Insight into Carotid Atherosclerotic Plaque Development with CT Angiography

Marjon van Gils

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Chapter 1

Introduction

Background

Ischemic stroke

Stroke is the leading cause of serious, long-term disability and an important cause of death in developed countries. Although the age-standardized incidence, prevalence, mortality rates and disability have declined the last decades, the global burden of stroke has continued to increase due to population growth and ageing. [1, 2]

The general aim of this thesis is to contribute to the reduction of the burden of disease by increasing our knowledge on the pathophysiology of atherosclerosis by means of advanced imaging of atherosclerosis in vivo.

Ischemic stroke is defined as a clinical syndrome of a sudden, focal or global neurological deficit, presumably of vascular origin, with intracranial haemorrhage excluded by imaging. It is confined to an area of the brain perfused by a specific artery, and according to the classic definition lasts longer than 24 hours or leads to death. In a transient ischemic attack (TIA) symptoms are reversible and last less than 24 hours.[3]

Whereas ischemic stroke can be caused by cardiac thrombo-emboli, small vessel disease or rare disorders like vasculitis or dissection, atherosclerotic disease - leading to thrombo-embolism or local occlusions - is by far the most important cause, counting for approximately 50% of ischemic strokes.[3]

Atherosclerosis

Atherosclerosis is a chronic immuno-inflammatory, fibro-proliferative disease of large and medium-sized arteries. It is characterized by a local thickening of the arterial wall due to a slow build-up of cholesterol, lipids, calcium and debris. This wall thickening is called an atherosclerotic plaque.[4, 5] Endothelial cells, leukocytes, macrophages and intimal smooth muscle cells play key roles in the pathogenesis of atherosclerotic plaque formation.[6, 7]

Different risk factors appear to accelerate this disease process driven by atherogenic lipoproteins.[2] Our limited ability to predict clinical disease based on cardiovascular risk factor profiles indicate that other factors like genetic susceptibility play a role. Moreover, susceptibility to atherosclerosis differs among arterial segments, with predilection for bends and bifurcations.[8] This implies that local hemodynamic conditions and resulting shear stress play a role in its development.[9-11]

Atherosclerotic plaque formation itself could be seen as a process of vascular aging. Although plaque burden can increase with significant reduction or obstruction of the vessel luminal diameter, it more often increases without compromising luminal diameter, because of outward remodelling.[12, 13] What makes atherosclerosis a challenging disorder is that it, after long periods of indolent growth, suddenly becomes complicated by plaque rupture with superimposed thrombosis and subsequent embolism and devastating consequences, such as stroke and myocardial infarction. Histology studies found about 75% of fatal coronary thrombi to be precipitated by plaque rupture.[7]

Ruptured plaques are histologically characterized by large lipid-rich cores, thin fibrous caps containing few smooth muscle cells and many macrophages, angiogenesis (intraplaque haemorrhage), adventitial inflammation, and outward remodelling.[7, 14-16] Rupture-prone plaques, or so-called 'vulnerable plaques', have certain patho-anatomical features, that could be detected in vivo by imaging.

Treatment and prevention of ischemic stroke

Management of ischemic stroke in the acute phase consists of surveillance on a stroke unit with optimization of vital functions, intravenous thrombolytics in patients presenting within 4,5 hours after onset of symptoms and endovascular thrombectomy in cases with a visible intracranial arterial occlusion on vessel imaging. The risk of recurrent stroke after a TIA or ischemic stroke is high.[17, 18] Secondary prevention is therefore very important. Medical treatment consists of platelet aggregation inhibitors (for example acetylsalicylic acid, dipyridamole) or coumarine derivatives (in cases of increased risk for cardiac embolism). Further, treatment of cardiovascular risk factors, such as diabetes, hypertension, hypercholesterolemia, and refraining from smoking, reduces the risk of recurrent events.[2, 19] In the secondary prevention of ischemic stroke, it is also important to focus on the causative factor. Imaging of the carotid arteries is therefore part of the work-up of every ischemic stroke patient.

Until now, the degree of stenosis of the carotid artery, caused by atherosclerosis, has been the only imaging-based risk factor for stroke that is used in clinical decision making. Large randomized clinical trials (the North American Symptomatic Carotid Endarterectomy Trial – NASCET, and the European Carotid Surgery Trial – ECST) have established the imaging criteria for surgical treatment of the carotid artery in stroke patients. Carotid endarterectomy (CEA) is considered indicated in symptomatic patients with high-grade stenosis (>70%) and in a selection of patients with moderate stenosis (50-69%) to reduce the risk of recurrent ischemic stroke.[20-23] However, since most persons with a high-grade carotid stenosis are asymptomatic, and only a minority of symptomatic patients has such a high-grade stenosis, this clinical decision model has considerable shortcomings. Plaque burden and numerous plaque-specific features are not yet taken into account in clinical decision making.

Ideally, we would be able to prevent (recurrent) ischemic strokes by early detection of atherosclerotic disease and intervention in the process of plaque development towards a vulnerable plaque and plaque rupture. Although epidemiological, histological and animal studies have helped to define mechanisms of atherosclerosis, a convincing model of plaque rupture, applicable in daily clinical practise, still does not exist. Current imaging techniques are promising tools for increasing our understanding of atherosclerosis progression in humans by providing a window to the human atherosclerotic process in vivo.[24]

Atherosclerosis imaging biomarkers

In the last decades, research has focused on increasing our knowledge of the pathophysiology of atherosclerosis by imaging atherosclerotic disease in vivo in different vessel beds. Atherosclerotic plaque in the carotid artery is mainly subject of investigation, since it is a large artery which is easily accessible for different imaging modalities, and the performance of plaque imaging techniques can be validated by histology because of the

availability of carotid endarterectomy (CEA) specimens. Moreover, serial in vivo imaging of the carotid artery allows the investigation of disease progression and the development of vulnerable plaques as well as the assessment of their determinants.

Advanced non-invasive or minimally invasive imaging techniques, such as duplex ultrasound (DUS), magnetic resonance imaging (MRI) and computed tomography angiography (CTA) enable carotid stenosis grading as well as visualisation of wall pathology. These techniques could provide imaging biomarkers of early and advanced atherosclerotic disease.[24]

Biomarkers are characteristics that are objectively measured as indicators of normal biological processes, pathological changes, or responses to therapeutic intervention.[25] An imaging biomarker is defined as an (anatomical or functional) feature detectable with an imaging technique that can indicate the presence or state of a disease. Except for detection of disease, imaging biomarkers can be used to predict risk of disease, classify the extent of disease, and grade its aggressiveness and prognosis. Although qualitative imaging features (either present or not present) can be used, accurate quantitative imaging biomarkers add valuable information as they enable monitoring of disease evolution and treatment response. Effective use of imaging biomarkers requires technological robustness, non-invasiveness, broad availability, standardization and validation. Standardization concerns data acquisition parameters and post-processing techniques to ensure reproducibility. Requirements for validation of imaging biomarkers, like sensitivity, specificity, precision and reproducibility) are high, especially when used in drug development trials. Biomarker s and changes in its values should correlate strongly with biological effects (histological validation) and clinical endpoints.[26]

Ultrasound was the first modality to replace digital subtraction angiography (DSA) for stenosis grading of the extracranial carotid artery in clinical practise. Measurement of the combined thickness of the intima and media layer (carotid intima-media thickness (cIMT)) as assessed with B-mode ultrasound, has been thoroughly investigated and is associated with cardiovascular events.[27, 28] The composition of atherosclerotic carotid plaques can also be evaluated with ultrasound. Echolucency of carotid plaques has been associated with unstable plaque and echolucent plaques have been associated with subsequent cerebrovascular events.[29]

Magnetic Resonance Imaging (MRI) has emerged as a valuable non-invasive technique to evaluate different carotid plaque features as imaging biomarkers of plaque burden, plaque composition and plaque activity. Its accuracy has been validated against histology with a high reproducibility.[30] Certain plaque features were found to stimulate plaque progression and instability.[31-34] Prospective MRI studies found thinned/ruptured fibrous caps, the presence of intraplaque haemorrhage, larger maximum percentage of LRNC and larger maximum wall thickness to be strongly correlated with occurrence of cerebrovascular events.[35-39] Carotid plaque composition can be accurately quantified by MRI.[40] Serial MRI plaque imaging has been upcoming in clinical trials of pharmaceutical compounds to better understand the pathogenesis of atherosclerosis.[31, 41]

CT imaging is widely used in the acute clinical work-up of stroke patients. First of all, CT of the brain is necessary to differentiate between haemorrhagic stroke and ischemic stroke in the acute setting, since both diseases require a completely different therapy. CT angiography is increasingly used for visualization of the intracranial and extracranial arteries, classically to assess carotid artery stenosis to indicate surgical intervention, but recently

also to diagnose possible acute occlusion of intracranial arteries, in order to initiate endovascular thrombectomy.[42] Multidetector CTA (MDCTA) allows fast and reliable evaluation of steno-occlusive disease of both intracranial and extracranial arteries, is readily available and can nowadays be performed using a relatively low radiation dose (1-5 mSv). CT angiography is therefore a potential imaging technique for the assessment of atherosclerotic plaque biomarkers. The technique has already been found to enable evaluation of lumen as well as plaque surface morphology[43, 44] and to enable quantification of plaque volume and plaque components, in good correlation with histology.[45-47]

We need better understanding of the development of carotid atherosclerosis and factors associated with plaque rupture and ischemic stroke.

The work in this thesis therefore focusses on:

- Quantification of imaging biomarkers of carotid atherosclerotic disease with CTA.
- The investigation of the role of carotid atherosclerotic plaque surface (i.e. ulceration) as an imaging biomarker of plaque vulnerability.
- The study of plaque development and its determinants using serial CTA imaging.

Quantitative imaging biomarkers of carotid atherosclerosis using CTA

To investigate the pathophysiology and development of atherosclerosis, accurate and robust measures of severity of stenosis, plaque burden and plaque features are necessary. Several technical imaging aspects should be taken into account, since they highly influence quantitative luminal and plaque measures. These considerations are explained in ***Chapter 2.1***.

DSA has been the gold standard for grading carotid artery stenosis according to the NASCET and ECST criteria. The inherent risk of the invasive nature of angiography has led to the introduction of less invasive diagnostic tools. Multi-detector CT Angiography has replaced the more invasive technique of DSA for the measurement of carotid stenosis in lots of clinical practises. Generally, 3D software is used to create multiplanar reformations (MPRs) and/or curved planar reformations (CPRs) in oblique planes parallel to the carotid lumen to seek the point of maximum stenosis, and the smallest diameter in the cross-sectional plane perpendicular to the central lumen line at that level is measured and compared to the reference diameter in the healthy distal internal carotid artery. In order to facilitate and automate this procedure, extensive efforts have been put in the development of (semi)-automated lumen segmentation methods and luminal stenosis assessment.[48, 49] The state-of-the-art CTA techniques to assess carotid artery stenosis are reviewed in detail in ***Chapter 2.1***.

Assessment and quantification of plaque volume and plaque composition with MDCT angiography has been validated in in vitro an in vivo studies, using histology as gold standard.[45, 46, 50] To obtain plaque volume measures, plaque boundaries should be defined to segment 3D plaque volume. This has been done manually, which is a very labour-intensive

task. Algorithms have been developed that combine (semi-)automatically obtained segmentations of the outer vessel wall and the lumen to create plaque segmentation. Plaque components within this segmented plaque are then specified and further segmented based on distinctive ranges of Hounsfield Units, distinguishing lipid, fibrous tissue and calcifications.[46, 50] In ***Chapter 2.1*** an in-depth overview on qualitative and (semi-automated) quantitative plaque assessment using CTA is provided.

Chapter 2.2 focusses on the performance of a (semi-)automated algorithm for plaque volume and plaque component measurements against manually derived measurements.

Carotid atherosclerotic plaque ulceration

Atherosclerotic carotid plaque ulceration is primarily diagnosed with conventional angiography and describes the extension of contrast media beyond the vascular lumen into the surrounding plaque.[51] MDCTA is also effective in the detection of carotid plaque ulceration, with a sensitivity and specificity of 94% and 99% respectively,[52] and is – due to its 3D data-acquisition and reconstruction- even superior to 2D DSA in detecting ulcerations.[53]

Atherosclerotic carotid plaque ulceration is considered to be a marker of previous plaque rupture,[51, 54] probably representing the heavily ruptured plaques, in which part of the LRNC is detached with downstream embolization. Further, it is an important predictor of ischemic stroke besides degree of stenosis.[55, 56] Non-lacunar stroke is thought to be most often caused by thromboembolism, whereas lacunar ischemic stroke would be caused by small vessel disease.[3] To evaluate the vulnerable plaque hypothesis we investigated the association between atherosclerotic plaque ulceration in the symptomatic artery and non-lacunar stroke, in comparison to lacunar stroke, in a large stroke population using MDCTA ***(Chapter 3.1)***.

In addition to its association with plaque rupture and ischemic stroke, carotid plaque ulcerations are likely to form an additional focal source of thromboembolism due to flow disturbances, causing recurrent ischemic events.[57, 58] The natural evolution of plaque ruptures and surface irregularities is not known. So far, knowledge on evolution after plaque rupture is based on histological analysis of coronary arteries in autopsy studies or carotid plaque specimens obtained from CEA. In CEA specimens for example, a strong negative association between time since stroke and prevalence of ruptured plaques was found which indicates a process of plaque healing.[59] These cross-sectional studies provide indirect evidence for temporal changes after plaque rupture. Serial imaging is the optimal method to study plaque surface morphology changes in vivo. MDCTA is able to identify and classify plaque ulcerations with an excellent interobserver agreement.[43] Therefore I used MDCTA to explore the natural history of ulcerated carotid plaques, by assessing the temporal changes in plaque surface morphology on serial scans in patients with TIA or stroke ***(Chapter 3.2)***.

According to the vulnerable plaque hypothesis, certain plaque characteristics render an atherosclerotic plaque prone to plaque rupture. Histological studies found plaque ruptures to be associated with large necrotic cores, inflammation and thin, fragile fibrous caps.[15] In addition, in severely stenosed carotid arteries, plaque ulceration on DSA has been associated with the presence of fibrous cap rupture, intraplaque haemorrhage, large lipid core and less fibrous tissue in carotid endarterectomy specimens.[51]

Imaging studies can investigate this hypothesis by evaluating both plaque composition and plaque ulceration in cross-sectional and serial studies. In an MR study, LRNC proportion of carotid plaques in arteries with a stenosis of 50-79% was found to be the strongest predictor of surface disruption.[33] ***Chapter 3.3*** presents the study that analysed the relation of plaque volume and plaque component proportions with plaque ulceration in a group of consecutive patients with AF, TIA or minor ischemic stroke, as assessed with MDCTA.

Serial carotid plaque imaging and determinants of atherosclerotic plaque changes

Serial quantitative imaging generates the opportunity to study temporal plaque changes in vivo, which provides insight into plaque development and its determinants, and could enable also the monitoring of treatment effects and prediction of plaque progression.

The Agatson score, a coronary artery calcification (CAC) measure, is a CT-derived imaging biomarker for coronary atherosclerosis burden, used in clinical decision making in cardiology. In accordance with this CAC score, carotid calcification has been used as a surrogate marker for carotid atherosclerosis in studies on risk prediction.[60, 61] Although some cross-sectional studies on asymptomatic subjects have demonstrated a relation between cardiovascular risk factors and presence or volume of calcification,[62-64] the natural history of carotid calcification in symptomatic patients has not been evaluated yet. Therefore, I investigated the growth pattern of calcifications in carotid arteries and the determinants of change using serial MDCTA imaging in patients with recent TIA or ischemic stroke ***(Chapter 4.1)***.

The clinical significance of carotid calcification, however, is not as clear as it is in the coronary arteries. Some studies suggest that carotid calcification burden is associated with increased stroke risk,[61, 65, 66] whereas others found a relatively high calcification content of carotid plaques to be associated with plaque stabilization.[67-70] The relative proportion of calcification within a carotid plaque seems to be important for risk evaluation.[71] This requires calcification volume measurements to be related to the total plaque volume.

Besides calcifications, other plaque characteristics assessed with plaque imaging have been associated with plaque instability and an increased risk of ischemic stroke, LRNC being an important feature.[33] Quantitative MDCTA plaque imaging enables the investigation of certain relative plaque components in comparison to total plaque volume. Furthermore, it enables the study of temporal changes in atherosclerotic disease and their determinants. In order to find out whether MDCTA and (semi-)automatically derived plaque segmentation can be used to quantify atherosclerotic plaque measures in vivo and track temporal plaque changes I first performed a pilot study in patients with TIA or ischemic stroke who underwent serial MDCT angiographies (***Chapter 4.2)***. In this pilot study, the carotid bifurcations of baseline and follow-up scans were registered using a semi-automated registration method. In this analysis a maximum coverage of the carotid plaque was evaluated. To improve the inclusion of baseline-follow-up pairs of carotid bifurcations and to enable comparison with results from the literature, the arteries in a larger group of patients were registered based on number of axial slices above and under the carotid bifurcation, according to methods used in serial MRI studies. In this study I investigated the determinants of plaque growth and plaque changes ***(Chapter 4.3)***.

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Chapter 2

Quantitative imaging biomarkers of carotid atherosclerosis using CTA

Chapter 2.1

Quantitative CT imaging of carotid arteries

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Background

Stroke is the second leading cause of mortality in the Western world after coronary heart disease. Although stroke death rate declined 44% in the last decade, the burden of disease remains high.[1] Of all strokes, 87% are ischemic, 10% are intra cerebral hemorrhage and 3% are subarachnoid hemorrhage strokes.[1] About 50% of the ischemic strokes are due to atherosclerotic disease, which is preferentially located in the carotid artery.[2]

Till now, the degree of luminal narrowing of the carotid arteries, caused by atherosclerosis, has been the only image-based risk factor for (recurrent) stroke that is used in therapeutic decision making. Large, randomized clinical trials (the North American Symptomatic Carotid Endarterectomy Trial (NASCET) and the European Carotid Surgery Trial (ECST)) have established the imaging criteria for surgical treatment in symptomatic patients. Carotid endarterectomy (CEA) is indicated for symptomatic patients with high-grade stenosis (>70%) and in selected patients with recent symptoms and moderate stenosis (50-69%).[3-5] In asymptomatic carotid artery stenosis, a modest benefit of CEA is described in selected patient groups (relatively young male patients) who had a severe stenosis.[6-8] However, most patients with a stenosis >70% are asymptomatic and most symptomatic patients have a carotid stenosis <70%, which suggest that other factors play an important role in the pathophysiological cascade of ischemic stroke. Especially in the group of patients with moderate carotid stenosis, it is of clinical importance to improve risk prediction.

The last decades, extensive research has been performed to increase our knowledge of the pathophysiology of atherosclerosis. Apart from luminal narrowing of the carotid artery resulting in blood flow compromise, rupture of the atherosclerotic plaque and subsequent thrombo-embolism is thought to result in ischemic events. Post-mortem histological studies of coronary and carotid arteries have found that certain atherosclerotic plaque characteristics increase the vulnerability of the plaque to rupture. Inflammation is the hallmark of vulnerability and plaques with active inflammation may be identified by extensive macrophage infiltration. Plaques with a thin cap of <100 mm and a lipid core accounting for >40% of total plaque volume are also considered highly vulnerable. Plaques with a fissured or ruptured cap are prone to thrombosis and thrombo-embolization.[9] Carotid plaque ulcerations on digital subtraction angiography (DSA) have been associated with plaque rupture [10] and with an increased risk of acute recurrent ischemic events.[11, 12] Advanced invasive and non-invasive imaging technologies enable the visualization of these atherosclerotic plaque characteristics in vivo.

DSA has long been the modality of choice for imaging carotid arteries, since it accurately visualizes the vascular lumen and its contours. However, DSA has several disadvantages; it is invasive, laborious, time intensive, and expensive. Moreover, DSA requires skilled operators and is therefore less readily available. More importantly, cerebrovascular DSA has a non-negligible morbidity and mortality, with a complication rate of 0.4-12.2% for neurological deficits.[13, 14] These drawbacks and the increasing interest in the arterial vessel wall have driven the use of other, less invasive modalities for imaging the carotid arteries.

Nowadays, non-invasive imaging techniques like duplex ultrasound (DUS), magnetic resonance imaging (MRI) and computed tomography (CT) not only enable grading of carotid stenosis but also provide a window to the atherosclerotic process in vivo.[15] They also allow for the quantification of plaque measures like plaque burden and plaque composition.

