stroke, MI, angina and claudication intermittent

Study IV

Searching the PubMed, 576 studies were identified in total and no additional studies were found in MEDLINE, EMBASE or in the Cochrane Center Register (Figure 15). In total nine studies (76, 127, 162-168) were included in the qualitative and all of them met the inclusion criteria to be quantitatively analyzed in a meta-analysis. Two of these studies had analyzed patients in two groups separately and we have included both groups in the meta-analysis.
The mean follow up period of patients was 7.2 months (range 1–12). Two different methods of quantifying plaque echogenicity were used in the included studies. GSM was used in five and IBS was used in the remaining four studies. All individual studies included in the meta-analysis showed a significant increase of plaque echogenicity after statin therapy. In the compared high (atorvastatin 80 mg/d) vs. low (atorvastatin 20 mg/d) statin therapy, the GSM was significantly increased more in the group receiving aggressive statin therapy. For each individual study we presented the percentage (%) change of the echogenicity from baseline and reported it as a mean difference %, which was finally used for pooled analysis (Table 12 and 13).

Meta-analysis results showed a consistent increase of carotid plaque echogenicity after statins therapy. Pooled weighted mean difference % (WMD) on plaque echogenicity after statins therapy was 29% (95% CI: 22%–36%), p < 0.001, I² = 92.1% (Figure 16). In a meta-regression analysis, the mean changes % of LDL, HDL and hsCRP from baseline were used as moderators to evaluate their association with
changes in plaque echogenicity. The increased plaque echogenicity with statins therapy was independent of LDL ($\beta = 0.32 \ (-0.28-0.94)$, $p < 0.29$) (Figure 17a) and HDL cholesterol ($\beta = 0.91 \ (-0.01-3.55)$, $p = 0.051$) (Figure 17b), but it was related to hsCRP changes from the baseline ($\beta = 1.01 \ (0.49-1.52)$, $p < 0.001$) (Figure 17c).

Patients were also divided into subgroups and analyzed based on treatment period. The effects of statins on carotid plaque was evident after the first month of treatment, however, this increase was higher in the following six and 12 months (Figure 18). Mean difference % was 16.2% (5.2–27.2%) vs. 30.4% (18.2–42.3%) vs. 35.4% (26.3–44.4%), $p = 0.03$, between 1, 6 and 12 months of treatment, respectively. In addition, it seems that the effect of statins on carotid plaque echogenicity is independent of cholesterol levels at baseline, since it was similarly increased in both groups. WMD% for hypercholesterolemic patients was 29.1 (21.3–36.8) and 28.8 (15.2–42.3) for non-hypercholesterolemic patients at baseline. We have meta-analyzed the effect of statins on LDL, HDL and hsCRP for the studies included in this paper, data presented in the Figures 19, 20, 21.

Table 12. Studies included in meta-analysis

<table>
<thead>
<tr>
<th>Author/Year</th>
<th>Population (n)</th>
<th>Mean Age±SD</th>
<th>Gender (male)</th>
<th>Hypercholesterolemic</th>
<th>Carotid stenosis</th>
<th>Echogenicity measured</th>
<th>Minor score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.Watanabe et al. 2005 (162)</td>
<td>30</td>
<td>69.9±8.8</td>
<td>63%</td>
<td>No</td>
<td>Moderate</td>
<td>IBS</td>
<td>RT</td>
</tr>
<tr>
<td>2.Yamagami et al. 2008 (163)</td>
<td>41</td>
<td>63.4±8.3</td>
<td>24%</td>
<td>Yes</td>
<td>Moderate</td>
<td>IBS</td>
<td>RT</td>
</tr>
<tr>
<td>3.Nakamura et al. 2008 (164)</td>
<td>33</td>
<td>60±9</td>
<td>25%</td>
<td>Yes</td>
<td>Moderate</td>
<td>IBS</td>
<td>RT</td>
</tr>
<tr>
<td>4.Kadoglou et al. 2008 (127)</td>
<td>113</td>
<td>63.6±9.9</td>
<td>67%</td>
<td>Yes</td>
<td>Moderate symptomatic</td>
<td>GSM</td>
<td>20</td>
</tr>
<tr>
<td>5.Yamada et al. 2009 (165)</td>
<td>40</td>
<td>71±8</td>
<td>90%</td>
<td>No</td>
<td>30-60%</td>
<td>IBS</td>
<td>RT</td>
</tr>
<tr>
<td>6.Kadoglou et al. 2009 (166)</td>
<td>67+46</td>
<td>66.7±7.3</td>
<td>40%</td>
<td>No</td>
<td>&gt;40%</td>
<td>GSM</td>
<td>20</td>
</tr>
<tr>
<td>7.Kadoglou et al. 2010 (167)</td>
<td>66+65</td>
<td>64.9±10</td>
<td>46%</td>
<td>Yes</td>
<td>30-60%</td>
<td>GSM</td>
<td>24</td>
</tr>
<tr>
<td>8.Della-Morte et al. 2011 (76)</td>
<td>40</td>
<td>&gt;45</td>
<td>NA</td>
<td>Yes</td>
<td>NA</td>
<td>GSM</td>
<td>15</td>
</tr>
<tr>
<td>9.Nohara et al. 2013 (168)</td>
<td>25</td>
<td>63.9±8.1</td>
<td>50%</td>
<td>Yes</td>
<td>NA</td>
<td>GSM</td>
<td>20</td>
</tr>
</tbody>
</table>
Table 13. Studies’ characteristics and statins effect on plaque echogenicity, LDL, HDL and hsCRP level on the blood