Using serial imaging, the early natural development of the atherosclerotic plaque can now be studied in vivo. Furthermore, it provides a tool to monitor changes in atherosclerotic plaque in response to secondary preventive therapies. The development of new pharmaceutical therapies is a slow and costly process, since the most reliable way to measure their clinical impact is to study its effect on clinical endpoints. The use of imaging biomarkers of atherosclerotic disease could speed up this process and reduce the number of subjects studied. For an effective use, imaging biomarkers should be derived in a robust, non-invasive way and the imaging modality should be broadly available.[16] Further, standardized image acquisition parameters and post-processing methods are required and the imaging biomarkers should be carefully validated and highly reproducible. The changes in an imaging biomarker should be correlated to the biological effect and the clinical endpoints.[16] Quantification, and especially automated quantification, of the degree of stenosis and atherosclerotic plaque measures is therefore important in the development of reliable surrogate endpoints for atherosclerosis.

Computed tomography angiography (CTA) is a potential imaging modality for monitoring atherosclerosis in vivo. It is a readily available and fast imaging technique causing minimal inconvenience for the patient. Although CTA involves potentially harmful ionizing radiation, the effective dose during a diagnostic CTA is relatively low (1-5 mSv).[17] The increased acquisition speed of multi-detector CT angiography (MDCTA) reduces motion artifacts. Current multi-detector row CTA enables fast vascular imaging from the aortic arch to the intracranial vessels. This enables simultaneous investigation of other vascular territories, which makes that MDCTA can compete with other non-invasive imaging techniques and is increasingly used in the clinical evaluation of stroke patients. In this chapter, the state-of-the-art CTA technique used to evaluate carotid artery stenosis and atherosclerotic plaque is described.

Luminal imaging using CTA

Technical aspects

In the early 1990s spiral CT was introduced, which enabled a volumetric data acquisition through continuous X-ray source rotation and simultaneous continuous table movement. Using this technique non-invasive imaging of blood vessels became widely available. The steady increase of the longitudinal coverage of the X-ray detectors, i.e. the number of slices, even further improved the feasibility of luminography.

Contrast material is necessary for the visualization of the lumen. Stenosis measurement relies upon the contrast difference between the lumen and its environment. Several technical factors should be taken into account when imaging vessel lumen using MDCTA.

The contrast difference between lumen and surrounding tissue is varying and depends mainly on the amount of lumen attenuation which is artificially increased by contrast material. The attenuation caused by contrast material can vary depending on patient-related factors like cardiac output and weight, and on scan parameters and contrast protocol-specific factors. Peak tube voltage (kVp) influences the difference in HU values between different tissues. The lumen contrast density increases as tube voltage decreases. The lumen enhancement pattern is determined by the injection volume, the injection

rate, and the iodine concentration in the contrast material.[18] Timing of contrast bolus arrival should be such that a maximum contrast density is achieved in the carotid artery with a concomitant low contrast density in the neighboring jugular vein. Use of a saline bolus chaser reduces the amount of contrast material needed by 20-40% and reduces the extent of perivenous artifacts due to high contrast density in the jugular vein.[18] Synchronization between passage of the contrast bolus and data acquisition can be achieved by real time bolus tracking at the level of the ascending aorta. Moreover, a craniocaudal scan direction also reduces contrast material-related perivenous artifacts.[19] The contrast injection protocol for carotid artery imaging is generally standardized with a fixed contrast volume of 80-125 mL (iodine concentration of >300 mg/mL), and a saline bolus chaser of 40 mL, both at an injection rate of 2-4 mL/sec. The disadvantage of intravenous contrast in CT angiography remains that its application is limited in patients with renal insufficiency and hyperthyroidism.

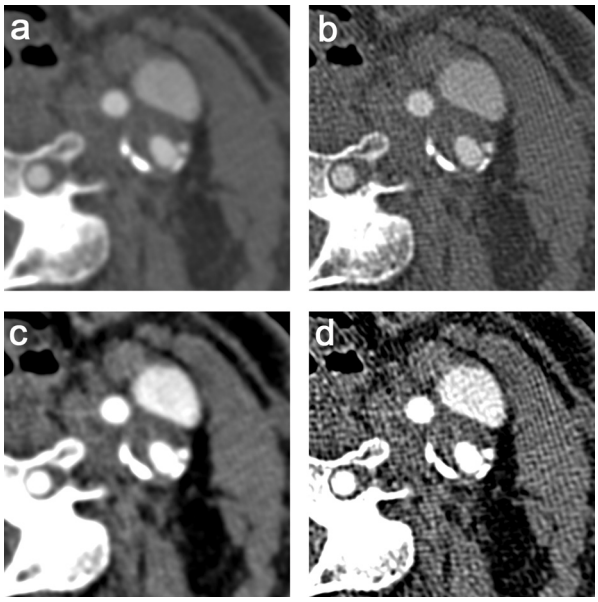

Figure 1. Influence of window-level setting and convolution kernels on the evaluation of lumen and plaque

Four axial MDCT images through the carotid bifurcation obtained with a smooth (a+c) or a sharp (b+d) kernel and with a larger (W1000 L200; a+b) or smaller (W400 L100; c+d) window width setting. A large window width (a+b) gives a better differentiation between lumen and neighboring calcifications, which mostly appear brighter. A smaller window width (c+d) enables visualization of the small density differences inside the non-calcified part of the plaque. A sharper reconstruction kernel (b+d) increases the contrast between the small dense calcifications and the surrounding structures, whereas a smoother kernel (a+c) leads to averaging of contrast differences, which gives a smoother appearance to the structures.

Because of the limited spatial resolution of the CT scanner partial volume averaging occurs, leading to the so-called blooming artifact. This is easily appreciated at the boundary of the enhanced lumen and the vessel wall where differences in density are large. In subtle cases this is reflected in a blurred interface between structures as well. Partial volume averaging may influence the appreciation of the real luminal dimensions and therefore the accuracy of the stenosis measurements. The extent of blooming also depends on the convolution kernel chosen in the filtered-back projection algorithm. Sharp convolution kernels increase the contrast of small dense structures as the blurring is reduced, whereas smooth kernels lead to averaging of contrast differences. The signal-to-noise-ratio on the other hand improves when applying a smooth kernel because the image noise is reduced.

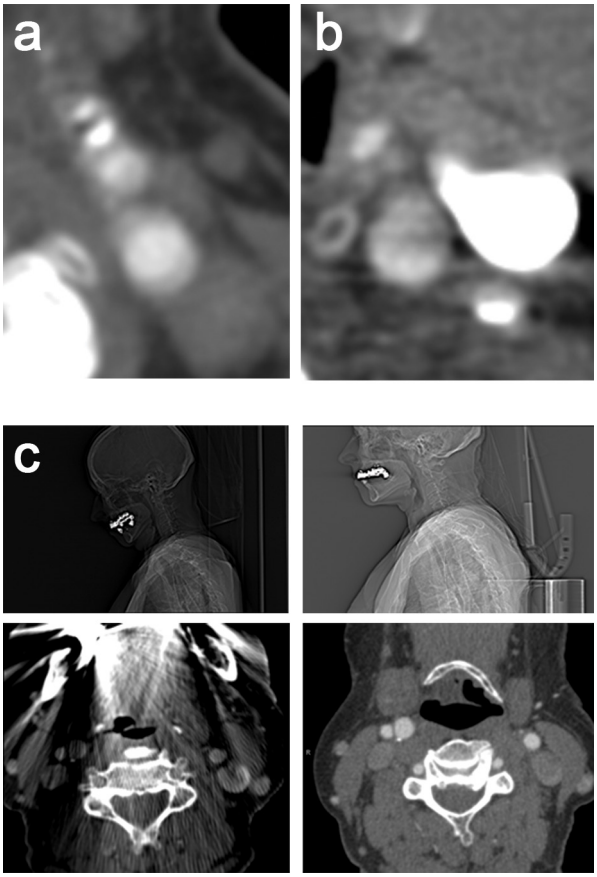

Figure 2. Influence of artifacts on MDCTA imaging

a) Axial image at a level above the carotid bifurcation showing motion artifacts due to swallowing. The tissue boundaries are heavily blurred. b) The dependent part of the jugular vein is filled with high density contrast material, which causes streaks of low attenuation, artificially introducing a low contrast area in the neighboring carotid artery and hampering visualization of its wall. c) Dental material can cause enormous streak artifacts (images on the left), impeding correct judgment of surrounding structures. A slight upward tilt of the chin moves these artifacts away from the region of interest and allows a normal visualization of the larger part of the carotid bifurcation (as shown on the right).

The appearance of the lumen-wall interface is influenced by adjustment of the window-level setting. Each lumen contrast opacification has been shown to theoretically have its own optimal window-level setting for which lumen measurements are most accurate.[20] When calcifications border the lumen, the two hyperdense structures may be difficult to differentiate from each other, impeding accurate lumen measurement. Normally, in CTA a large window width (500-1000 HU) is used, which can be adjusted by the reader dependent on the lumen attenuation and the presence of calcifications near the lumen in order to improve the visual differentiation between dense structures. Figure 1 illustrates the influence of window-level setting and convolution kernels on the evaluation of the lumen.

In MDCTA images a challenge is formed by the artifacts from extra luminal dense structures like dental material, bone, and atherosclerotic calcifications which might obscure a clear visualization of the lumen. Correct head positioning with a slight tilt of the head and an upright position of the chin reduces the effect of beam hardening artifacts from dental material at the level of the carotid bifurcation, the predilection place for atherosclerotic disease in the carotid artery (Figure 2). As described, convolution kernels and window-level settings highly influence the appearance of high density calcifications. In addition, with a fixed window-level setting, calcification volumes appear smaller in higher kVp-settings.[21]

From the cross-sectional source images, 2D or 3D image reconstructions can be created which aid in the identification and measurement of the maximal stenosis. Multiplanar reconstructions (MPR) and curved planar reconstructions (CPR) provide 2D images of any predefined plane and enable accurate stenosis measurement. For creating a longitudinal

view of the artery, CPR has the advantage over MPR that it corrects for vessel curvature outside of the plane. Shaded surface display (SSD), volume rendering (VR) and maximum intensity projection (MIP) are all 3D techniques with their own strengths and weaknesses. In SSD all pixels with densities below a certain threshold are excluded and the remaining data are viewed as if their surfaces are illuminated by a point source. VR utilizes the image intensities directly, by assigning opacity and color coding, to create 3D reconstructions. Both techniques are less useful for carotid artery stenosis measurements. MIPs are created by projection of the maximum intensity pixels from a 3D data set on a predefined 2D plane and give a simple overview of the vessel and its stenosis. However, this technique is limited in arteries with atherosclerotic calcifications, since calcifications in the vessel wall can easily cover the contrasted lumen causing overestimation of the degree of luminal stenosis. In addition, bony structure like the spine, thyroid cartilage, cricoid and hyoid might interfere with a clear overview of the artery in 3D post-processing techniques (Figure 3).

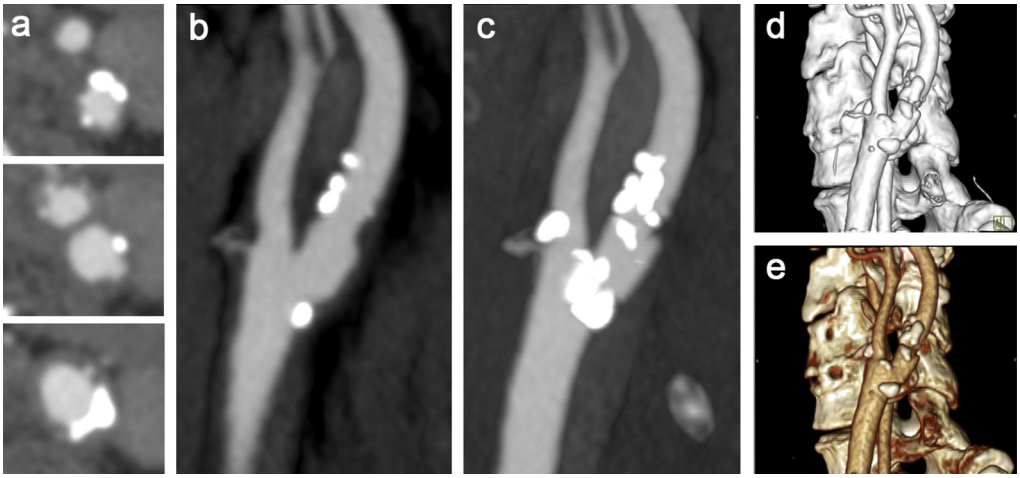

Figure 3. Different post processing techniques in MDCTA images of a moderately stenosed carotid artery

a) Axial slices through the common carotid artery (lower image), the level of the carotid bifurcation (middle) and a level above the bifurcation (upper image). b) Multiplanar reformat (MPR) in the sagittal plane visualizing the atherosclerotic plaque around the bifurcation. c) Maximum intensity projection (MIP, 8.8 mm) in the same plane. Over projection of calcifications hampers a clear visualization of the lumen. d) Volume rendering (VR) shows a 3D reconstruction of the carotid artery. e) Shaded surface display (SSD) of the same carotid bifurcation. Both last techniques suffer from over projection of calcifications.

New techniques have been investigated that might solve the problem of artifacts from bone and calcifications on images. Matched mask bone elimination (MMBE) is a technique for the automated removal of bone pixels from CTA data sets. Preceding to the CT angiography a nonenhanced data set is acquired on which the bone pixels are identified. The corresponding pixels on the registered CT angiography are assigned an arbitrarily low value and MIP images free from overprojecting bone can then be obtained.[22, 23] Whereas for MMBE, acquisition and registration of two separate datasets is necessary, in dual-energy CT (DECT) two image data sets can be simultaneously acquired with different tube voltages (for example 80 and 140 kVp). Tissues can be differentiated by analysis of their attenuation differences depending on the tube voltage. The attenuation difference is es-

pecially large in materials with a high atomic number, such as iodine. Bone and calcifications, which show a smaller attenuation difference, can therefore be differentiated from iodine in the carotid lumen. As a result, calcifications can be removed from the contrast filled lumen, enabling quantification of carotid stenosis in heavily calcified arteries.[24] However, because in both techniques an additional rim around the calcified pixels is removed due to blooming artifacts, overestimation of the grade of stenosis can still be introduced.

Stenosis Measurement

The accuracy of the stenosis measurement is important, seen its role in clinical decision making about carotid endarterectomy. Traditionally, the stenosis in the carotid artery was assessed using intra-arterial digital subtraction angiography (DSA), which is still considered the gold standard. The degree of stenosis was defined as the residual lumen at the stenosis as a percentage of the normal lumen in the distal internal carotid artery (according to the NASCET criteria) or as the residual lumen as a percentage of the estimated original diameter of the artery at the level of the stenosis (according to the ECST criteria). In the large symptomatic carotid surgery trials, conventional DSA was performed in two or three projections (lateral, postero-anterior, and/or oblique) which were investigated for the most severe stenosis. Whereas rotational DSA, using multiple planes, showed to provide a benefit in detecting the smallest diameter in a stenosed artery compared to conventional DSA,[25] the association between the severity of stenosis and stroke risk and therefore the indication for surgical intervention remained based on conventional DSA.

The volumetric CTA datasets allow for MPRs and MIPs in any plane and therewith provide much more information on the lumen and its morphology than conventional DSA. The residual lumen is almost never circular and DSA performed in a limited number of projec-

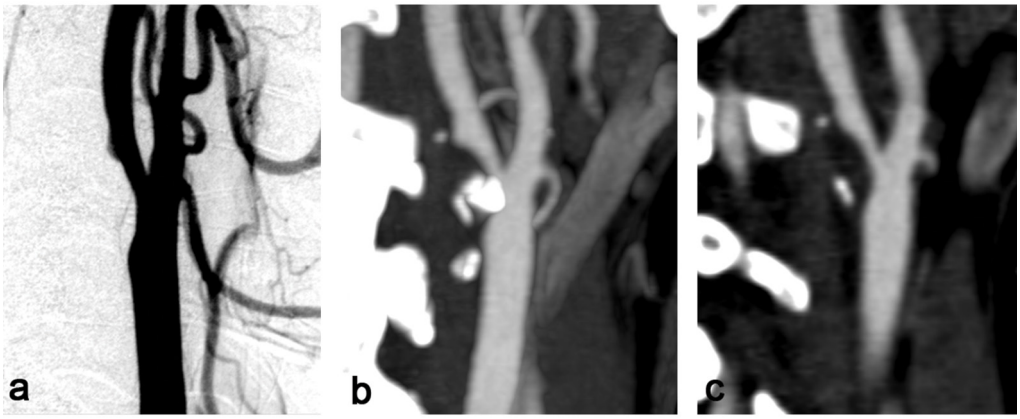

Figure 4. Assessment of carotid stenosis with DSA and MDCT angiography

a) Digital subtraction angiography (DSA) of a right carotid artery shows a 50% stenosis at the level of the bifurcation. b) A maximum intensity projection (MIP, 6 mm) of MDCTA images of the same artery. MIP has the disadvantage of overprojection of calcifications over the lumen, causing overestimation of stenosis measurement. c) A multiplanar reformatted image (MPR, 1 mm) in the same plane as the MIP in (b); the problem of overprojection does not occur here. Using MPR reconstruction of 3D data the point of maximum stenosis can be found easier compared to using DSA.

tions does not always reveal the narrowest lumen. Analysis of 3D information therefore may provide a more realistic way to assess the true maximum stenosis.

In case CTA replaces DSA in clinical decision making, stenosis measurements on CTA should be performed in a comparable way i.e. measuring the diameter of the remaining lumen at the level of the maximal stenosis and of the normal lumen distal to the stenosis. This can be done in several ways using different post-processing techniques. Although with MIP reconstructions images comparable to those in DSA can be obtained, this technique is limited in calcified plaques and it is recommended not to use MIP images for stenosis measurements in arteries with calcifications (Figure 4). Generally, one uses 3D software to create MPRs and/or CPRs in oblique planes parallel to the carotid lumen to seek the point of maximum stenosis and measures the smallest diameter in the cross-sectional plane perpendicular to the central lumen line at that level. Figure 5 shows this method of stenosis measurement using 3D software. The reference diameter is measured in the same way at a level above the carotid bulb where the lumen walls run parallel to each other (i.e. the healthy distal carotid artery).

When using the ECST criteria to assess the degree of stenosis, CTA directly enables visualization of the outer vessel wall, whereas on DSA the vessel diameter has to be estimated by delineating the projected lumen contour. Therewith, CTA takes into account the changes in vessel diameter caused by vascular remodeling, whereas this phenomenon is ignored when measured on DSA. This might cause differences in ECST stenosis measurements between CTA and DSA.

Diagnostic accuracy

Several diagnostic studies have been performed which compared single slice CTA with DSA in the assessment of carotid stenosis. From a meta-analysis of studies published between 1990 and 2003, single slice CTA has been shown to have a pooled sensitivity of 85% and a pooled specificity of 93% for detection of a 70-99% stenosis. Sensitivity and specificity for detection of an occlusion were 97% and 99%, respectively.[26] Another systemic review reported a pooled sensitivity of 95% and a specificity of 98% for the detection

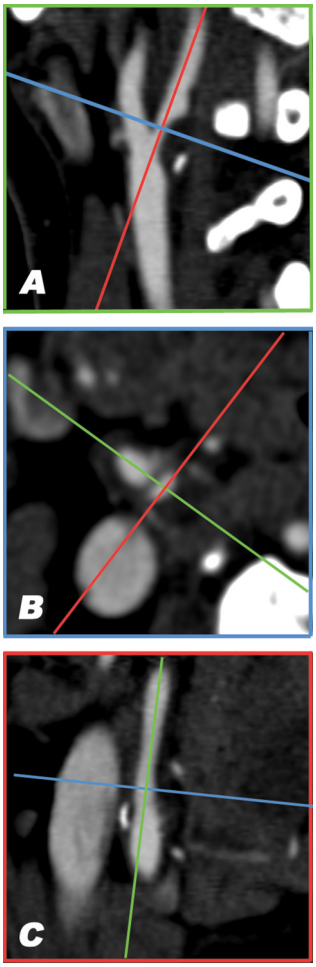

Figure 5. Stenosis measurement in MDCT angiography using 3D software
Multiplanar reformatted images are created in planes parallel and perpendicular to the lumen axis; the smallest lumen diameter in the cross-sectional plane can then be measured using calipers. A) A sagittal view of the carotid bifurcation. The blue and red lines correspond to the planes that are depicted in B and C, respectively. A large atherosclerotic plaque is visible at the origin of the internal carotid artery, causing a high-grade stenosis. B) The cross-sectional image perpendicular to the central lumen line at the level of the smallest vessel diameter. The residual lumen has an oval shape. C) The view perpendicular to those in A and B. In this plane the stenosis is not very prominent.

of a 70-99% stenosis.[27] The latter study also found that CTA was sensitive (95%), but slightly less specific (92%) in depicting stenosis >30%. In 2006, Wardlaw and colleagues performed a meta-analysis comparing non-invasive imaging techniques with intra-arterial angiography. They found only 11 studies on CTA, published between 1980 and 2004, that explicitly met the Standards for Reporting of Diagnostic Accuracy (STARD) criteria [28] and they reported a sensitivity of 77% and a specificity of 95% for diagnosing 70-99% stenosis using CTA.[29] The authors warned for the methodological shortcomings of many studies evaluating diagnostic imaging. They concluded that the existing data might support the cautious use of non-invasive imaging to diagnose 70-99% stenosis, but that more data are needed from carefully designed trials to determine true sensitivity and specificity of non-invasive imaging techniques in routine clinical practice, especially for 50-69% stenosis, or when used in combination.[29] In 2009, Chappell and colleagues performed an individual patient data meta-analysis to find clinically significant estimates of the accuracy of non-invasive imaging in diagnosing severe and moderate symptomatic artery stenosis.[30] They also concluded that existing primary studies provide limited data and that the literature overestimates the accuracy of noninvasive imaging techniques. The small CTA dataset included in this analysis revealed a sensitivity and specificity of 65% and 56% for detection of 70-99% stenosis, respectively.[30]

A difficulty in the evaluation of the accuracy of stenosis measurement using non-invasive imaging techniques is that both acquisition and post-processing procedures evolve rapidly. Although multidetector CTA is now widespread and is expected to improve diagnostic accuracy, this has barely been tested. Only one study compared MDCTA with DSA and found MDCTA to have a high specificity and a high negative predictive value for significant carotid disease.[31] Since DSA is not routinely used anymore in clinical practice, the assessment of new non-invasive imaging techniques against DSA cannot be justified ethically anymore. Therefore there is an increasing need for practical, reliable methods for evaluating new technologies, for example by standardized comparison with other non-invasive tests or test phantoms.

Both aforementioned systemic reviews[26, 27] did not provide enough evidence to draw robust conclusions about the diagnostic accuracy of the different post-processing techniques, although stenosis assessment using axial slices and MIPs seemed to be better than when using VR and SSD.[27] Most studies did not report on the exact –combinations of- reformatting techniques used, which hampers a solid meta-analysis. More recent studies comparing the post-processing techniques in MDCTA revealed that stenosis measurements on axial source images are highly reproducible and accurate and that the additional use of MPRs or other reconstructions is not necessary, but might aid in finding the location of the maximum stenosis.[32-34]

(Semi)-automated quantification of luminal measures

Manual lumen segmentation and stenosis quantification is laborious and suffers from inter and intra observer variability. Consequently much work has been performed on the development of (semi)-automated lumen quantification. The majority of publications with respect to lumen quantification focus on the segmentation of the lumen while the assessment of the severity of luminal stenosis is addressed by few.

Lumen segmentation methods have been reviewed and grouped according to the mathematical framework used [35] or categorized with respect to (1) the way vessel geometry and appearance are modeled, (2) the image features which are used for vessel extraction

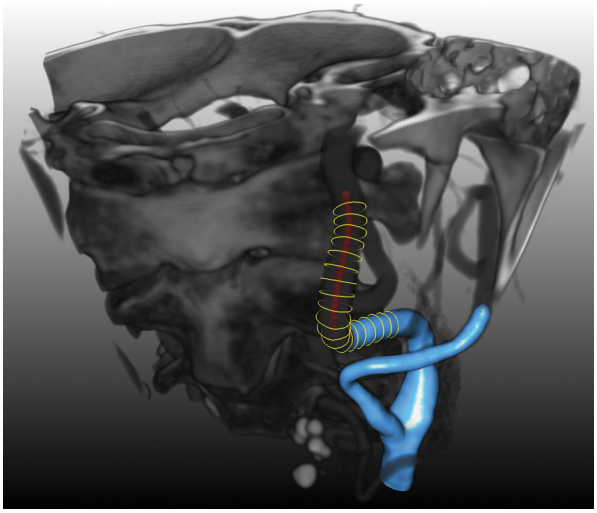

Figure 6. 3D-segmentation of the lumen of a carotid artery

An example is shown of a carotid artery lumen segmentation using three different segmentation representations. The red dots indicate a centerline through the centroids of the vessel cross-sections. The yellow 'circles' show the lumen contours perpendicular to the centerline. The blue surface shows an interpolated surface through the yellow contours.

and (3) the methodology used in vessel extraction.[36]

Some of the published methods have been tailored to or evaluated on carotid CTA images.[37-40] Reported values vary highly. However the comparison of these methods is hampered by the fact that they all use different imaging data and evaluation measures, like Dice similarity coefficient, mean surface distances, or visual inspection. In addition, most studies were performed on small and selected data sets. Figure 6 illustrates a 3D lumen segmentation of a carotid bifurcation.

To facilitate an objective comparison of carotid artery segmentation and stenosis quantification algorithms, the Carotid Bifurcation Algorithm Evaluation Framework was set up in 2009 (http://cls2009.bigr.nl/).[41] This framework consists of a publically available image database, annotated data for training and evaluation and standardized evaluation measures. Till date 9 algorithms have been evaluated by the framework, of which only one is fully automatic, whereas the others require three initialization points. The three best performing methods evaluated by the framework have dice similarity coefficients of 0.92, 0.88 and 0.90, mean surface distances of 0.18, 0.54 and 0.17 mm and Hausdorff distances of 1.5, 4.4 and 1.7 mm, respectively.[41] Figure 7 shows three examples of lumen segmentations with three different dice values.

These three best performing methods are based on three different approaches, i.e. graph cut, level set and active surface algorithms.[41]

In the graph cut framework voxels are assigned to vessel lumen or background by considering all image voxels as nodes in a 3-dimensional graph, and creating an optimal surface which separates (cuts) the foreground (lumen) from the background. To compute this optimal cut the image gradient can be used.

In the level set framework, the vessel surface is represented implicitly by the zero level lines (zero level set) of an embedding function (similar as e.g. sea level in a height map). This embedding function is then changed (evolved), implicitly resulting in deformation zero level set. This representation has the advantage that the zero level set can change topology (Figure 8). The evolution of the embedding function should ensure that the zero level set halts at the vessel lumen boundary. This is achieved by defining a speed function derived from the image data. Both the initial segmentation and the design of the speed image are the key ingredients in the design of a level set-based segmentation method.

Active surfaces are a generalization of active contours (also called snakes). Using active surfaces the segmentation is also the result of the evolution of an initially segmented surface. However changing topology is much harder to model in this framework. The segmentation is modeled as a surface on which forces are acting which causes the evolution of the segmentation. This evolution can be constrained by properties of the used surface representation.

Although considerable research has been performed on vessel lumen segmentation, only few researchers have published on automatic vessel stenosis.[42-44] Also, approaches differ widely in the evaluation that has been performed, both with respect to evaluation measures and number of data sets used.

The evaluation framework discussed previously also allows objective comparison of performance in stenosis quantification. To date only three stenosis grading methods have been evaluated using this framework, also indicating that this field has received less attention.[41] Clinically, the minimal diameter is often used to calculate the stenosis degree. However, the minimal diameter of a non-elliptical shape is not uniquely defined and is therefore prone to measurement errors and is hard to measure automatically. The evaluation framework evaluates two stenosis measures: an area based measure which compares the area of the lumen at the stenosis to the area of a distal vessel part and a measure that compares the minimal diameter at the two positions. In the framework, the diameter-based stenosis degree is defined by the smallest line that divides the cross-sectional area in two equal parts. Using automated lumen segmentation the minimal diameter can easily be replaced by the lumen cross-sectional area. This is a much more accurate measure for the obstruction of

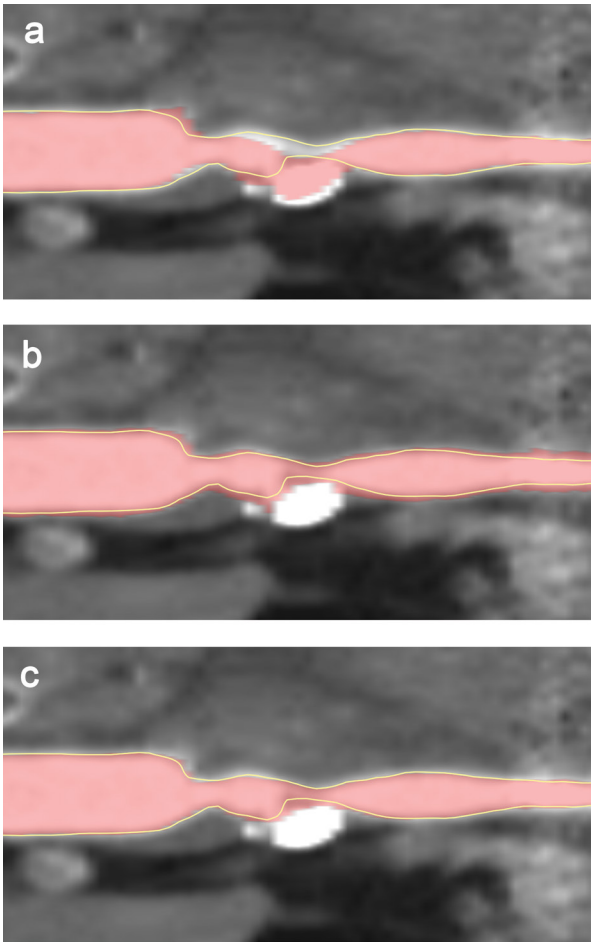

Figure 7. (Semi-) automated lumen segmentations of a carotid artery of different qualities

Shown are curved multiplanar reformats (CMPR) of a carotid artery with a calcified atherosclerotic plaque that causes a high-grade stenosis. A visual impression is shown of the reference standard (yellow line) based on manual annotations by three observers and automated lumen segmentations (in red) that have different qualities: a) with a bad Dice similarity index (SI) of 0.881, b) a moderate Dice SI of 0.884 and c) a good Dice SI of 0.945. The Dice similarity indices are calculated on the whole volume of which the shown CMPR is just a single plane.

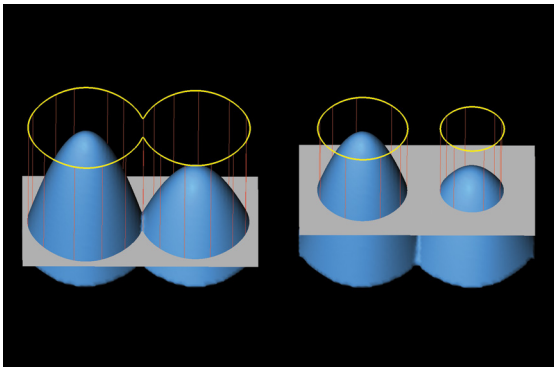

Figure 8. Level set method for lumen segmentation

Using the level set framework a segmentation (indicated by the yellow contours) is seen as the zero level (grey plane) of an embedding function in a higher dimension (blue surface). If the embedding function changes as is indicated by the two blue surfaces which are slightly different, the zero level (segmentation) changes. Using this framework a segmentation can easily change topology. The segmentation on the left has one object (contour) while the segmentation on the right consists of two distinct objects.

the blood flow as carotid arteries, especially at the site of atherosclerotic plaque, generally do not have circular luminal cross-sections and also do not run exactly perpendicular to the axial plane of the CT scan. Zhang et al. investigated the use of area measurements and found that assessment of area stenosis was highly reproducible and correlated better with diameter stenosis on DSA than did diameter stenosis as assessed on CTA.[45] The average error in assessing carotid artery stenosis of the best stenosis grading method according to the evaluation framework is 16.9% for area-based and 17.0% for diameter-based measurements.[41]

Besides stenosis grading, lumen segmentation also enables the extraction of other quantitative measures. The extracted lumen model can e.g. be used for Computation Fluid Dynamic calculations to assess the shear stress in the atherosclerotic carotid bifurcation[46] and the quantification of geometric parameters such as vessel tortuosity and bifurcation angles.[47]

Plaque imaging using CTA

Technical aspects

Assessment of different atherosclerotic plaque components in CTA relies on the differences in linear attenuation coefficient expressed in Hounsfield Units (HU) of the plaque components. Plaque component differentiation is highly dependent on scan parameters.

Reconstruction of thin slices is very important for plaque evaluation. Thin slices allow for datasets with isotropic and higher resolution and therefore enhance the differentiation of plaque components. Especially for plaque components of which HU values are close, like lipid and fibrous tissue, thin slices are crucial.

Where blooming artifacts from vessel wall calcifications can hamper correct stenosis measurements, it also causes a problem in the evaluation of plaque, since it interferes with optimal plaque characterization of the non-calcified part of the plaque. The finite spatial resolution of CT causes partial volume averaging and therefore blooming artifacts. Blooming of calcifications leads to overestimation of calcifications and inability to

evaluate the atherosclerotic regions that border the calcifications. Moreover, the calcification volume appears larger when using lower kVp settings.[21]

For accurate differentiation of plaque components a high signal-to-noise ratio (SNR) is necessary. Image noise depends mainly on the product of the tube current and rotation time (mAs), the tube voltage and reconstruction kernel. Because the atherosclerotic plaque is a relative small structure, a thin slice thickness and a small field-of-view are required. These result in a decrease of SNR, which should be compensated by a higher radiation exposure.

High intraluminal contrast material density may influence the density measurements in the plaque. Ex vivo studies in coronary arteries revealed that intraluminal attenuation strongly affects the measured attenuation of the plaque.[48] This can be explained by partial volume effects, but also by the entrance of contrast material in the plaque via the vaso vasorum. It is unknown yet if this effect is also seen in the larger carotid artery, but it underlines the necessity of standardized scan protocols, especially since plaque enhancement in CTA is thought to be associated with increased risk for neurological events.[49, 50]

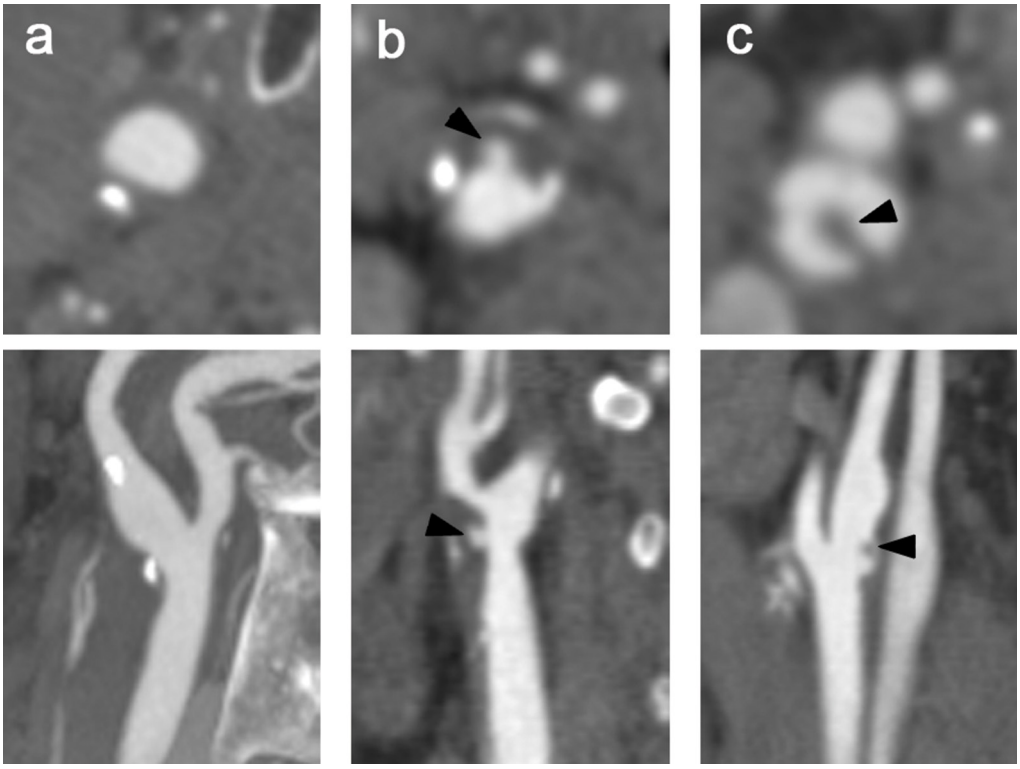

Figure 9. Assessment of plaque surface morphology using MDCT angiography

Cross-sectional images (upper panels) perpendicular to the central lumen line and multiplanar reformats (lower panels) of carotid bifurcations. a) An atherosclerotic carotid plaque with a smooth surface. b) A plaque at the level of the carotid bifurcation with an ulceration (arrowhead). c) An ulcerated plaque with thrombus material (arrow head) that protrudes into the lumen.

Another technical aspect influencing accurate differentiation of plaque components in CTA is the convolution kernel used for reconstruction of the image dataset. The convolution kernels allow for influencing the image characteristics; a smooth algorithm will reduce spatial resolution, image noise and image contrast for tiny structures, whereas a sharp algorithm has the opposite effect. Plaque characterization and quantification of the different plaque components based on measurement of HU densities is thus highly influenced by the convolution kernel used. Smooth kernels hamper the correct differentiation between tissues with small differences in density, as is the case for lipid and fibrous tissue. In contrast, sharp kernels increase contrast differentiation, but also lead to an increase in calcium size and low-intensity rings around calcifications (edge-enhancement artifacts), which hamper further plaque interpretation. Intermediate reconstruction kernels turned out to allow optimal plaque interpretation.[21]

The window-level setting also influences the visualization of the different plaque components. Whereas a large window-width is used in luminography to differentiate lumen from calcifications that border the lumen, a small window-width is necessary to enhance the small differences in HU density inside the non-calcified plaque (Figure 1).

Diagnostic accuracy

Plaque surface morphology

The accuracy of DSA in the detection of plaque ulceration, as compared to macroscopic surgical observations, has been found to be low (sensitivity 46% and sensitivity 74%).[51] CTA allows for analysis of the plaque surface (Figure 9) and the differentiation between ulcerations and irregularities and has been demonstrated to perform better than DSA.[52] A validation study, comparing single slice CTA with histological specimens reported a sensitivity and specificity of 60% and 74%, respectively.[53] However, MDCTA has been found to have a high sensitivity and specificity (94% and 99%, respectively) in detecting plaque ulcerations compared to surgical observation.[54] Assessment of plaque ulceration on MDCTA is highly reproducible ($k > 0.86$).[55, 56]

Plaque composition

The first validation studies compared 3 mm single slice CT images with histological sections from carotid endarterectomy specimens and did not show clear-cut results. Whereas two studies reported that calcifications, lipid and fibrous tissue could be differentiated based on density measurements[57, 58], another study concluded that single slice CT was not sufficiently robust to reliably characterize plaque composition and plaque morphology.[53] The introduction of multidetector CT enabled a more detailed analysis of the atherosclerotic carotid plaque composition and the differentiation of plaque components.