<table>
<thead>
<tr>
<th>Author/Year</th>
<th>Study design</th>
<th>Statin/dose</th>
<th>Follow up months</th>
<th>% Change echogenicity</th>
<th>% Change LDL</th>
<th>% Change HDL</th>
<th>% Change hsCRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.Watanabe et al. 2005</td>
<td>Randomized case-control trial</td>
<td>Pravastatin</td>
<td>6</td>
<td>14.1±3.3</td>
<td>24.5±6.4</td>
<td>10.2±6.0</td>
<td>45.0±58.3</td>
</tr>
<tr>
<td>(162)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.Nakamura et al. 2008</td>
<td>Randomized case-control trial</td>
<td>Pitavastatin 4 mg</td>
<td>12</td>
<td>32.1±5.9</td>
<td>37.8±12.4</td>
<td>9.3±2.0</td>
<td>43.7±51.5</td>
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<tr>
<td>(164)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>3.Yamagami et al. 2008</td>
<td>Randomized case-control trial</td>
<td>Simvastatin 10 mg</td>
<td>1</td>
<td>10.6±4.3</td>
<td>34.2±18.4</td>
<td>0</td>
<td>43.0±119.8</td>
</tr>
<tr>
<td>(163)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.Kadoglou et al. 2008</td>
<td>Open-label prospective trial</td>
<td>Atorvastatin</td>
<td>6</td>
<td>36.0±15.2</td>
<td>41.7±19.9</td>
<td>4.5±2.4</td>
<td>58.9±34.0</td>
</tr>
<tr>
<td>(127)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.Yamada et al. 2009</td>
<td>Randomized case-control trial</td>
<td>Simvastatin</td>
<td>6</td>
<td>17.0±5.9</td>
<td>44.0±23.9</td>
<td>0</td>
<td>42.1±94.6</td>
</tr>
<tr>
<td>(165)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>6a.Kadoglou et al. 2009</td>
<td>Open-label prospective trial</td>
<td>Atorvastatin</td>
<td>6</td>
<td>36.8±9.8</td>
<td>38.6±20.0</td>
<td>13.4±6.6</td>
<td>78.3±74.9</td>
</tr>
<tr>
<td>(166)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6b.Kadoglou et al. 2009</td>
<td>Open-label prospective trial</td>
<td>Atorvastatin+ CAS</td>
<td>6</td>
<td>48.4±18.6</td>
<td>33.3±15.0</td>
<td>4.4±2.2</td>
<td>52.1±39.5</td>
</tr>
<tr>
<td>(166)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7a.Kadoglou et al. 2010</td>
<td>Randomized case-control trial</td>
<td>Atorvastatin 10-20 mg</td>
<td>12</td>
<td>32.6±11.7</td>
<td>64.5±23.6</td>
<td>5.5±2.6</td>
<td>52.9±55.2</td>
</tr>
<tr>
<td>(167)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>7b.Kadoglou et al. 2010</td>
<td>Randomized case-control trial</td>
<td>Atorvastatin 80 mg</td>
<td>12</td>
<td>51.4±18.4</td>
<td>54.2±37.2</td>
<td>10.3±6.0</td>
<td>65.0±80.0</td>
</tr>
<tr>
<td>(167)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.Della-Morte et al. 2011</td>
<td>Prospective pilot study</td>
<td>NA</td>
<td>1</td>
<td>21.9±4.8</td>
<td>51.4±31.0</td>
<td>2.0±1.1</td>
<td>NA</td>
</tr>
<tr>
<td>(76)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.Nohara et al. 2013</td>
<td>Prospective open label, blinded-endpoint</td>
<td>Rosuvastatin</td>
<td>12</td>
<td>16.9±33.1</td>
<td>50.1±22.9</td>
<td>8.1±3.6</td>
<td>NA</td>
</tr>
<tr>
<td>(168)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
### Figure 16. Effects of statins on plaque echogenicity. Note: Kadaglou 2009 has divided and analyzed patients in two groups: the first group (*) was on statins, but underwent contralateral carotid artery stenting (CAS), and the second group (**) was treated only with statins. Kadaglou 2010 has divided and analyzed patients into two groups: the first group (^) received atorvastatin 10–20 mg, and the second group (^^) atorvastatin 80 mg.
**Figure 17.** Meta-regression. Regression of LDL (a), HDL (b) and hsCRP (c) changes on plaque echogenicity after statins therapy.