Several validation studies have been performed, in carotid arteries as well as in coronary arteries. In coronary studies, intravascular ultrasound (IVUS) is used as a gold standard. In carotid studies, the availability of histological carotid plaque specimens from carotid endarterectomy enables reliable validation against histology. An additional advantage is that the characterization of the separate plaque components can be performed easier and in more detail in the larger carotid arteries. In 2005, we performed an ex vivo validation study, in which CEA specimens were scanned and the images were compared with

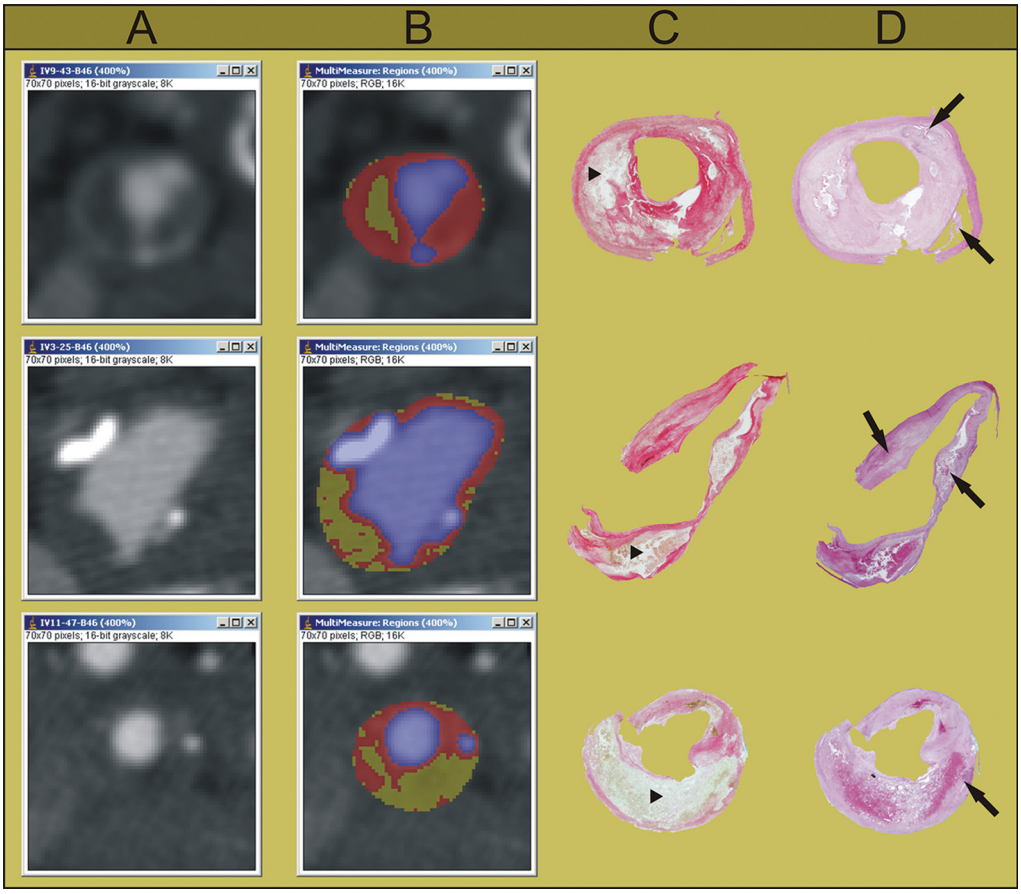

Figure 10. Hounsfield attenuation based differentiation of plaque components – validation against histological sections

Column A: axial MDCT images of a carotid artery with atherosclerotic plaque. Column B: MDCT plaque composition based on HU differences. Column C+D: Corresponding histological sections with Sirius Red (SR) and haematoxylin eosin (HE) staining, respectively. Blue regions in the MDCT plaque composition images correspond well with lumen and calcifications on HE stained histological sections (arrows). The red regions correspond well with the red collagen-rich regions in the SR stained sections. Yellow regions correspond with the lipid core (i.e. lipid, hemorrhage and necrotic debris) (arrowhead) regions on histology (the non-red regions on the SR stained sections that are not calcified areas on the HE stained sections). (Reprinted with permission from de Weert TT, Ouhlous M, Meijering E, et al (2006) In vivo characterization and quantification of atherosclerotic carotid plaque components with multidetector computed tomography and histopathological correlation. Arterioscler Thromb Vasc Biol 26 (10):2366-2372)

the histological slices (Figure 10). The CT value of lipid-rich regions differed significantly from that of fibrous-rich regions (45±21 HU versus 79±20 HU, $p<0.001$). An ROC-analysis revealed 60 HU as the optimal cut-off point for differentiation between lipid and fibrous tissue, with a sensitivity of 89% and a specificity of 93%.[21] The study was repeated in vivo, in which the CT values for lipid and fibrous-rich tissue were 25 ± 19 HU and 88 ± 18 HU, respectively. Again an optimal threshold value of 60 HU was found, with a sensitivity and specificity of both 100%.[59] Calcifications are easily detected on CT images as high density

structures and equivalent to coronary calcium scoring in electron beam CT, 130 HU is generally taken as a threshold for differentiating calcifications.

Wintermark and colleagues performed a validation study in which they compared in vivo MDCTA images with histological sections for the non-calcified plaque components and with ex vivo MDCTA images for the calcifications.[56] They found the following scan-parameter dependent cut-off values, determined as the half way HU attenuation value between the average HU values of each plaque component: 39.5 HU between lipid-rich and connective tissue, 72.0 HU between connective tissue and hemorrhage and 177.1 HU between hemorrhage and calcifications. They further compared the CT classification with the histological classification of type of atherosclerotic plaque and stage of lesion development according to the system derived from the AHA classification and found an overall agreement of 72.6% (unweighted κ of 67.6%).[56] The concordance for calcifications was perfect, whereas the reliability of the identification of the non-calcified plaque components was limited due to overlap of the values between the soft components. However, CTA showed good correlation with histology for larger lipid cores and larger hemorrhages. Further they demonstrated that CTA performed well in measuring fibrous cap thickness (R^2 = 0.77, p<0.001).[56]

Quantification of plaque components

Calcifications in the vessel wall can easily be measured in a quantitative way. Agatston and colleagues were the first to quantify coronary calcifications with electron beam CT.[60] As a default, the threshold to differentiate calcification is ³130 HU in non-contrast CT scans. Although the Agatston score as a quantification tool can be used in carotid arteries, other scoring methods like a volume score are more frequently used.[61] However, when CTA images are used, the threshold has to be higher in order to automatically differentiate calcifications from the bordering luminal contrast. Another possibility is to first delineate the inner and outer borders of the carotid plaque and subsequently discriminate calcifications using a HU threshold of 130 HU within the plaque. In this way, 3D volumetric MDCTA datasets also allow for quantification of the soft plaque components.

Annotation of the luminal area can be done (semi-)automatically based on thresholds that separate the bright, contrast filled lumen from the lower density plaque. However, calcifications bordering the lumen might be included in the lumen segmentation and therefore manual correction is necessary. Using lumen and outer vessel wall contours, the plaque area can be calculated by subtracting lumen area from vessel area.

Validation studies of plaque area measurements with histology as gold standard are hampered by the fact that histologic preparation leads to shrinkage of the specimens. Nevertheless, strong correlations have been found between ex vivo and in vivo MDCTA and histology for the assessment of plaque area (r^2 = 0.81 and 0.73, respectively).[21] Interobserver reproducibility of plaque area measurements with MDCTA was good (coefficient of variation of 10%).[59]

The CT value thresholds in HU that differentiate between plaque components create the opportunity to quantify plaque composition. The aforementioned in vivo validation study, comparing MDCTA images with histological specimens, showed that area measurements of calcifications were overestimated by MDCTA, however the correlation with histology was good (R^2= 0.74). The correlation between MDCTA and histology for fibrous

area measurements was also good (R^2 = 0.76), but was poor for lipid (R^2 = 0.24). Further investigation however showed that this correlation improved in mildly calcified plaques and non-calcified plaques (R^2 = 0.77 and 0.81, respectively). The intraobserver variability of area measurements of the different plaque components was low, with a coefficient of variation of 8%, 11% and 15% for calcifications, fibrous tissue and lipid respectively.[59]

Plaque volume and plaque component volumes can be calculated by multiplying area measurements with slice increment and the number of slices in the range of interest. In an in vivo study in 56 patients, plaque volume and plaque component volumes could be assessed in a reproducible way. The difficulty of defining the transition of normal wall into atherosclerotic plaque contributes highly to the interobserver variability. Consensus about the longitudinal dimension of the plaque improved the reproducibility of plaque volumes strongly.[62]

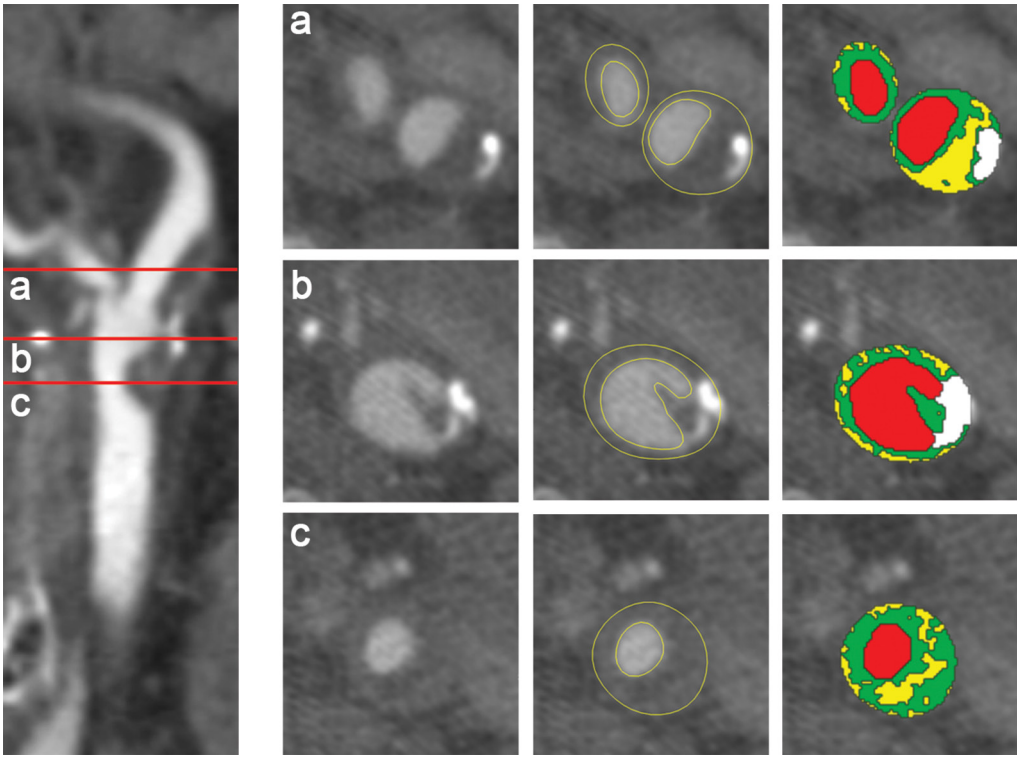

Figure 11. (Semi-) automated segmentation of lumen and plaque components

(Semi-) automated segmentation of lumen and plaque is performed on axial images within a predefined range. Contours of lumen and plaque are generated and can be manually adjusted if necessary. Within the plaque area (outer vessel wall contour minus lumen contour) the plaque components are differentiated based on Hounsfield Unit thresholds (lipids: <60 HU, fibrous 60-130 HU, calcification >130 HU). The color overlay shows the different structures; lumen = red, lipid = yellow, fibrous = green, calcification = white. This figure shows a CPR image of a carotid bifurcation on the left and plaque segmentation on axial slices at three levels: a) through the internal and external carotid artery, showing a large, mainly non-calcified atherosclerotic plaque in the internal carotid artery; b) through the distal common carotid artery just below the bifurcation on which an ulcerated surface is visible and c) through the common carotid artery at the level of the smallest vessel diameter.

(Semi)automated plaque measurements

Manually assessing lumen and vessel contours is a time-consuming task and is highly influenced by the window-level setting. Segmentation of the outer vessel wall and subsequent automated plaque characterization has received considerably less attention than luminal analysis.

The challenge of automated analysis of carotid atherosclerotic plaque lies in the difficulty of defining the outer vessel wall. This is a challenging task due to the low and varying contrast between the plaque and its surrounding soft tissue. Vukadinovic et al. developed a semi-automated method to segment the outer vessel wall[63], which only required clicking initialization points for lumen segmentation and clicking seed points for defining the range of interest for plaque segmentation. The method uses a level-set framework for vessel lumen segmentation,[37] followed by classification of calcium objects using a set of image features related to the appearance, shape and size of bright objects in the CTA data set. Subsequently, image voxels are identified as lying inside or outside the vessel wall, using a same set of image features. Finally, the outer vessel wall is determined by fitting an ellipsoid that utilizes the information from the calcium and inner/outer vessel classification step. After generation of the inner and outer vessel wall contours, the plaque components (lipid, fibrous tissues and calcifcations) are automatically differentiated based on the aforementioned HU-thresholds. Figure 11 shows the results of (semi) automatically generated plaque segmentations.

The method has been trained and tested on manually annotated MDCTA datasets of the carotid arteries and validated against manually segmented carotid arteries. The average Dice similarity index was 91%, which was comparable to the similarity index between two observers.[63] Subsequently, the performance of this plaque segmentation method in quantifying plaque volume and plaque component volumes was studied by comparing measurement error of the automated method using manual contours as a reference standard with the interobserver variability in manual annotations. The differences between the automated method and the manual observers were comparable to the interobserver variability.[64]

Although the method is highly automated some observer interventions are still needed and the outer vessel wall segmentations should still be checked manually for erroneous inclusion or exclusion of calcifications, and therewith over-segmentation or under-segmentation of the plaque. Taken these manual interventions into account, the intraobserver and interobserver reproducibility of the semiautomated method is better as compared to the reproducibility of manual annotating, with intraclass correlation coefficients of 0.93/0.84 for plaque volume and 0.86-1.00 / 0.76-0.99 for plaque component volumes (unpublished data).

Applications of quantitative atherosclerotic measures

MDCTA quantitative plaque measures have been used in several studies to investigate atherosclerotic development. Rozie et al. investigated in a cross-sectional study the correlation between cardiovascular risk factors and plaque volume and plaque composition. They found that plaque volume and severity of stenosis were just moderately correlated, which means that plaque volume could be an additional predictor for ischemic stroke.

An increasing plaque volume was associated with increased lipid and calcium proportion and a decreased fibrous proportion. Age and smoking were independently related to plaque volume. Patients with hypercholesterolemia had a significantly higher contribution of calcifications and a significantly lower contribution of lipid in the atherosclerotic plaque.[65] Another study investigated whether plaque features could be correlated with the presence of ulcerations, which is thought to be a marker of plaque rupture.[10] It was demonstrated that degree of stenosis, plaque volume and the proportion of lipid-rich necrotic-core were associated with the presence of carotid plaque ulcerations.[66] In another cross-sectional study, carotid atherosclerotic plaque features were identified that were significantly different in acute carotid stroke patients compared to non-stroke patients and on the infarct side compared to the contralateral side in stroke patients. These features included increased vessel wall, thinner fibrous caps, greater number of lipid cores and their location closer to the lumen. The number of calcium clusters was a protective factor.[67]

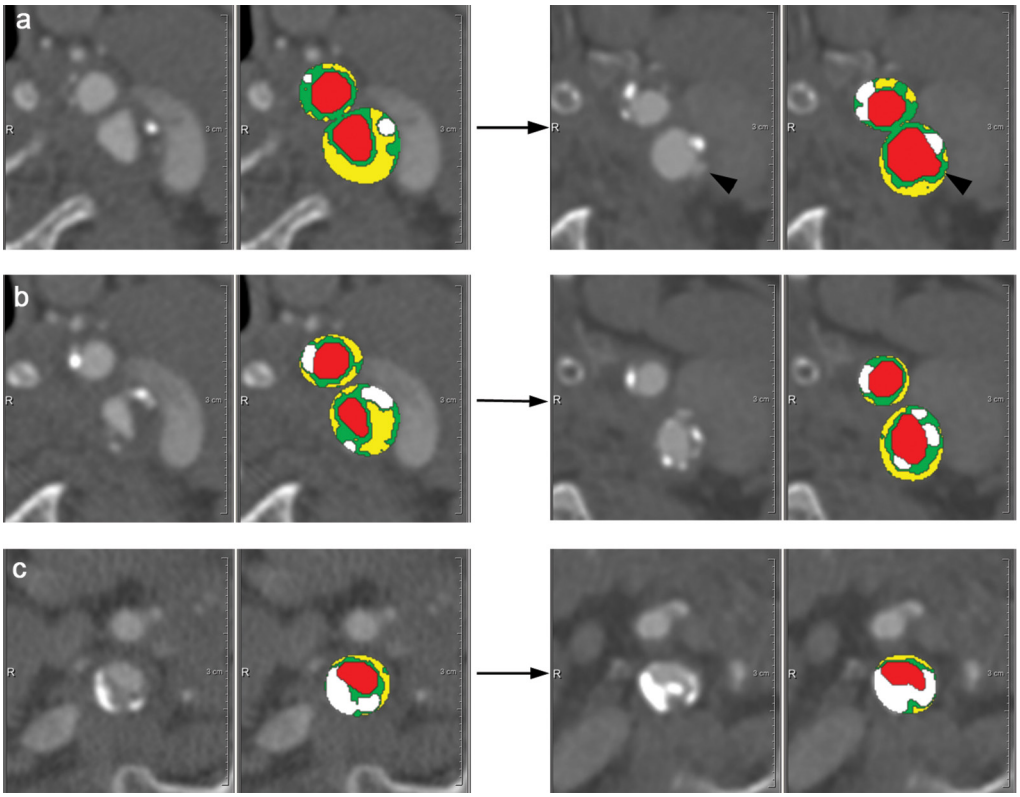

Figure 12. Serial quantitative plaque imaging

Serial plaque imaging in a 67 year old male patient (a+b) and a 62 year old male patient (c). On the left, axial MDCTA images and the plaque color overlays of the baseline scan and on the right the corresponding images of the follow-up scan. a) Predominantly non-calcified plaque at a level just above the bifurcation. At follow-up imaging after 6 years an ulceration is visible (arrow head). b) Images from the same artery at a more distal site. a+b) At follow-up a decrease in plaque volume and a change in plaque composition is demonstrated. c) Internal carotid artery demonstrating an increase in calcification of the vessel wall at follow-up (after 6 years).

Numerous studies report on the role of plaque calcifications in plaque stability. The findings are not conclusive. A recent systemic review suggests that clinically symptomatic plaques have a lower degree of calcifications than asymptomatic plaques.[61] The percentage calcification within a plaque of a stenosed artery, rather than the absolute volume seems to be associated with plaque stability.[68] This underscores why the (automated) quantification of the relative contribution of the plaque components might add to the improvement of stroke risk prediction.

The MDCTA plaque imaging studies performed so far had a cross-sectional design. The ability to quantify atherosclerotic features in vivo creates the potential to further explore atherosclerotic plaque development by prospective, serial in vivo imaging of the carotid plaque (Figure 12). Currently, a prospective serial MDCTA plaque imaging study is being performed, which investigates the temporal changes in plaque burden and plaque composition and its determinants in TIA and stroke patients.

Summary and future directions

Currently, MDCTA is a non-invasive imaging technique frequently used in clinical practice for the assessment of carotid stenosis grading and is replacing the more invasive technique of DSA. MDCTA can perfectly be combined with a native CT scan and a perfusion CT scan of the brain in the evaluation of stroke patients.

Although plaque imaging is currently only used in research settings, the same MDCTA data set might provide further clinically important information on the atherosclerotic plaque burden, plaque surface morphology and plaque composition for a more individualized risk prediction. Large, prospective studies demonstrating significant associations between CTA-based risk factors and (recurrent) events are yet lacking. Future research should focus on the role of plaque measures in stroke risk prediction and on the use of MDCTA-based quantified plaque measurements in monitoring efficacy of medical therapy. Large –preferably multicenter– studies, should pay attention to the standardization of data acquisition and post processing across centers and imaging time points, since several technical parameters highly influence quantification of plaque features as is broadly depicted in this chapter. More effort should further be put in the development of robust, accurate, automated quantification of plaque measures in order to minimize measurement variability.

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Chapter 2.2

Automated versus manual segmentation of atherosclerotic carotid plaque volume and components in CTA: associations with cardiovascular risk factors

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Abstract

The purpose of this study was to validate automated atherosclerotic plaque measurements in carotid arteries from CT angiography (CTA). We present an automated method (three initialization points are required) to measure plaque components within the carotid vessel wall in CTA. Plaque components (calcifications, fibrous tissue, lipids) are determined by different ranges of Hounsfield Unit values within the vessel wall. On CTA scans of 40 symptomatic patients with atherosclerotic plaque in the carotid artery automatically segmented plaque volume, calcified, fibrous and lipid percentages were 0.97 ± 0.51 cm^3, 10 ± 11%, 63 ± 10% and 25 ± 5%; while manual measurements by first observer were 0.95 ± 0.60 cm^3, 14 ± 16%, 63 ± 13% and 21 ± 9%, respectively and manual measurement by second observer were 1.05 ± 0.75 cm^3, 11 ± 12%, 61 ± 11% and 27 ± 10%. In 90 datasets, significant associations were found between age, gender, hypercholesterolemia, diabetes, smoking and previous cerebrovascular disease and plaque features. For both automated and manual measurements, significant associations were found between: age and calcium and fibrous tissue percentage; gender and plaque volume and lipid percentage; diabetes and calcium, smoking and plaque volume; previous cerebrovascular disease and plaque volume. Significant associations found only by the automated method were between age and plaque volume, hypercholesterolemia and plaque volume and diabetes and fibrous tissue percentage. Significant association found only by the manual method was between previous cerebrovascular disease and percentage of fibrous tissue. Automated analysis of plaque composition in the carotid arteries is comparable with the manual analysis and has the potential to replace it.