**Figure 18.** Analysis of studies based on treatment period in months. The effect of statins on plaque echogenicity was obvious from the first month after treatment, and the effect was progressive on the following six and 12 months (m).
Figure 19. Effect of statins on LDL

Figure 20. Effects of statins on HDL

Figure 21. Effects of statins on hsCRP
Reproducibility of the measurements

Study I

The interobserver variability was expressed by intra-class correlation coefficient, for GSM it was 0.928 (95% CI 0.899–0.950) and for JBA it was (0.927; 95% CI 0.848–0.965). Furthermore, the interobserver agreement for the presence of JBA and Geroulakos plaque type between the same two independent observers was also satisfactory (K = 0.948, p < 0.0001 and α = 0.843, p < 0.001).

Study II and III

There was a good agreement between the two observers for both intima–media complex and plaque measurements. 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; 0.783–0.980). Also for the plaque measurements the intra-class correlation was 0.921 (95% CI; 0.89–0.942) and for JBA it was 0.910 (95% CI; 0.871–0.960) p < 0.001. The Bland–Altman test for the intima-media measurement is presented in Figure 22. There was not any proportional bias in our measurements; mean difference between the two observers' measures was not significantly different from zero.

![Figure 22](image)

**Figure 22.** a. Bland–Altman plot for IMT measurements. In linear regression analysis t-test was not significant (p = 0.178), there was no proportional bias. Bias = 0.0015, upper limits of agreement (LOA) = 0.016, and lower LOA = −0.0135.

b. Bland–Altman plot for IM-GSM. In linear regression analysis t-test was not significant (p = 0.121), there was no proportional bias. Bias = 1.27, upper LOA = 12.8 and lower LOA = −10.3.
DISCUSSION

Study I

The results of this study show that the contralateral to symptomatic carotid plaques have similar morphological and textural features to the symptomatic ones but more vulnerable features than the asymptomatic arteries.

It is generally believed that the pattern of atherosclerosis is unpredictable in patients with carotid artery disease. In our cohort, the symptomatic group had more aggressive disease, in the right and left carotid systems, in the form of irregular contour, more echolucent and with signs of inconsistent texture, suggesting vulnerable plaque nature compared to those seen in asymptomatic patients. Although the symptoms were unilateral, our results clearly show that very similar disease severity affected the contralateral carotid artery. However, the more frequently present JBA in the symptomatic side represents an additional evidence for plaque instability which could also help in better patient risk stratification. In addition, such findings confirm 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 and suggest that adding assessment of JBA to plaque GSM could be a better indicator for identifying high risk patients. Previous histological and imaging studies suggested that vulnerable patients may have multiple unstable lesions, other than the culprit ones, either distally or even in another territory (1, 169). These claims thus support our results. Likewise, biochemical findings suggest that aggressive inflammatory reactions might explain the development of symptoms in an arterial system (170, 171), which is otherwise similar to another that is asymptomatic (172).

Asymptomatic carotid arteries had less vulnerable plaque features as shown by higher GSM, and GSM of the JBA, and less frequently mild and markedly irregular plaques. It has been shown that videodensitometric computer analysis of carotid images can identify potentially unstable plaques, which are less echogenic than stable asymptomatic densely fibrotic plaques (63). Surface plaque irregularities, GSM and JBA have been evaluated previously in many carotid artery studies as an attempt to correlate with histology and to predict future cerebrovascular events (75, 78, 173). Studies that compared US findings with histology showed that the presence and
extend of JBA correlated with vulnerable phenotype of the carotid plaque and that low GSM is associated with large hemorrhagic areas, while lower GSM values located in the juxtaluminal position (close to the lumen) are associated with predominant necrotic cores (66). To our knowledge this is the first study that provides information on plaque vulnerability in contralateral carotid arteries as assessed by morphological and textural features.

**Study II**

The results of this study show that IMT measured in common carotid artery correlated with severity of plaque stenosis and irregularities in the carotid bifurcation and the internal carotid artery (ICA). Also, its echogenicity (IM-GSM) correlated with textural features of distal carotid plaques. IM-GSM was related to plaque echogenicity, even after adjusting for IMT and other conventional clinical risk factors (age, sex, hypertension, diabetes, LDL and HDL). Furthermore, patients with bilateral carotid disease had clear evidence for higher IMT and lower GSM values as well as more features for vulnerable plaque at the bifurcation and ICA.

The generalized inflammatory nature of atherosclerosis was confirmed in many studies (32). Increased common carotid IMT was considered a sign of early atherosclerotic changes and has been shown to predict cardiovascular and cerebrovascular events (63, 158) that result from bifurcation and proximal internal lesions in patients with known atherosclerotic disease. Also, a low IM-GSM was associated with increased cardiovascular morbidity and mortality (95, 174). However, the evidence of a potential relationship between measurements in two different segments of carotid artery, arterial wall measurements in CCA with plaque features in bifurcation and ICA has only been reported as modest (94, 175). The results of this study again support the concept of diffuse carotid disease, with significant stenosis at the site of bifurcation having its roots clearly defined at the proximal segment of the artery. These findings were confirmed in other arterial systems as well, as the severity of the disease in proximal segments of the coronary artery were closely related with severity of the disease parts at the branching points (176). Furthermore, the exaggerated features of plaque vulnerability in patients, with bilateral carotid disease compared to those with unilateral disease support this interpretation. We did not find any relationship between IMT in the CCA and textural plaque features, neither
between IM-GSM and morphological features, suggesting that these two measures may have just a complementary role in determining disease vulnerability.

**Study III**

This study demonstrates that IMT and IM-GSM measured in common carotid artery are associated with atherosclerosis disease affecting the other vascular systems, particularly coronary arteries but not atherosclerosis related lower extremity disease. Only IM-GSM was consistently different between patient groups according to the number of diseased arterial systems. It was progressively increased with increasing number of arterial systems affected by atherosclerosis. It was lower in patients with previous symptoms in one vascular system compared to truly asymptomatic patients and even lower in those with atherosclerosis in multi-system arterial disease. The conventional measure of the carotid arterial wall, IMT, was less sensitive in showing similar differences between groups.