Introduction

One of the major causes of death in the western world is atherosclerotic disease, which manifests itself as ischemic heart disease and ischemic stroke.[1] The amount of atherosclerotic disease in carotid arteries is normally expressed by the severity of luminal narrowing. Risk of (recurrent) stroke is related to the severity of stenosis.[2] However, the presence of a large atherosclerotic plaque is not always associated with luminal narrowing[3], which demonstrates that luminal narrowing alone is probably not a reliable marker of atherosclerosis. In addition, studies on carotid atherosclerotic plaque show that plaque morphology and composition are also important in the risk assessment of patients with carotid artery stenosis.[4-6]

Atherosclerotic plaque volume and composition can be determined with Magnetic Resonance Imaging (MRI)[7-10] and CT angiography (CTA).[11-14] CTA has established itself as an accurate modality to assess the presence of atherosclerotic disease and to grade the severity of stenosis.[15] Carotid plaques with a thin fibrous cap and a large lipid core are also considered to increase the risk for stroke,[16,17] while plaques with high calcium content, especially when located superficially, are thought to be associated with a lower risk for stroke.[18]

Manual measurement of plaque volume and the contribution of the different plaque components to the plaque volume in MRI or CTA data is a very labor intensive task. Several methods address the segmentation of the outer vessel wall and plaque components in both MRI and CTA data in carotid arteries[19-24] as well as coronary arteries.[25,26] We previously developed an algorithm to automate the plaque measurements in CTA imaging data of the carotid arteries. In this algorithm we combined outer vessel wall segmentation[27] with lumen segmentation.[28] Once the outer vessel wall and lumen were segmented, the plaque components were segmented using distinctive ranges of Hounsfield Unit (HU) values.[11] However, with this algorithm the outer vessel wall was automatically segmented only in common carotid artery, while atherosclerotic disease is commonly present in both the common and internal carotid artery.

The purpose of this study was to develop and evaluate a method to obtain automated measurements of plaque volume and its components at the carotid bifurcation and to demonstrate that this method has potential to replace the manual measurements in terms of accuracy in plaque volume and plaque component characterization. Furthermore, we investigated whether in group associations studies similar trends can be found with automated processing. Hereto, the method validation consists of (1) evaluation of the accuracy by comparing differences between method and manual tracings with variability of manual measurements of different observers and (2) comparison of the associations between cardiovascular risk factors and plaque features as assessed with manual segmentation and automated segmentations.

Materials and methods

Study population

From November 2002 to December 2005, patients with amaurosis fugax, TIA or minor ischemic stroke (Rankin score ≤3) were consecutively enrolled in the study cohort and clinical and research data were derived in a standardized way. Multi-detector CT angiography (MDCTA) of the carotid arteries was performed as part of a research protocol, approved by the Institutional Review Board. All patients gave written informed consent. All patients underwent neurological examination on admission and symptoms and risk factors were reported. Subsequently, all carotid arteries of those patients with symptoms in the anterior circulation were evaluated for the presence of atherosclerotic plaque. This validation study of the automated plaque segmentation has a retrospective study design. The main test set contained the symptomatic carotid artery from 90 randomly selected patients (63% male, mean age 67 ± 11 years) from the group of patients with atherosclerotic plaque in the symptomatic carotid artery. The symptomatic carotid artery was the artery ipsilateral to the ischemic hemisphere, which was based on clinical symptoms and findings on MDCT of the brain. A subset of 40 datasets, which has a similar distribution of stenosis degrees as the full set, was used for the interobserver study.

Training set for automated method

The parameter settings for the automated method were previously trained on 40 manually annotated datasets, which are not part of the 90 datasets for which the method is evaluated. Furthermore this training was manually annotated by a different observer than the two observers who annotated the imaging data reported on this study. Hence, a possible bias of the method to one of the observers is prevented.

Scan protocol and image reconstruction

CTA of the carotid arteries was performed on a 16-slice MDCT system (Siemens, Sensation 16, Erlangen, Germany) with a standardized optimized contrast-enhanced protocol (120 kVp, 180 mAs, collimation 16 x 0.75 mm, table feed 12 mm/rotation, pitch 1).[29] All patients received 80 ml contrast material (Iodixanol 320 mg/mL, Visipaque, Amersham Health, Little Chalfont, UK), followed by 40 ml saline bolus chaser, each at an injection rate of 4 mL/s. Synchronization between the passage of contrast material and data acquisition was achieved by real-time bolus tracking at the level of the ascending aorta. Image reconstructions were made with a 120-mm field of view, a matrix size of 512 x 512, a slice thickness of 1.0 mm, an increment of 0.6 mm, and an intermediate reconstruction filter (B46f).

Plaque volume and composition measurements

To define different plaque components by using different HU ranges, it is sufficient to have a segmentation of the carotid artery outer vessel wall and the lumen.[11]

Automated Segmentation of the outer vessel wall and lumen

An automatic method using a three-point initialization was used to segment the outer vessel wall of the carotid artery in CTA.[27,30] First, the vessel lumen was segmented using a level set approach,[28] using an initialization point in the common, internal and external carotid artery. Subsequently, using a set of image features, calcium objects which are part of the vessel wall were detected using a GentleBoost framework.[27] Calcium object classification is used as a preprocessing step for the outer vessel wall segmentation since it is a much easier task than outer vessel segmentation and it can improve the accuracy of outer vessel wall segmentation. In the third step probability images were created that indicate the likeliness of a voxel lying within or outside the vessel, using the same GentleBoost framework. Each voxel is represented by a set of descriptive features: distance of the pixel to the lumen center and a set of contextual features. Contextual features in this case are radial image intensity profiles emanating from the lumen center. These profiles are extracted from the original image, the image smoothed with 2D Gaussian filters at different scales and directional 2D, Gaussian derivatives also at different scales. Based on this set of features, a GentleBoost classifier is trained to classify each pixel as being inside or outside vessel wall. The classifier provides a confidence measure which reflects the likelihood that a pixel lies inside or outside the vessel. Finally, ellipsoids are fitted using both the calcium and vessel classification results.

Manual Segmentation of the outer vessel wall and lumen

Images of carotid arteries that contained plaque were analyzed using custom-made quantitative image analysis software, developed using MeVisLab (MeVis Research, Bremen, Germany). Using this software, regions of interest (ROI) were manually drawn over the outer vessel wall contour in consecutive axial MDCTA images (Figure 1). Since the observers placed the ROI over the outer vessel wall contour, the ROI consisted of both plaque and lumen. The window/level setting was fixed at 1200/800 HU for all measurements. To assess the border between lumen and atherosclerotic plaque it was necessary to draw a second ROI close to the lumen in each image. Normally, the lumen area was then automatically differentiated from atherosclerotic plaque based on the adjusted cut-off value. But in those plaques in which calcifications bordered the lumen and the two dense structures merged with each other, lumen area and calcifications had to be separated by manual drawing. One observer (S.R.) who was blinded to other clinical information, manually drew lumen and outer vessel wall contours. A second observer (M.G.) performed the manual segmentations in the interobserver dataset. A third observer (T.W.) performed manual annotation of the training set on which our automated method was trained.

Calculation of the volume of plaque and the components

Having the carotid artery vessel wall and lumen segmented, different HU ranges were used to define different plaque components. The cut-off point for the distinction between calcifications and fibrous tissue was set at 130 HU, the value currently used for calcium scoring. The cut-off point for the distinction between fibrous tissue and lipid was set at 60 HU as determined in previous studies.[11] We adjusted the cut-off point for the distinction between atherosclerotic plaque and vessel lumen for each patient on the basis of the full-width-half-maximum principle (mean lumen attenuation plus mean fibrous tissue attenuation (≈88 HU) divided by two). The pixels surrounding the vessel lumen, with a

density between 130 HU and the adjusted cut-off value, were considered to be fibrous tissue.

The plaque volume and the volume of the plaque components were calculated by multiplying the number of pixels of the total atherosclerotic plaque or its components, with the pixel size and the slice increment. The proportion of plaque components was calculated as the ratio of volume of the component to the total plaque volume multiplied by 100. As an example, in Figure. 1 a cross-sectional slice with outer vessel wall and all three plaque components in different colors automatically segmented and manually segmented by two observers is shown.

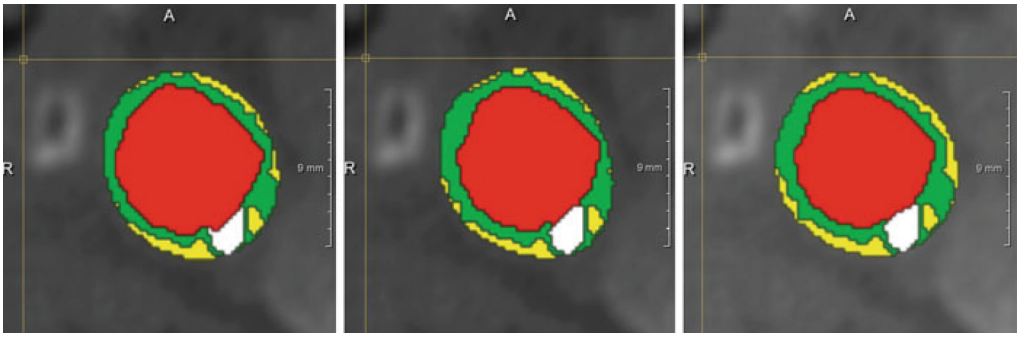

Figure 1. Different plaque components on one cross sectional slice segmented by the automated method (left), observer S.R. (center) and observer M.G. (right). Red = lumen, Green = fibrous, yellow = lipids, white = calcium

Cardiovascular risk factors

We obtained clinical measures and information on risk factors and medication during the patient's visit at the outpatient clinic. Subjects were categorized as currently, ever, or never smoking. Hypertension was defined as systolic blood pressure over 140 mmHg and/or diastolic blood pressure over 90 mmHg during two episodes of at least 15 min of continuous noninvasive blood-pressure measurement and/or treatment with antihypertensive medication. Blood pressure-lowering drugs comprised ACE inhibitors, calcium-antagonists, beta-blockers, and diuretics. Hypercholesterolemia was defined as fasting cholesterol over 5.0 mmol/l and/or use of cholesterol-lowering drugs. Diabetes was defined as fasting serum glucose levels over 7.9 mmol/l, nonfasting serum glucose levels over 11.0 mmol/l, or use of antidiabetic medication. Information was collected on previous cardiovascular events and conditions (myocardial infarction, atrial fibrillation, angina pectoris, chronic heart failure, coronary artery bypass grafting) and previous cerebrovascular events.

Statistical analysis

Both for automated and manual measurements (2 observers), plaque and plaque component volumes and proportions were presented on 40 datasets with mean ($^{+}/_{-}$ SD). The differences between automated and manual measurements and those between the measurements of two observers are presented with mean values and standard deviations.

The differences between the automated and manual measurements (2 observers) on 40 datasets were plotted against the mean value of the measurements (Bland–Altman plot), they were evaluated with paired Student t test and the correlation between the measurements by the two methods was evaluated by Pearson's correlation coefficient (Rp). Bland–Altman plots are plotted in Excel. Limits of agreement are calculated as average difference ± 1.96 * standard deviation of the difference.

The differences between automated and manual measurements on 90 datasets (1 observer) are presented with mean values and standard deviations and the correlation was evaluated by Rp.

Both for automated and manual measurements (1 observer) we determined the associations between cardiovascular risk factors and PV and plaque composition on 90 datasets using univariable linear regression. Multivariable analysis was not performed for two reasons. Firstly, the focus of the study was to show that automated method can replicate the associations between manually measured plaque components and cardiovascular risk factors. Second, we did not focus on the assessment of independent associations between risk factors and plaque characteristics as this was not possible due to limited sample size. Because the distribution of plaque volume was skewed, we used a log10 transformation prior to statistical analysis. Similarly, for proportion of plaque components we used square root (sqrt) transformation.

P values ‹ 0.05 were considered statistically significant. Statistical analyses were performed using SPSS software (version 15.0, Inc., Chicago, Illinois).

Results

Baseline clinical characteristics

The baseline characteristics are presented in Table 1.

Table 1. Demographic and clinical characteristics of 90 patients

Characteristics	N=90
Age [years; mean ±SD]	67±11
Male sex [%]	63
Hypertension [%]	84
Hypercholesterolemia [%]	85
Diabetes Mellitus [%]	24
Smoking: current or past [%]	44
Previous cardiac disease [%]	35
Previous cerebrovascular disease [%]	31

Plaque measurements: comparison to interobserver variability

Table 2 shows lumen, plaque volume, plaque component volumes and plaque component proportions assessed by the automated method and by the manual methods (two observers) in 40 datasets. The differences between the automated and the manual method and between the manual measurements of two observers are also shown. The differences between automated method and both observers were in the same range as the differences between observers. The difference between the automated method and first observer manual measurements of PV, calcified, fibrous and lipid percentages were 0.02 ± 0.24 cm^3, -4 ± 6%, -0 ± 8% and 4 ± 7%, respectively. The differences between automated method and second observer manual measurements of PV, calcified, fibrous and lipid percentages were -0.09 ± 0.43 cm3, -1 ± 4%, 2 ± 8% and -1 ± 9%. respectively. The differences between two observers manual measurements of PV, calcified, fibrous and lipid percentages were 0.11 ± 0.29 cm3, 3 ± 5%, 2 ± 6% and -5 ± 7%, respectively. Similar differences were found between automated plaque measurements and manual measurements performed on 90 datasets by observer S.R.

Figure 2 shows regression plots and Pearson's correlation coefficient (Rp) between automated and manual measurements by both observers of plaque features on 40 datasets. For PV, calcium, fibrous and lipid contribution correlation coefficients between automated and manual measurements of observer S.R. and M.G. were: 0.92 and 0.83; 0.94 and 0.94; 0.79 and 0.73 and 0.57 and 0.52, respectively. Correlation coefficients between automated and manual measurements by observer S.R. on 90 datasets of PV, calcium, fibrous and lipid contributions were 0.89, 0.86, 0.77 and 0.55, respectively.

From the Bland–Altman plots, in Figure 3, it can be observed that the difference between automated and manual measurements of plaque volume increases when the volume increases. Similarly, the difference between automated and manual measurements of plaque components proportions increase with the components proportions.

Table 2. Plaque features measured by automated method and by two observers in 40 datasets with the differences and coefficients of variation.

40 datasets	μ (A) ± SD	μ (Obs 1) ± SD	μ (Obs 2) ± SD	μ (Diff (A,Obs1)) ± SD	μ (Diff (A,Obs2)) ± SD	μ (Diff (Obs1,Obs2)) ± SD
Plaque volume [mm^3]	965±511	946±595	1052±754	19±235	-87±432	-106±288*
Lumen volume [mm^3]	1030±577	1040±562	1052±567	-10±84	-22±73	-12±27*
Calcium volume [mm^3]	129±178	155±197	136±174	-26±50*	-6±45	19±31*
Fibrous volume [mm^3]	591±271	564±302	602±336	26±101	-10±165	-37±112*
Lipid volume [mm^3]	244±144	225±215	314±348	18±133	-69±272	-88±190*
Calcium proportion [%]	10±11	14±16	11±12	-4±6*	-1±4	3±5*
Fibrous proportion [%]	63±10	63±13	61±11	-0±8	2±8	2±6*
Lipid proportion [%]	25±5	21±9	27±10	4±7*	-1±9	-5±7*

μ- mean, A – automated method, Obs1 – first observer, Obs2 – second observer, SD – standard deviation, Diff – difference, CoV – coefficient of variation, * - t-test P-value < .05

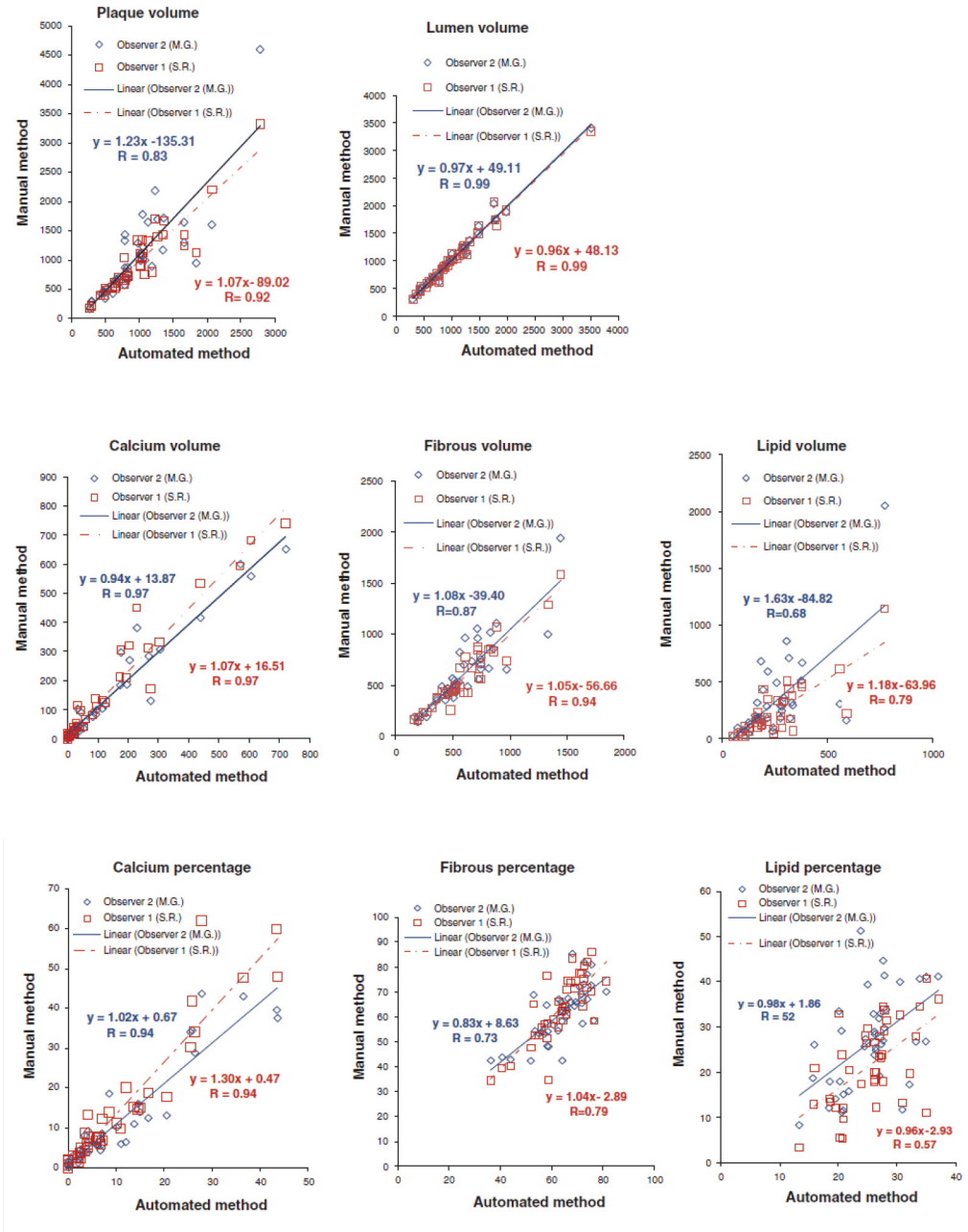

Figure 2. Regression plots showing the comparison between automated and manual measurements in 40 datasets

Risk factors associations with plaque features: comparison between manual and automated measurements

Table 3 shows the associations between cardiovascular risk factors and automatically assessed and manually assessed plaque volume and plaque component percentages on 90 datasets. Mostly, similar associations were found with automatically and manually assessed plaque features and cardiovascular risk factors.

Older patients had significantly larger PV measured by the automated method, whereas the association with manually assessed PV was not significant (P = 0.065). Male patients had significantly higher plaque volume for both automated and manual plaque measurements. Patients with hypercholesterolemia had significantly lower plaque volume when measured with the automated method, and not when measured manually. Patients who were smokers and patients who had a history of cerebrovascular disease had significantly higher plaque volume for both automated and manual plaque measurements.

For both automated and manual plaque measurements older patients had a significantly higher calcium contribution and a significantly lower fibrous contribution. Male patients had a significantly higher lipid contribution for both automated and manual plaque measurements. Patients with diabetes had a significantly higher calcium contribution for both automated and manual plaque measurements and a significantly lower fibrous contribution when measured with the automated method and not when measured manually.

Patients with a history of cerebrovascular disease had a significantly lower fibrous contribution when measured manually and not when measured automatically.