Atherosclerosis as an inflammatory disease, although its worst consequences are derived by affecting coronary and carotid arteries, it could affect any arterial bed. Several studies have revealed a clinically important relationship between the two arterial systems, coronary and carotid. IMT and especially plaque presence in the carotid arteries is an important predictor of coronary artery disease in the future. Patients with previous stroke have been shown to be at risk of suffering another stroke and even higher risk of suffering a future MI (1). In addition, patients with generalized atherosclerotic disease, having multiple systems affected have been considered as “vulnerable patients”. In general, almost a quarter of patients with symptomatic disease in one vascular system stand a 25% risk for developing MI, stroke or vascular death compared to >40% in those having multiple diseased arterial systems (119). The latter group therefore, can be considered vulnerable in view of the high risk they carry for vascular events. It was only them, who had the lowest IM-GSM, meaning an echolucent intima-media complex, supporting this measure as a much more sensitive indicator for differentiating patients as they went from asymptomatic to symptomatic with single arterial system affected and finally to symptomatic multi-system disease.
IM-GSM is a newly introduced measure of the carotid arterial wall, like the GSM of the plaque; a low IM-GSM is associated with a soft intima-media layer of the common carotid artery. IM-GSM can add incremental information over and above conventional risk factors for optimum patient’s risk stratification. An echolucent IM-complex (low IM-GSM) was associated with a three-fold greater risk of all-cause mortality and an eight-fold greater risk of cardiovascular mortality (174).

Only few head-to-head comparisons between IMT and IM-GSM were previously attempted. While, increased IMT was related to increased systolic blood pressure and to male gender, low values of IM-GSM related to low levels of HDL-C and increased levels of inflammatory markers (CRP). IMT, itself, has been demonstrated to correlate with the severity of arterial stenosis and plaque irregularity, whereas IM-GSM was highly related to plaque textural features at the bifurcation and ICA (94) with echolucent plaques being more vulnerable than the echo dense ones. It remains to be confirmed in prospective studies if the combined means of assessing carotid disease IM thickness and its GSM represent a comprehensive approach for accurate estimation of overall arterial disease risk patients could carry.

Study IV

Different features that characterize the atherosclerotic plaque could be recognized using carotid ultrasound. Echolucent (soft) plaques represent the ones associated with potential cerebrovascular complications (155), and echogenic (hard) plaques represent more stable fibro-calcific phenotype.

Statins therapy is a well-established treatment for atherosclerosis and its complications. Their beneficial clinical effect, in the form of reduced events, e.g., stroke and coronary syndromes, is through lowering LDL-cholesterol levels (134) and their anti-inflammatory effect (128). Plaque echogenicity increased after statin therapy represents an early effect that could be used for monitoring individual patient’s response to therapy. There is evidence confirming that the changes in plaque volume in patients using statins appear later, after changes in echogenicity. This is not a unique feature of carotid disease, but also coronary plaques, which have been shown to demonstrate quantitative regression in volume within months of statins therapy.
In this study, using meta-regression analysis, the effect of statins on plaque echogenicity was shown to be independent of changes in LDL and HDL levels, but was related to changes of hsCRP levels. This finding is in accordance with another study that confirmed that the plasma lipid levels or their genetic determinants do not influence histologically evaluated carotid plaque composition.

In addition, we confirmed that the increased echogenicity was higher in patients treated for a longer period, again irrespective of the cholesterol level at baseline. Current data indicate that effects of statins on carotid plaque are dose-dependent, higher statins doses have a more potent effect on increasing plaque echogenicity compared to smaller doses. Our analysis shows that ultrasound carotid imaging plays a pivotal role in early and potential continuous monitoring of such an effect.

**Clinical Importance**

**Study I**

In this study we confirmed the concept of widespread atherosclerosis pathology that may have important therapeutic implications, as these individuals should receive aggressive secondary prevention treatment in addition to the revascularization, being at high risk of recurrent events. Our results highlight the importance of more detailed scanning of other non-culprit arteries located in the contralateral side. Improvement of imaging modalities that could identify histologically confirmed vulnerable plaque features e.g. hemorrhage and neovascularization, disease progression could be better monitored and the risk of stroke better controlled.

**Study II**

The well-established carotid IMT sensitivity in predicting vascular events can be extended to clinical prediction of stenosis severity in the distal segments. The newly introduced measure of carotid wall echogenicity (IM-GSM) is a promising technique for identifying distal carotid plaque features of stability and potential vulnerability. Because plaque features were different to those related to IMT, integrating both measurements, IMT and IM-GSM in a model that could increase the predictability of future events and thereby be used as a better marker of disease vulnerability than each
measure individually should increase the accuracy of the investigation.

**Study III**

Carotid IM-GSM is a novel technique for identifying patients at higher risk for future ischemic events, particularly those with multi-system disease. Identifying such patients should warrant aggressive medical management of modifiable risk factors. However, to justify such implementation in clinical practice, these results need to be reproduced prospectively and also the technique needs to be confirmed as a reproducible one among a wide range of operators.