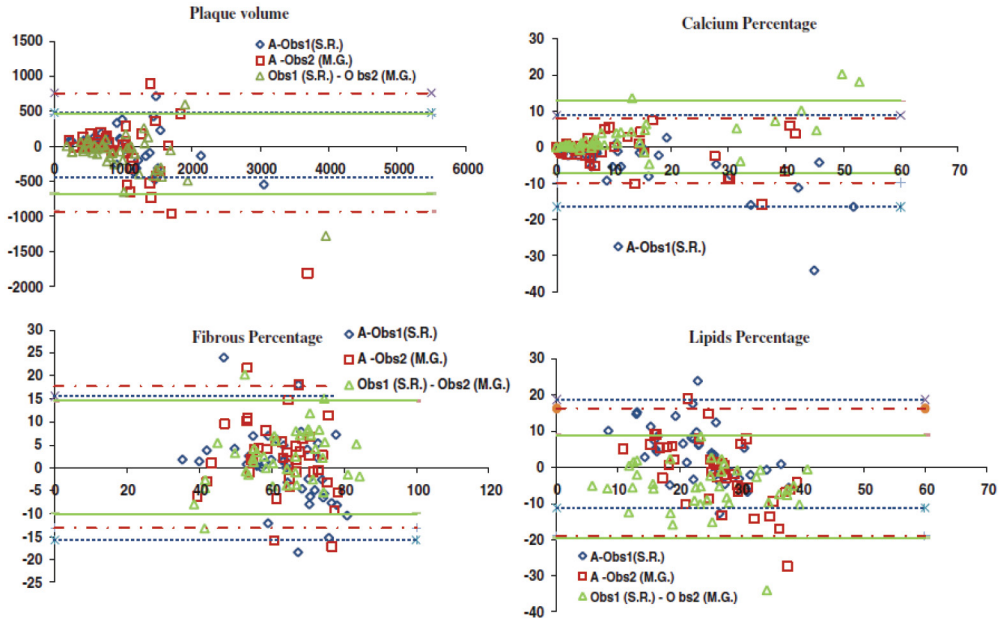

Figure 3. Bland-Altman plots of plaque volume and plaque components percentages assessed by automated method and manual method by two observers in 40 datasets

Table 3. The associations between risk factors and PV and plaque component percentages assessed by automated method (A) and manual method (M) in 90 datasets

90 Datasets	PV(A) B (P value)	PV (M) B (P value)	Calc% (A) B (P value)	Calc% (M) B (P value)	Fib% (A) B (P value)	Fib % (M) B (P value)	Lip % (A) B (P value)	Lip % (M) B (P value)
Age	0.004 (0.029)*	0.005 (0.065)	0.048 (0.004)*	0.056 (0.001)*	-0.013 (0.033)*	-0.021 (0.005)*	-0.006 (0.25)	-0.006 (0.56)
Gender	0.113 (0.010)*	0.168 (0.003)*	0.182 (0.64)	0.192 (0.64)	-0.196 (0.16)	-0.323 (0.062)	0.234 (0.048)*	0.560 (0.024)*
Hypercholesterolemia	-0.120 (0.049)*	-0.054 (0.49)	-0.728 (0.17)	-1.056 (0.60)	0.268 (0.16)	0.152 (0.53)	-0.064 (0.71)	0.505 (0.14)
Hypertension	0.052 (0.39)	0.048 (0.52)	0.315 (0.54)	0.224 (0.68)	-0.005 (0.98)	-0.044 (0.85)	-0.106 (0.52)	-0.138 (0.68)
Diabetes Mellitus	0.025 (0.62)	0.018 (0.78)	1.163 (0.006)*	1.231 (0.007)*	-0.396 (0.010)*	-0.317 (0.10)	-0.103 (0.46)	-0.403 (0.15)
Smoking (ever)	0.092 (0.031)*	0.113 (0.038)*	0.137 (0.72)	0.094 (0.82)	-0.048 (0.72)	-0.093 (0.58)	0.015 (0.90)	0.221 (0.36)
Previous cardiac disease	0.053 (0.24)	0.075 (0.19)	0.593 (0.12)	0.634 (0.13)	-0.095 (0.50)	-0.264 (0.13)	-0.199 (0.11)	-0.058 (0.82)
Previous cerebrovascular disease	0.122 (0.008)*	0.138 (0.019)*	0.489 (0.22)	0.812 (0.056)	-0.234 (0.11)	-0.465 (0.009)*	0.047 (0.71)	0.076 (0.77)

The associations were assessed by linear regression and represented by coefficient of regression (B) and P value

Significant P values (<0.005), *PV* plaque volume, *A* automated method, *M* manual method, *B* regression coefficients, *Calc* calcium, *Fib* fibrous, *Lip* lipid

Discussion

In this study we presented a method for automated plaque volume and plaque composition assessment. Furthermore, we evaluated its accuracy (i) with respect to manual tracings, and (ii) its ability to replicate associations between plaque characteristics and cardiovascular risk factors.

With respect to segmentation accuracy, we showed that the differences in estimating plaque volume and plaque components between our automated method and expert observers are in the same range as interobserver variability. The results show some bias between the observers and between observers and the method. All automated volume measurements values are larger than volumes measured by observer S.R. and smaller than the volumes measured by observer M.G. with the exception of calcium volume and fibrous contribution. All the volumes and proportion differences between two observers were statistically significant, although these differences were small. The statistically significant difference is a consequence of a persistent, albeit small, oversegmentation of most plaque components by observer M.G. compared to observer S.R. The differences between automated method and observer S.R. were significant for calcium volume and proportion and lipid proportion. None of the differences between automated method and observer M.G. were significant. The differences between automated method and observer S.R. were larger than the differences between automated method and observer M.G. for more measurements: calcium volume, fibrous volume, calcium proportion and lipid proportion. In a previous interobserver study of plaque and plaque components assessment with CTA, in which three observers manually annotated 46 CTA datasets,[14] the differences of plaque volume and all plaque components were significant between at least one pair of observers.[14] Pearson's correlation coefficients between automated and manual measurements of both observers for PV and plaque contributions were mostly similar as shown in Figure 2.

In a second evaluation, we showed that associations between cardiovascular risk factors and automated plaque measurements were mostly similar to the associations found with manual plaque measurements. Seven associations were significant for both the automated and manual plaque measurements; one was significant for the automated method and almost significant for manual method and three were significant for only one of the methods. The associations that were found to be significant for both manual and automated measurements have also similar correlation coefficient values.

To our knowledge, this is the first study that compares cardiovascular risk factors associations with manually and automatically assessed plaque volume and plaque components. When comparing our results with results of a previous study in which the association between cardiovascular risk factors and manually assessed plaque volume and plaque components was evaluated in 57 symptomatic carotid arteries,[31] we found more associations. This can possible be explained by the larger number of datasets that is used in our study. In the previous study age and smoking were related to plaque volume, which is confirmed in our current study. In the previous study, patients with hypercholesterolemia had significantly less lipid and more calcium; these associations were not confirmed. A reason for this could be that automated results are the least similar to manual ones in case of lipids contribution (Rp = 0.55). Calcium proportion is shown to be underestimated by the automated method.

In previous studies from our institute on CT based plaque assessment, [11, 29, 14] different datasets were used than in this paper. The 57 datasets used to relate manually derived

plaque measurements to cardiovascular risk factors in[31] are a subset of the 90 datasets used in this paper. Compared to this work, we thus both extended the dataset, and we investigated the influence of automated plaque assessment, in order to try to replace laborious manual plaque segmentation.

Manual quantification takes on average around 30 min per carotid artery. This time period depends on the lesion length. The automated method takes on average around 6 min on a single CPU 2GHZ, RAM 24 GB computers. Manual plaque segmentation is not applicable in clinical setting, because it is too time-consuming. Automated plaque assessment already is considerably faster and improved implementation and hardware could reduce processing time such that it would become acceptable in clinical workflow. Also, this automated tool can be used in larger datasets for the investigation of associations between plaque volume/composition and risk factors or recurrent ischemic events and for longitudinal studies on plaque imaging.

Study limitations

A limitation of our study is that we do not have a definite gold standard. The scarcity of histological carotid plaque specimens hampers the validation of automated plaque volume and composition assessment on a sizeable dataset. We therefore compared results from automated plaque segmentation with those from manual plaque segmentation—a method previously validated against histology—thus indirectly evaluating the performance of our automated method.

A second limitation of our study is that we used CT data from a single vendor, collected at a single site. Our automated method might perform less on data from different CT-scanners, different sites or using different image protocols. However, the underlying method is generic and if required can be tuned to different systems by using new training on data.

A third limitation concerns the plaque segmentation method that uses distinctive ranges of Hounsfield Unit (HU) values.[11] There is some overlap between the HU of lipid and fibrous tissue and no distinction can made between lipid and intraplaque heamorrhage, which is thought to be an important feature in plaque vulnerability assessment as well.[9]

As a final limitation, the comparison of cardiovascular risk factors associations with manually and automatically segmented plaque components does only indicate that the automated method can be used to find similar associations in a group study. It does not provide information on the method applicability on a single subject.

Conclusion

We presented an approach to automatically segment outer vessel wall and plaque of carotid artery in CTA and to automatically assess plaque volume and plaque components. The results were validated with respect to manual tracings and interobserver variability.

Furthermore, the associations between cardiovascular risk factors and plaque volume and plaque component contributions assessed by our automated method and a manual

method were compared. We have shown that the difference between our automated method and the observers is in the range of the variability of the observers, and hence can be applied for automated analysis in large studies.

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Chapter 3

Carotid atherosclerotic plaque ulceration

Chapter 3.1

Atherosclerotic plaque ulceration in the symptomatic internal carotid artery is associated with non-lacunar ischemic stroke

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Abstract

Background and Purpose Atherosclerotic carotid plaque ulceration is considered a marker of previous plaque rupture and subsequent thromboembolism. It can be accurately detected with multidetector CTA. We hypothesized that atherosclerotic plaque ulceration is associated with non-lacunar ischemic stroke rather than lacunar stroke.

Methods Prospectively, 750 consecutive patients with transient ischemic attack or ischemic stroke symptoms in the anterior cerebral circulation were evaluated for the presence of atherosclerotic plaque ulceration in the symptomatic carotid artery with multidetector CTA. Patients with stroke due to cardiac embolism or other specific etiologies and patients with amaurosis fugax were excluded. Ischemic strokes in the remaining 534 patients were classified as non-lacunar (n=236) or lacunar (n=298) based on clinical symptoms and multidetector CT of the brain. Ulceration was defined as extension of contrast material beyond the vascular lumen into the surrounding plaque.

Results Plaque ulceration in the symptomatic carotid artery was more common in non-lacunar strokes (n=47, 20%) as compared to lacunar strokes (n=20, 7%; $P<0.001$). After adjustment for age, gender, cardiovascular risk factors, and degree of stenosis, ulcerations were independently associated with non-lacunar stroke compared to lacunar stroke (odds ratio, 2.70; 95% confidence interval, 1.43-5.09).

Conclusions Atherosclerotic carotid plaque ulceration is associated with non-lacunar ischemic stroke, independent of the degree of carotid stenosis. These results suggest that non-lacunar stroke and lacunar stroke are caused by different pathophysiological mechanisms.

Introduction

Whereas lacunar strokes are associated with local occlusive disease of the deep perforating arteries at the base of the brain,[1] large deep and non-lacunar ischemic strokes are frequently caused by thromboembolism from extracranial arteries or the heart.[2] The association of atrial fibrillation and carotid stenosis with non-lacunar stroke[3] supports the assumption that this is attributable to (thrombo-)embolism. Atherosclerotic carotid plaque ulceration is considered to be a marker of previous plaque rupture and an influential predictor of ischemic stroke besides degree of stenosis.[4,5] Plaque rupture with subsequent thrombus formation and embolisation of plaque material or thrombus into the intracranial circulation may cause non-lacunar stroke.

Multidetector computed tomography angiography (MDCTA) has been demonstrated to be effective in the detection of carotid plaque ulceration, with a sensitivity and specificity of 94% and 99% respectively.[6] CT angiography is superior to digital subtraction angiography in detecting ulcerations of the carotid atherosclerotic plaque.[7]

In the current study, the association between atherosclerotic plaque ulceration in the symptomatic carotid artery and non-lacunar stroke was evaluated by means of MDCTA in a large population of patients with ischemic stroke. If non-lacunar stroke is associated with thromboembolism, then it may also be associated with atherosclerotic plaque ulceration in the symptomatic carotid artery. To test this hypothesis, we compared the prevalence of plaque ulceration by means of MDCTA between patients with lacunar and non-lacunar stroke.

Materials and Methods

Study Population

From a prospective registry of 911 consenting patients with amaurosis fugax, transient ischemic attack (TIA) or minor ischemic stroke (Rankin score <4) who underwent MDCTA of the carotid arteries, we selected all patients (n=750) with symptoms in the anterior circulation. Patients were enrolled from a specialized TIA/stroke outpatient clinic or the neurology ward. All patients underwent an interview, neurological examination, electrocardiography and laboratory analysis on admission. Medical history and cardiovascular risk factors were thereby recorded. On admission, patients underwent MDCT of the brain and MDCTA of the carotid arteries in a single session. In 3 patients the carotid arteries could not be analyzed because of scan artifacts. Patients with a likely cardiac etiology (n=96) or other specific etiology 9n=20) according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria were excluded.[8] Subsequently, patients with amaurosis fugax (n=79) were excluded. Amaurosis fugax is often associated with nonatherosclerotic, primary neurological or ophthalmologic orirgin, and it conveys a different cerebrovascular prognosis.[9] The remaining 534 patients were included in the study.

Cardiovascular Risk Factors

Hypercholesterolemia was defined as fasting cholesterol >5.0 mmol/l or treatment with cholesterol-lowering medication. Hypertension was defined as a systolic blood pressure >140 mmHg or a diastolic blood pressure >90 mmHg, during two episodes of at least 15 minutes of continuous non-invasive blood pressure measurement or treatment with antihypertensive medication. Diabetes was defined as fasting serum glucose levels >7.9 mmol/l, HbA1c >6.5%, or use of antidiabetic medication.

MDCT and MDCTA Data Acquisition

Imaging was performed with a 16-slice MDCT scanner (Sensation 16, Siemens) or a 64-slice MDCT scanner (Sensation 64, Siemens) with a standardized optimized contrast-enhanced protocol (120 kVp, 180 mAs, collimation 16 x 0.75 mm or 64 x 0.6 mm, pitch ≤1).[10,11]

The MDCT brain scan ranged from the foramen magnum to the vertex. Image reconstructions were made with a 220 mm field of view, matrix size 512 x 512, (real in-plane resolution 0.5 x 0.5 mm), slice thickness 3 to 4.5 mm and with an intermediate reconstruction algorithm.

The MDCTA scan ranged from the ascending aorta to the intracranial circulation (2 cm above the sella turcica). All patients received 80 ml contrast material (Iodixanol 320 mg/ml, Visipaque, Amersham Health), followed by a 40 ml saline bolus chaser, both with an injection rate of 4 ml/sec. Synchronization between the passage of contrast material and data acquisition was achieved by real time bolus tracking at the level of the ascending aorta. Image reconstructions were made with a 100 mm field of view, matrix size 512 x 512 (real in-plane resolution 0.6 x 0.6 mm), slice thickness 1.0 mm, increment 0.6 mm and with an intermediate reconstruction algorithm.[12]

MDCT and MDCTA Data Analysis

Relevant cerebral infarctions on MDCT of the brain related to the stroke symptoms were classified as non-lacunar infarction or lacunar infarction. A non-lacunar infarction was defined as an infarction with involvement of the cerebral cortex or a large deep infarction > 1.5 cm. A lacunar infarction was defined as an infarction in the deep brain structures (grey or white matter) with a size ≤ 1.5 cm.

The MDCTA images were sent to a stand-alone workstation (Leonardo; Siemens Medical Solutions) with dedicated 3-dimensional analysis software for further analysis. The symptomatic carotid bifurcation was evaluated with multi-planar reformatting software, which allows reconstruction of sagittal, coronal, and oblique views from axial sections. Two experienced investigators blinded to clinical data and MDCT of the brain analyzed the MDCTA images. Discrepancies were solved by consensus.

First, the degree of stenosis in the symptomatic carotid artery was determined according to the North American Symptomatic Carotid Endarterectomy Trial criteria[13] on multiplanar reformatting images perpendicular to the central lumen line. Second, the symptomatic carotid artery was evaluated for the presence of occlusion, atherosclerotic plaque and atherosclerotic plaque ulceration. Presence of atherosclerotic plaque was defined as

thickening of the vessel wall and/or the presence of calcification. Plaque ulceration was defined as extension of contrast media beyond the vascular lumen into the surrounding plaque.

Stroke Type and Etiology

All patients were analyzed for ischemic stroke etiology. The presence of a likely cardiac etiology or other specific etiology according to the TOAST criteria was determined.[8] For the purposes of this study, stroke was defined as ischemic stroke or transient ischemic attack. Based on clinical symptoms, strokes were classified as non-lacunar stroke of lacunar stroke in consensus by 2 experienced neurologists. In patients with a relevant infarction on MDCT of the brain, classification was corrected for imaging results. Non-lacunar ischemic stroke was defined as either ≥2 of the following symptoms: (1) higher cerebral dysfunction (e.g. dysphasia, dyscalculia, visuospatial disorder); (2) homonymous visual field defect; and (3) ipsilateral motor or sensory deficit, *or* higher cerebral dysfunction alone or a motor or sensory deficit more restricted than those classified as lacunar (e.g. confined to one limb, face, or hand but not the complete arm). Lacunar ischemic stroke was defined as a pure motor stroke, pure sensory stroke, sensory-motor stroke, dysarthria clumsy hand syndrome, or ataxic hemiparesis without brainstem symptoms.[14]

Statistical Analysis

Data are presented as means ± SD, medians with interquartile range, or number of patients (%). Differences between categorical data were analyzed with a $\chi 2$ test or Fisher Exact Test when appropriate. Differences between continuous data were analyzed with a Mann-Whitney test.

The association between degree of stenosis and plaque ulceration was evaluated in a multivariable logistic model adjusted for age and sex. The association between age, gender, cardiovascular risk factors, degree of stenosis and plaque ulceration with non-lacunar stroke (with lacunar stroke as reference) was first evaluated in a univariable logistic regression model. Thereafter, a multivariable logistic regression analysis was performed to identify variables independently associated with non-lacunar stroke after adjustment for all variables. Patients with a symptomatic occluded carotid artery, in which assessment of atherosclerotic plaque morphology was not possible, were not included in this analysis.

A multivariable logistic regression analyses was repeated with non-lacunar infarctions (versus lacunar infarctions) on MDCT of the brain to confirm the association of plaque ulcerations with clinically defined stroke subtype. Adjustments were made for age and gender plus the 6 variables, with the strongest association in the univariable analysis. Statistical analyses were performed using SPSS software (version 15.0, SPSS). $P<0.05$ was considered statistically significant.

Results

Patients Characteristics

Excluded patients with a likely cardiac etiology or other specific etiology (n=116) had less hypertension (56% versus 71%, *P*=0.002) and smoked less often (28% versus 39%, *P*=0.02) than patients included in the analysis. In this group, plaque ulceration in the symptomatic carotid artery was present in 9 patients (8%). Excluded patients with amaurosis fugax (n=79) smoked less often (28% versus 39%, *P*=0.04) and less often had a previous ischemic stroke (5% versus 14%, *P*=0.02) than included patients. Plaque ulceration in the symptomatic carotid artery was present in 7 patients (7%) with amaurosis fugax.

In the remaining 534 patients, non-lacunar stroke was present in 236 patients and lacunar stroke was present in 298 patients. The mean age of the study population was 62±13 years and 56% of the patients were male. In 355 (66%) patients the index event was an ischemic stroke, whereas 179 (34%) patients had a transient ischemic attack. Patients with non-lacunar stroke were significantly older than patients with lacunar stroke (64 versus 61 years; *P*=0.003; Table 1). The prevalence of other cardiovascular risk factors was similar in the 2 groups.

Table 1. Characteristics of patients with non-lacunar and lacunar ischemic stroke

	Non-lacunar Stroke n=236 (44%)	**Lacunar Stroke n=298 (56%)**	***P*-value**
Age (years)	64±13	61±13	0.003
Male Sex	128 (54%)	170 (57%)	0.52
Hypercholesterolemia	174 (74%)	215 (72%)	0.68
Hypertension	167 (71%)	212 (71%)	0.92
Diabetes Mellitus	50 (21%)	55 (18%)	0.43
Smoking	82 (35%)	126 (42%)	0.08
Peripheral Arterial Disease	17 (7%)	17 (6%)	0.48
Previous Ischemic Stroke	31 (13%)	42 (14%)	0.75
Previous TIA	36 (15%)	42 (14%)	0.71
Previous Intracerebral Hematoma	4 (2%)	5 (2%)	0.99
History of Ischemic Heart Disease	37 (16%)	42 (14%)	0.61

Data are means±SD or number of patients (%).