**Study IV**

Changes of carotid plaque composition has been shown to occur long before changes of plaque volume, supporting the use of carotid ultrasound analysis of plaque echogenicity as a marker of plaque stability in response to statins therapy. In addition, the effects of statins on the plaques were progressive and independent of baseline cholesterol levels. Based on these data, echogenicity evaluation by carotid ultrasound could contribute to the concept of “individualized therapy” with statins, especially in low/intermediate risk asymptomatic patients, where it might support treatment adjustments for targeting better clinical outcome.

**Limitations**

**Study I**

The most important limitations of this study are the small number of patients analyzed, particularly asymptomatic patients, also being a retrospective study with its known limitations. Another limitation is the lack of body mass index data and of the detailed information for the presence and characteristics of atherosclerotic disease in other arterial systems e.g. coronary, intra-cerebral and peripheral vascular diseases. The data in this retrospective study was extracted from analog storage and the images used for analysis were slightly clouded. However, applying standard image despeckle-filtering methodology prior to the image analysis minimized the noise (178). In general, the calculated features were robust, however, the entropy was not,
and the remaining noise may have influenced these values, giving a higher variance, and weakening potential additional differences between groups.

**Study II**

This is a retrospective study, which is known for its limitation. Confirmation of our findings in prospective evaluation should strengthen their regular use in clinical practice. Our findings are limited to asymptomatic patients; however, exclusion of symptomatic patients could have contributed to avoid any potential erroneous measurements resulting from plaque rupture or a clot adherent to the plaque. The carotid scans were performed by a number of operators, but we believe that they all had the experience to run a clinical service independently.

**Study III**

The first limitation of this study is its retrospective nature. Secondly, the information about previous symptoms in different arterial systems was acquired from patients’ medical records, and not by direct imaging of the arterial systems. In addition, information on the duration of individual risk factors that could have influenced the results was not available. Assessing of the arterial wall thickness and plaque analysis of the lower extremity arteries together with any non-invasive imaging modality to assess directly the coronary artery plaque burden would have been of great interest, however, it was not possible with the retrospective nature of the study.

**Study IV**

A systematic review based on relevant key words might have missed some relevant publications, however, two investigators blinded to each other’s means of search checked the search. Another limitation was the low number of patients included in studies and that variable doses were used in different studies. Studies included had different follow-up periods, and in most studies, statins dosage was ranged, thus limiting the possibility of assessing the dose effect on plaque echogenicity using the meta-regression analysis.
CONCLUSIONS

Study I

Symptomatic patients with carotid stenosis have profound features of vulnerable plaques in both sides, suggesting increased relative vulnerability of the contralateral plaques. These findings support the concept of generalized atherosclerotic pathology rather than incidental unilateral disease, and hence emphasize the need for more aggressive treatment for secondary atherosclerosis prevention.

Study II

Common carotid IMT correlates with distal bifurcation and internal carotid stenosis, even in patients with established disease in distal segments. In addition, proximal IMT echolucency (low IM-GSM) is associated with distal arterial plaque vulnerability. Since, adding textural features of the plaque have been shown to have incremental information over and above the degree of stenosis alone, we suggest that the combination of IMT and IM-GSM evaluation could have a complementary role in identifying vulnerable patients needing aggressive plaque stabilization therapy.

Study III

Carotid IMT was higher and IM-GSM lower in patients with symptomatic nearby arterial territories but not in those with atherosclerosis disease relied in distal arterial systems, e.g. lower extremity. It was only the IM-GSM that was able to differentiate between the severity of atherosclerotic arterial territories, based on the number of systems involved, suggesting a better surrogate for monitoring systemic burden of atherosclerosis.

Study IV

This systematic review and meta-analysis support the use of echogenicity assessment by carotid ultrasound as a marker of plaque stability in response to statins therapy. These changes were showed to be independent of plaque area or volume, suggesting that they might reflect an early effect before anatomical response and plaque shrinking is detected. The effects of statins on the plaques were progressive and independent of baseline cholesterol levels. Applying this method in monitoring...
patients with carotid stenosis might support treatment adjustment for targeting better clinical outcome.
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