Plaque Surface Morphology in the Symptomatic Carotid Artery

In 362 of the 534 patients (68%), atherosclerotic plaque was observed in the symptomatic carotid artery. In 23 patients (4%), the symptomatic carotid artery was occluded and in 149 patients (28%) the symptomatic carotid artery was normal. Plaque ulcerations were observed in 67 symptomatic carotid arteries (13%). The likelihood of plaque ulceration in the symptomatic carotid artery was increased with a higher degree of stenosis after adjustment for age and gender (OR 1.6; 95% CI, 1.4-1.7).

Risk Factors for Non-lacunar Ischemic Stroke

Occlusions of the symptomatic carotid artery were significantly more prevalent in patients with non-lacunar stroke as compared to patients with lacunar stroke (8% versus 1%; *P*<0.001; Table 2). Normal symptomatic carotid arteries were significantly less prevalent in patients with non-lacunar stroke as compared to patients with lacunar stroke (20% vs 34%; *P*<0.001; Table2).

Table 2. MDCTA Plaque characteristics of the symptomatic carotid artery in patients with non-lacunar and lacunar ischemic stroke

	Non-lacunar Stroke n=236 (44%)	**Lacunar Stroke n=298 (56%)**	***P*-value**
Atherosclerotic Plaque	169 (72%)	193 (65%)	0.09
Occluded carotid artery	19 (8%)	4 (1%)	<0.001
Normal carotid artery	48 (20%)	101 (34%)	<0.001
Degree of Stenosis			
0%	121 (56%)	207 (70%)	<0.001
1-29%	38 (18%)	57 (19%)	0.36
30-49%	18 (8%)	17 (6%)	0.37
50-69%	22 (10%)	5 (2%)	<0.001
≥70%	18 (8%)	8 (3%)	0.01
Plaque Ulceration	47 (20%)	20 (7%)	<0.001

Data are number of patients (%).

Stenosis of ≥50% of the symptomatic carotid artery in patients with non-lacunar stroke was significantly more prevalent than in patients with lacunar stroke (*P*<0.001). Plaque ulceration in the symptomatic carotid artery was more common in non-lacunar strokes (n=47; 20%) as compared to lacunar strokes (n=20; 7%; *P*<0.001). In patients with plaque ulcerations no significant differences in number of ulcerations per plaque, location of the ulceration and type of ulceration were observed between patients with non-lacunar and lacunar stroke.

Risk factors associated with non-lacunar stroke (as opposed to lacunar stroke) in univariable and multivariable analysis are provided in Table 3. In univariable analysis age, degree of stenosis and plaque ulceration were found to be significantly associated with non-lacunar stroke, whereas smoking was found to be associated with lacunar stroke. Risk factors independently associated with non-lacunar stroke in multivariable analysis were degree of stenosis (OR, 1.19; 95% CI, 1.07-1.32) and plaque ulceration (OR, 2.70; 95% CI, 1.43-5.09).

As illustrated by the forest plot in the Figure, multivariable analysis revealed an independent association between plaque ulceration and non-lacunar stroke after adjustment for age and gender; age, gender and cardiovascular risk factors; degree of stenosis alone and age, gender, all cardiovascular risk factors, and degree of stenosis.

Table 3. Univariable- and multivariable OR for association between non-lacunar stroke (versus ischemic lacunar stroke) and cardiovascular risk factors, degree of stenosis, and plaque ulceration

	Univariable Analysis	**Multivariable Analysis**
Age (per decade)	1.23 (1.08-1.40)*	1.11 (0.96-1.29)
Male	0.84 (0.59-1.20)	0.70 (0.48-1.02)
Hypercholesterolemia	1.02 (0.69-1.51)	0.86 (0.56-1.33)
Hypertension	0.98 (0.66-1.44)	0.75 (0.48-1.15)
Diabetes Mellitus	1.27 (0.82-1.95)	1.24 (0.77-1.98)
Smoking	0.68 (0.47-0.98)*	0.73 (0.49-1.10)
Peripheral Arterial Disease	1.04 (0.49-2.19)	0.81 (0.36-1.87)
Previous Ischemic Stroke	0.89 (0.53-1.49)	0.81 (0.46-1.42)
Previous TIA	1.17 (0.71-1.94)	1.05 (0.61-1.82)
Previous Intracerebral Hematoma	1.09 (0.29-4.09)	1.07 (0.27-4.27)
History of Ischemic Heart Disease	1.23 (0.75-2.00)	1.09 (0.63-1.89)
Degree of Stenosis (per 10%)	1.27 (1.16-1.40)*	1.19 (1.07-1.32)*
Plaque Ulceration	3.79 (2.17-6.61)*	2.70 (1.43-5.09)*

Data are OR (95% CI). In multivariable analyses, adjustments were made for all variables.

*Significant relation.

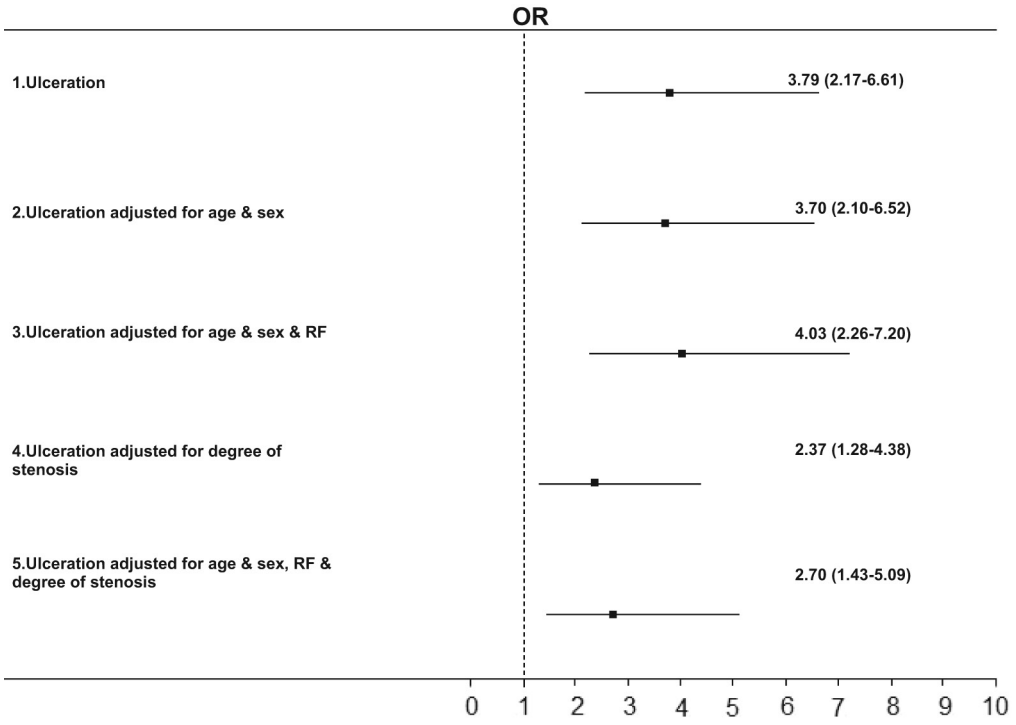

Figure. Symptomatic carotid plaque ulceration is independently associated with non-lacunar stroke after correction for age, sex, cardiovascular risk factors (RF) and degree of stenosis.

Risk Factors for Non-lacunar Infarction on MDCT of the brain

An infarction on MDCT of the brain was found in 162 patients (30%), including 82 patients with non-lacunar infarction and 80 patients with lacunar infarction. The median time between the index event and acquisition of the MDCT of the brain was 5 days (interquartile range 1-14 days). In patients with non-lacunar infarction as compared to patients with lacunar infarction, a higher prevalence of atherosclerotic plaques (n=60; 73% vs n=65; 81%) and occlusions (n=10; 12% vs n=1; 1%; *P*=0.02), was observed in the symptomatic carotid artery. Plaque ulceration in the symptomatic carotid artery was more common in non-lacunar infarction (n=23; 28%) as compared to lacunar infarction (n=7; 9%; *P*=0.002).

Risk factors associated with non-lacunar infarction (as opposed to lacunar infarction) in univariable and multivariable analysis are provided in Table 4. Atherosclerotic plaque ulceration was independently associated with non-lacunar infarction in multivariable analysis (OR 3,88; 95% CI, 1.39-10.84).

Table 4. Univariable- and multivariable OR for association between CT-confirmed non-lacunar infarctions (versus lacunar infarctions) and cardiovascular risk factors, degree of stenosis, and plaque ulceration

	Univariable Analysis	Multivariable Analysis
Age (per decade)	0.90 (0.69-1.18)	0.80 (0.60-1.10)
Male	1.16 (0.61-2.20)	0.88 (0.43-1.80)
Hypercholesterolemia	0.70 (0.32-1.53)	0.76 (0.33-1.78)
Hypertension	0.60 (0.28-1.31)	0.45 (0.19-1.09)
Diabetes Mellitus	1.48 (0.72-3.03)	2.04 (0.92-4.55)
Smoking	0.95 (0.50-1.80)	
Peripheral Arterial Disease	0.97 (0.35-2.67)	
Previous Ischemic Stroke	1.47 (0.64-3.39)	1.44 (0.58-3.60)
Previous TIA	0.99 (0.38-2.58)	
Previous Intracerebral Hematoma	1.10 (0.07-17.89)	
History of Ischemic Heart Disease	1.11 (0.45-2.75)	
Degree of Stenosis (per 10%)	1.23 (1.05-1.44)*	1.18 (0.98-1.43)
Plaque Ulceration	4.83 (1.92-12.12)*	3.88 (1.39-10.48)*

Data are OR (95% CI). In multivariable analyses, adjustments were made for age, gender, hypercholesterolemia, hypertension, diabetes mellitus, previous ischemic stroke, degree of stenosis, and plaque ulceration.

*Significant relation.

Discussion

In the current study patients with ischemic cerebrovascular symptoms were classified according to presumed stroke etiology. Besides degree of stenosis, atherosclerotic carotid plaque ulceration was shown to be independently associated with non-lacunar stroke. This relation between plaque ulceration and clinically defined non-lacunar stroke was confirmed by an independent association between the presence of atherosclerotic carotid plaque ulceration and non-lacunar infarction on MDCT of the brain.

Etiology of ischemic stroke subtypes

The association of various risk factors with subtypes of ischemic stroke has been studied extensively.[3] Differences in cardiovascular risk factor profile would support a distinct arterial pathological process underlying different types of stroke. Previous studies have suggested lacunar strokes to be predominantly associated with hypertension and diabetes mellitus.[15,16]

However, in a review by Jackson et al,[3] only a marginal increase was shown in the prevalence of hypertension in patients with lacunar stroke as compared to non-lacunar stroke.

In the present study, a risk factor-free stroke subtype classification was used and stroke subtypes were differentiated based on clinical symptoms and MDCT imaging of the brain. In concordance with Jackson et al, no significant difference in cardiovascular risk factors was observed between patients with non-lacunar and lacunar ischemic stroke. To further elucidate the pathophysiological mechanisms associated with subtypes of ischemic stroke, we evaluated the relation between parameters of carotid atherosclerosis as assessed by MDCTA of the symptomatic carotid artery.

Relation of Carotid Artery Stenosis and Plaque Ulceration with Non-lacunar Stroke

The relation of degree of stenosis and plaque ulceration with any ischemic stroke has been previously studied using conventional angiographic studies. Previous studies relating degree of stenosis with specific stroke subtypes have identified an association with non-lacunar stroke.[3,17,18] Accordingly, in the current study a significant relation was observed between the severity of carotid stenosis and presence of non-lacunar stroke. Plaque ulceration in the symptomatic carotid artery of patients with ischemic stroke has been shown to be independently associated with an increased risk of recurrent ipsilateral ischemic stroke in patients using medical treatment in the North American Symptomatic Carotid Endarterectomy Trial and European Carotid Surgery Trial (ECST) study.[4,5] However, no previous studies have evaluated the association of plaque ulceration with non-lacunar stroke in particular.

Atherosclerotic carotid plaque ulcerations are thought to be a marker of previous plaque ruptures.[19] After plaque rupture, thrombogenic material is exposed to blood initiating platelet aggregation and thrombus formation, ultimately leading to thromboembolism or local carotid artery occlusion.[20] Emboli from ruptured atherosclerotic carotid plaques may occlude the intracranial cerebral arteries, resulting in ischemia of cortical and subcortical brain tissue. Accordingly, Lovett et al[21] observed a strong correlation between atherosclerotic plaque ulceration in the carotid artery and histological characteristics of plaque instability including plaque rupture, intraplaque hemorrhage and large lipid core. The results of the present study support this underlying mechanism by revealing an independent association between plaque ulceration in the symptomatic carotid artery and non-lacunar stroke. In addition, current results suggest a different pathophysiological mechanism in non-lacunar and lacunar stroke subtypes.

Clinical and Research Implications

Recent research on atherosclerosis has shifted from severity of stenosis toward parameters of plaque vulnerability. Plaque surface morphology and plaque ulcerations may be a reflection of plaque vulnerability. Plaque surface evaluation of the symptomatic carotid artery has been recommended in an algorithm for clinical decision making concerning carotid endarterecomy.[22] That particular algorithm is based on the results of the North American Symptomatic Carotid Endarterectomy Trial and ECST data, in which plaque surface morphology was evaluated using conventional arterial angiography. However, in current clinical practice conventional angiography is increasingly replaced by non-invasive imaging modalities such as MDCTA and MRA. Both techniques provide accurate non-invasive evaluation of carotid artery stenosis. In addition, MDCTA provides supplementary information on plaque surface morphology as compared to MRA.[7] Accordingly,

for individual risk stratification assessment of plaque surface morphology could be based on MDCTA rather than conventional angiography. This study further supports the importance of etiologic stroke subtype assessment in stroke patients and the evaluation of plaque morphology in patients with non-lacunar stroke. Furthermore, future studies evaluating the relation between atherosclerotic disease in the carotid bifurcation and clinical events or brain tissue damage should take into account the heterogeneity of stroke etiologies.

Study Limitations

The current study is based on carotid analysis by MDCTA, and findings were not confirmed by histological specimens. Thus far, MDCTA has been validated for detection of plaque ulceration in one stuy.[6] The good accuracy observed in that particular study remains to be confirmed. The applied stroke subtype classification was based on clinical symptoms and corrected for relevant infarctions seen on MDCT of the brain. In patients with a transient ischemic attack in whom the diagnosis is often solely based on the patients' history, subtype classification may be less accurate. MRI of the brain, especially when combined with diffusion-weighted imaging, can better-detect and localize infarcts resulting in an improved classification of stroke subtypes. Furthermore, stroke classification based on clinical symptoms alone may lead to misclassification of stroke subtype in 25% of the patients.[23] In the current study, the clinical classification of stroke subtypes was corrected for relevant infarctions seen on MDCT of the brain. This refinement has likely improved the accuracy of stroke subtype classification. However, relevant infarctions on MDCT of the brain were absent in 70% of the patients. Accordingly, an overall misclassification of stroke subtype may be possible in up to ≈20% of the study population. Of note, the current study results were confirmed by a subanalysis in patients with relevant brain infarction on MDCT, in which an independent association was found between carotid plaque ulceration and non-lacunar infarction. It should be acknowledged that given the limited total number of relevant infarctions, this analysis was based on a small number of ulceration (23 in non-lacunar infarctions compared to 7 in lacunar infarctions).

Finally, the present study has a cross-sectional design. Therefore, the prognostic value of carotid plaque ulcerations in different stroke subtypes remains to be determined in follow-up studies.

Conclusion

Atherosclerotic plaque ulceration of the symptomatic carotid artery is strongly related to non-lacunar ischemic events as compared to lacunar ischemic events, independently of severity of stenosis. This finding was confirmed by an independent association between the presence of atherosclerotic carotid plaque ulceration and non-lacunar infarctions on MDCT of the brain. These results indeed suggest that non-lacunar ischemic stroke and lacunar stroke are caused by different pathophysiological mechanisms. Plaque ulceration is an important factor to evaluate in future prognostic and therapeutic studies of patients with carotid atherosclerotic disease.

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Chapter 3.2

Evolution of atherosclerotic carotid plaque morphology: Do ulcerated plaques heal? A serial Multidetector CT Angiography study

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Abstract

Background Atherosclerotic carotid plaque rupture may lead to thrombo-embolization, causing transient ischemic attack or ischemic stroke. Carotid plaque ulceration on angiography is associated with plaque rupture. Although healing of ruptured plaques has been described in coronary arteries, little is known about the natural development of plaque ulcerations in carotid arteries. We therefore explored the evolution of carotid plaque surface morphology with serial multidector CT angiography (MDCTA).

Methods From a registry of patients with transient ischemic attack or minor ischemic stroke, we selected 83 patients who had undergone serial MDCTA of the carotid arteries. Arteries subjected to revascularization procedures between the two scans were excluded (n=11). Plaque surface morphology was classified as smooth, irregular or ulcerated on both baseline and follow-up MDCTA. Progression (i.e. development of irregularities or ulceration) and regression (i.e. disappearance of irregularities or ulceration) in morphology were evaluated.

Results The mean time interval between the MDCTA scans was 21±13 months. At baseline, 28 (18%) arteries were normal, 124 (80%) contained atherosclerotic plaque and 3 (2%) were occluded. Plaque surface morphology was smooth in 86 arteries (55%), irregular in 23 (15%) and ulcerated in 15 (10%). At follow-up, surface morphology was unchanged in 88% of arteries, had progressed in 8% and regressed in 4%. Most importantly, plaque morphology remained unchanged in most ulcerated plaques (10/15; 67%). One ulcerated plaque had progressed, whereas 4 had regressed. New ulcerations had developed in 2 non-ulcerated plaques.

Conclusion MDCTA allows evaluation of temporal changes in atherosclerotic carotid plaque morphology. Plaque surface morphology remained unchanged in most arteries. Carotid ulcerations persist for a long time, and may remain a potential source of thromboembolism.

Introduction

Carotid plaque ulceration is associated with plaque rupture,[1] which is an important element in the cascade between vulnerable plaque and the occurrence of thromboembolic events.[2,3] In addition to this, carotid plaque ulcerations may be an additional cause of embolic stroke due to local flow disturbances.[4-6] While there is evidence of a healing process of plaque ruptures in the coronary arteries which contributes to the degree of luminal narrowing,[7,8] little is known about the healing process of plaque ruptures in carotid arteries.

Current knowledge about the evolution of atherosclerotic plaque rupture is mainly based on histological analysis of coronary arteries in autopsy studies[9,10] or carotid plaque specimens obtained from carotid endarterectomy.[11-13] However, for extensive investigation of the temporal changes of plaque surface characteristics and their relation with recurrent thromboembolic events, longitudinal noninvasive serial imaging studies are required. Computed tomography angiography has been demonstrated to be superior to angiography in detecting plaque ulcerations[14] and multidetector CT angiography (MDCTA) has been found to have a high sensitivity and specificity (94% and 99%, respectively) in detecting plaque ulcerations.[15] Therefore MDCTA is an appropriate noninvasive imaging technique for studying plaque ulceration. As MDCTA is able to identify and classify plaque ulcerations with a very good interobserver agreement,[16] it is also suitable for serial imaging.

To explore the natural history of ulcerated plaques and to assess whether plaque ulcerations heal, we studied the temporal changes in plaque surface morphology on serial MDCTA in patients with transient ischemic attack (TIA) or minor ischemic stroke.

Materials and Methods

Study population

From November 2002 to January 2007, 911 consecutive patients with amaurosis fugax, TIA or minor ischemic stroke (Rankin score < 4) who underwent MDCTA of the carotid arteries were registered. From November 2002 until December 2004, MDCTA was performed as part of a research protocol approved by the Institutional Review Board. All patients gave written informed consent. Onwards of January 2005, MDCTA was performed as part of standard clinical workup. Patients were enrolled from the neurology department's specialized TIA/stroke outpatient clinic or neurology ward and underwent neurological examination on admission; symptoms, risk factors and medication use were reported. Follow-up MDCTA scans of the carotid arteries were registered.

For the present study we selected the patients from our registry who had undergone more than one MDCTA of the carotid arteries in the period November 2002 to December 2008 (n=84). One patient was excluded because of poor image quality caused by dental artifacts on both MDCTA scans. If more than two MDCTA scans per patient had been performed, the one with the largest lead time before any surgical treatment was evaluated in this study. Arteries subjected to revascularization procedures between the two scans were excluded (n=11).The present study was approved by the Institutional Review Board.

Cardiovascular risk factors

Hypercholesterolemia was defined as fasting cholesterol > 5.0 mmol/L or treatment with cholesterol-lowering medication. Hypertension was defined as a systolic blood pressure > 140 mmHg and/or a diastolic blood pressure >90 mmHg during two episodes of at least 15 minutes of continuous non-invasive blood pressure measurement, or treatment with antihypertensive medication. Diabetes was defined as serum glucose levels > 7.9 mmol/L, HbA1c > 6.5%, or use of anti-diabetic medication. Smoking was categorized as either current smoking or non-smoking. Information was collected on previous peripheral vascular disease (intermittent claudication and/or ankle/arm systolic blood pressure ratio < 0.85; or a history of related leg amputation, reconstructive surgery or angioplasty), previous ischemic TIA or stroke, and previous cardiovascular disease (myocardial infarction, angina pectoris, coronary artery bypass graft) or otherwise previous cardiac disease (atrium fibrillation, chronic heart failure).

MDCTA data acquisition

Imaging was performed on a 16-slice multidetector CT scanner (Siemens, Sensation 16, Erlangen, Germany) or a 64-slice MDCT scanner (Siemens, Sensation 64) with a standardized, optimized contrast-enhanced protocol (120 kVp, 180 mAs, collimation 16 x 0.75 or 32 x 2 x 0.6 mm, pitch ≤1).[17,18] The scan range extended from the ascending aorta to the intracranial circulation. All patients received 80 mL of contrast material (iodixanol 320 mg/ml, Visipaque, Amersham Health, Little Chalfont, UK), followed by 40 mL saline bolus chaser, both at an injection rate of 4 mL/sec. Real-time bolus tracking at the level of the ascending aorta was used to synchronize passage of contrast material and data acquisition. Image reconstructions were made with a 120 mm FOV, a matrix size of 512 x 512, a slice thickness of 1.0 mm, a slice increment of 0.6 mm, and intermediate reconstruction algorithms (B30 and B46).

Analysis of the atherosclerotic plaque

The MDCTA images were sent to a workstation (Leonardo-Siemens Medical Solutions, Forchheim, Germany) equipped with dedicated 3-D analysis software. Multi-planar reformatting software was used to evaluate both carotid bifurcations in multiple reformations and different planes. Each carotid artery was evaluated for the presence of an atherosclerotic plaque, defined as the presence of thickening of the vessel wall and/or a calcification in the wall. Atherosclerotic plaque surface morphology was evaluated and classified as smooth, irregular or ulcerated. Plaques were classified as ulcerated if contrast material extended into the plaque, being visible in at least two perpendicular planes. The number of ulcerations per carotid artery was recorded. Irregularities were described as prestenotic or poststenotic dilatation and/or irregular plaque-surface morphology without extension of contrast material into the plaque. Plaques that were not ulcerated or irregular were classified as smooth. The interobserver agreement for the presence of plaque ulceration as described above was found to be good (kappa=1.00; 95% CI 0.86-1.00).[16]

In addition, plaque density was evaluated as previously described by Saba et al.[19] In atherosclerotic carotid plaques, the mean Hounsfield unit attenuation was measured within manually drawn regions of interest covering the plaque on three consecutive axial images at the level of largest plaque burden. Plaque density was classified as soft, mixed

or calcified, according to cut-off values derived from a method validated by Schroeder et al[20]: soft plaques with mean density values < 50 HU, intermediate plaques with mean density values between 50 and 119 HU and calcified plaques with a mean density value ≥ 120 HU.

The number of calcifications in the investigated plaques was manually counted within a range of 3 cm above to 3 cm under the bifurcation point. An automated calcium scoring tool was used to assess calcium volumes within this same range, using a cut-off value of 600 HU to differentiate calcifications from contrast material in the lumen.

Assessment of changes in plaque morphology

The atherosclerotic plaque morphology at baseline and follow-up MDCTA were evaluated separately, with a time interval of more than one month between the two assessments. Two experienced observers blinded to clinical data analyzed the multi-planar reformation images. Discrepancies were resolved by consensus. Additionally, temporal changes in morphology between baseline and follow-up MDCTA scans were verified by a consensus reading where necessary. Changes in atherosclerotic plaque morphology were considered as progression if irregularities, ulceration or occlusion had developed. Regression was defined as disappearance of ulceration or irregularities.

Statistics

Where appropriate, data are presented descriptively as numbers and percentages and means ± standard deviations. We evaluated the association between cardiovascular risk factors and baseline plaque morphology and between cardiovascular risk factors and temporal change in plaque morphology. Differences between categorical data and continuous data were analyzed with a χ^2 test and a Mann-Whitney test or a Student *t* test, respectively. Temporal changes in continuous data on plaque characteristics were analyzed with a paired-samples *t* test.

Results

In total, 83 patients who had undergone a baseline MDCTA as well as a follow-up MDCTA evaluation of the carotid arteries were included. The mean time period between the serial scans was 21 ± 13 months. The mean age in the study population was 60 ± 12 years, and the majority of patients were male (64%; Table 1). In 75 patients (90%), the indication for follow-up MDCTA was recurrent TIA or ischemic stroke; in 39 (47%), the recurrent event was attributed to the same vascular distribution as the previous attack, and in 36 (43%) a different vascular territory was involved. In eight patients (10%), a follow-up MDCTA was performed for pre- and post interventional indications or for neurological symptoms that could not be attributed to a specific vascular territory.

Of the 166 carotid arteries in 83 patients, 11 arteries were excluded, because of treatment by carotid endarterectomy (n=3) or because of stent placement (n=8) in the period between the baseline and follow-up MDCTA. One-hundred-fifty-five carotid arteries were available for analysis of changes in atherosclerotic plaque characteristics on follow-up MDCTA.

Table 1. Baseline characteristics of the study population

	Total Patients (N=83)
Age (years, mean ± SD)	59.8 ± 12.4
Gender (% male)	53 (64%)
Risk factors (%):	
- previous cerebrovascular disease	36 (43%)
- previous cardiovascular disease	16 (19%)
- peripheral vascular disease	6 (7%)
- hypertension	61 (73%)
- hypercholesterolemia	64 (77%)
- diabetes mellitus	22 (27%)
- smoking	29 (35%)
Cerebrovascular symptoms (%):	
Amaurosis fugax	8 (10%)
TIA	33 (40%)
Minor stroke	42 (51%)
Symptomatic artery (%):	
Carotid	68 (82%)
Vertebrobasilar	15 (18%)

Table 2 shows plaque characteristics of the carotid arteries on baseline and follow-up MDCTA scans. At baseline, 28 (18%) carotid arteries were normal, 124 (80%) contained atherosclerotic plaque, and 3 arteries were occluded. The majority (n=86, 55%) of vessels had a smooth plaque surface, whereas 23 (15%) had irregular plaque surfaces. Fifteen of the 155 vessels (10%) had an ulcerated plaque surface; most arteries contained one ulceration. These ulcerated plaques were present in 15 patients. Most of the plaques (119/124; 96%) were mixed or calcified plaques, since the majority of carotid plaques (102/124, 82%) contained at least some calcifications. The mean HU density of the 124 plaques at baseline was 179 ± 147 HU, which increased to 216 ± 187 HU over time (p=0,001). The number of calcifications did not significantly change over time (4.9 ± 3.5 at baseline versus 4.6 ± 3.2 at follow-up, p=0.21), whereas calcium volume increased significantly (44.7 ± 72.1 mm^3 at baseline versus 62.8 ± 92.2 mm^3 at follow-up, p<0.001).

Changes in surface morphology between the baseline and the follow-up MDCTA are presented in Table 3. On the follow-up MDCTA the surface morphology was unchanged in 137 (88%) of the carotid arteries, whereas 12 (8%) had progressed and 6 (4%) had regressed. The 12 arteries that had progressed were present in 10 patients. The morphology of most ulcerated plaques (10 out of 15) had not changed over a mean time period of 20 ± 15 months, as illustrated by an example in figure 1. A second ulceration evolved in one ulcerated plaque (over 21 months), whereas a new ulceration developed in two non-ulcerated

Table 2. Plaque characteristics of 155 carotid arteries on baseline and on follow-up MDCTA

	Baseline MDCTA	Follow-up MDCTA
Occlusion	3 (2%)	4 (3%)
No plaque	28 (18%)	24 (15%)
Plaque	124 (80%)	127 (82%)
Plaque surface morphology		
Smooth plaque surface	86 (55%)	87 (56%)
Irregular plaque surface	23 (15%)	26 (17%)
Ulcerated plaque	15 (10%)	14 (9%)
Number of ulcerations		
1	12	11
2	2	2
3	1	1
Plaque density *		
Soft plaque	5 (4%)	6 (5%)
Mixed plaque	51 (41%)	46 (36%)
Calcified plaque	68 (55%)	75 (59%)
Calcifications **		
Mean number of calcifications	4.9 ± 3.5	4.6 ± 3.2
Mean calcium volume (mm^3)	44.7 ± 72.1	62.8 ± 92.2

* Assessed in carotid arteries containing plaque

** Assessed in carotid arteries containing plaques with calcification at baseline (n=102)

plaques (over 29 and 40 months). Four ulcerated plaques regressed (over 3, 9, 14 and 44 months), of which an example is shown in figure 2.

We did not find any associations between cardiovascular risk factors and baseline plaque morphology and between cardiovascular risk factors and temporal change in plaque morphology. There was no relation between changes in plaque morphology and the occurrence of TIA or ischemic stroke at baseline or follow-up (Table 4).

Discussion

This is the first MDCTA study that longitudinally evaluates the natural evolution of carotid plaque surface morphology. Our results show that plaque surface morphology remains largely unchanged, and that regression and progression occur in just a minority of plaques after a time period of 21 ± 13 months. Most importantly, plaque ulcerations do not disappear; 10 out of 15 ulcerated plaques had a similar appearance after 20 ± 15

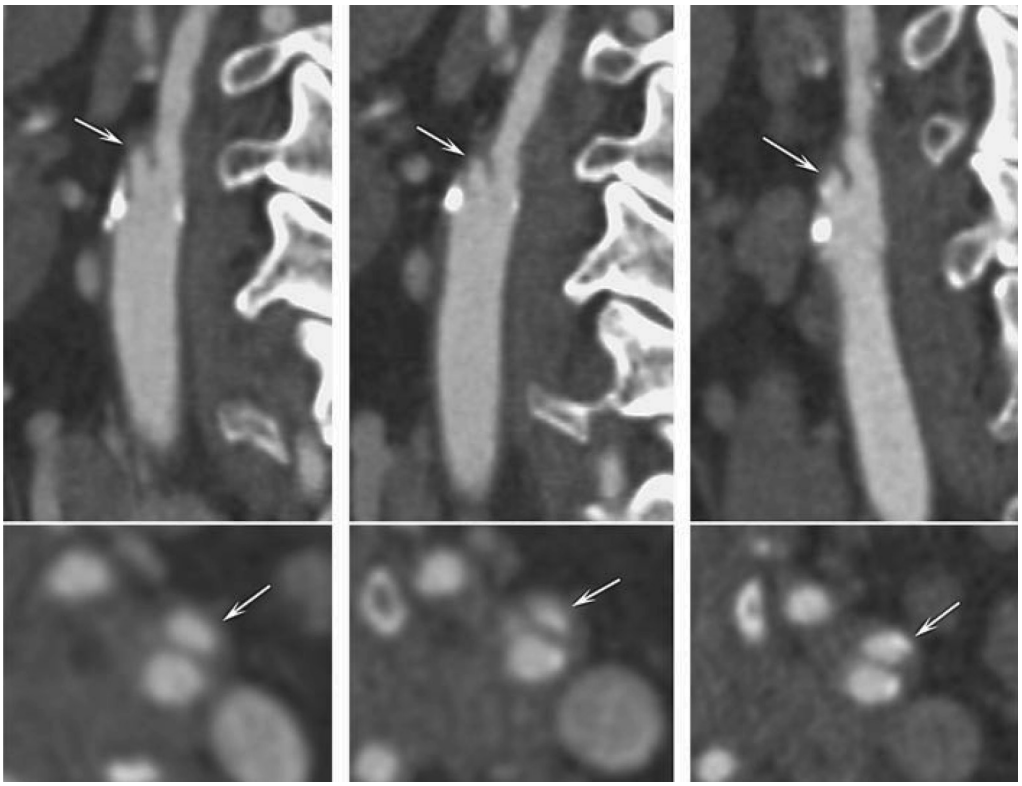

Figure 1. Multiplanar reformat images (on top) and axial images (below) showing a carotid bifurcation of the same patient with plaque ulceration (arrows) in the proximal internal carotid artery. The time delay between the MDCTA scans was 11 months (a → b) and 13 months (b → c) respectively. No change in appearance of plaque morphology is found.

months. Mean plaque density increased over time, while the number of calcifications did not change significantly. The increased density can be explained by the increase in volume and density of the calcifications.

Carotid plaque ulceration is associated with fibrous cap rupture,[1] which is an important cause of thromboembolic events.[3,12] Current imaging modalities allow the visualization of carotid plaque ulceration, which is associated with cap rupture and other features of plaque instability.[1] The site of ulceration probably represents part of the plaque where fibrous cap and part of the necrotic core has disappeared and has most probably embolized.[13] However, our results show that plaque ulceration on MDCTA images does not necessarily suggest that the plaque rupture is recent.

Since a healing process of ruptured coronary plaques has been described in histological studies,[7,8] our finding of temporarily unchanged carotid plaque ulcerations on serial MDCTA imaging was unexpected. In coronary arteries a mechanism of plaque healing has been reported in which layers of collagen, proteoglycan-rich matrix and smooth muscle cells overly the ruptured cap and contribute to increased luminal narrowing.[7,8,13] One case report has described the concept of rupture and healing in a carotid atherosclerotic plaque visualized with magnetic resonance imaging (MRI).[21] We therefore expected that

Table 3. Changes in plaque surface morphology between baseline and follow-up MDCTA in 155 carotid arteries

Plaque Morphology	Baseline MDCTA	Follow-up MDCTA	Carotid arteries (N)
Unchanged	No plaque	No plaque	24
N=137 (88%)	Smooth	Smooth	81
	Irregular	Irregular	19
	1 Ulceration	1 Ulceration	8
	2 Ulcerations	2 Ulcerations	1
	3 Ulcerations	3 Ulcerations	1
	Occlusion	Occlusion	3
Progression	No plaque	Smooth	4
N=12 (8%)	Smooth	Irregular	4
	Smooth	1 Ulceration	1
	Irregular	Occlusion	1
	Irregular	1 Ulceration	1
	1 Ulceration	2 Ulcerations	1
Regression	1 Ulceration	Irregular	3
N=6 (4%)	2 Ulcerations	1 Ulceration	1
	Irregular	Smooth	2

carotid plaque ulcerations might fill and disappear. Our findings may be explained by the differences in vessel size and haemodynamic forces in carotid arteries compared to coronary arteries, or by the possibility that ulcerations reflect the more severely ruptured plaques. It is uncertain which time interval should be regarded as sufficient to demonstrate long term changes in plaque morphology. However, we consider a mean follow-up period of almost two years sufficient to evaluate changes, since healing of ulceration as assessed with MRI has been reported to occur within two months.[21]

Our results are indirectly supported by some observations that also question the concept of healing of carotid plaque ulcerations. Whereas a histological study found plaque ulcerations to be more common in symptomatic carotid arteries than in asymptomatic arteries (36% versus 14%, $p<0.0001$), the prevalence of ulceration in the ipsilateral and contralateral carotid artery in symptomatic patients was the same.[22] Likewise, an MDCTA study reported that the prevalence of complicated plaques (i.e. irregularities and ulcerations) was 25% in symptomatic carotid arteries and 18% in asymptomatic carotid arteries.[16] Whereas ulcerations seem to correlate with symptomatic arteries, they are also present in asymptomatic arteries. This might be explained either by the fact that not every plaque rupture leads to thrombosis and embolization, or that the embolized particles dissolve or cause clinically silent infarctions. Another explanation of the incidental detection of ulcerations in asymptomatic arteries may be that plaque ulcerations heal very slowly or do not disappear at all. The findings correspond to our own: ulcerations can persist unchanged for a long time in both symptomatic and asymptomatic carotid arteries.

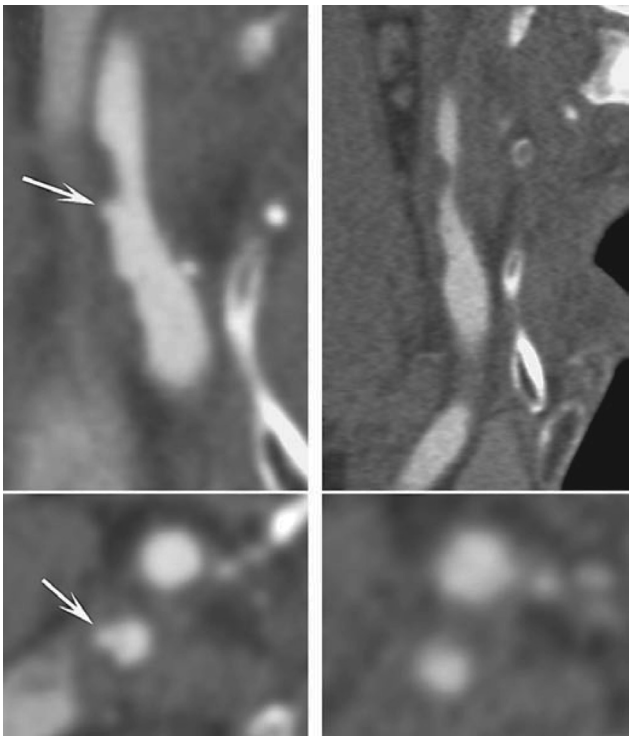

Figure 2. Multiplanar reformat images (on top) and axial images (below) of a carotid bifurcation with a time delay between the MDCTA scans of 9 months. The plaque ulceration (arrows) disappeared over time, leaving a smooth plaque surface.

Persisting plaque ulceration may be a potential imaging-based stroke-risk predictor. The presence of plaque ulceration on angiography has been found to be associated with recurrent stroke in patients with a previous ischemic cerebral event.[23] The question is whether persisting ulcer cavities should be regarded merely as a scar after a previous plaque rupture or also as a potential source of thromboembolization. Indeed, some local factors at the level of ulceration have been found to play an important role in the development of new thrombi. Firstly, plaque ulcerations may induce thrombus formation by disturbing the blood flow. In-vitro flow experiments with ultrasound showed that the flow downstream from ulcerated, moderate stenotic, carotid plaques was more disturbed than from non-ulcerated plaques.[24] Similarly, an experimental slipstream visualization technique showed that the introduction of an ulceration into a stenotic carotid bifurcation produced observable flow disturbances.[4] Further, color flow Doppler ultrasound, which uses detection of vortices, is useful in detecting ulcerated plaques.[25] Moreover, local flow disturbance caused by ulcerations is likely to favor thrombo-genesis.[5,6] Secondly, plaque ulcerations may induce thrombus formation by possessing an erosive surface. Whereas histological studies describe plaque healing as the deposition of a collagen matrix superimposing the ruptured cap,[7] they have not described re-endothelialisation. Future studies should examine whether the surfaces of persistent ulcerations are prothrombotic.

The clinical and pathophysiological importance of the distinction between ulcerations and irregularities is unknown. There is no evidence that ulcerated plaques on angiographic criteria are any more likely to lead to thrombus formation than irregular plaques. Rothwell et al.[26] found that plaque-surface irregularities were highly predictive of ipsilateral ischemic stroke and significantly associated with macroscopic surface ulceration and thrombus formation. Our results show that 19 out of 23 irregular plaques on the baseline MDCTA remained unchanged on follow-up MDCTA. Our considerations with regard to the consequences of persisting ulcerations might also apply to plaque irregularities.

The strength of this serial imaging study is that it presents a way to non-invasively study the development of atherosclerotic plaques in human beings. Serial MDCTA provides ad-

Table 4. Relation between changes in plaque morphology and symptomatology of the carotid arteries. Shown are the number of carotid arteries presenting with a particular plaque morphology on baseline and on follow-up MDCTA and the number of these arteries that became symptomatic or asymptomatic (AS = asymptomatic, S = symptomatic).

Baseline	Follow-up	N	AS → S	AS → AS	S → AS	S → S
no plaque	no plaque	24	4	11	4	5
smooth	smooth	81	14	37	15	15
irregular	irregular	19	5	7	2	5
1 ulceration	1 ulceration	8	1	4	3	0
2 ulcerations	2 ulcerations	1	0	1	0	0
3 ulcerations	3 ulcerations	1	1	0	0	0
occlusion	occlusion	3	0	1	0	2
	Total unchanged	**137**	**25**	**61**	**24**	**27**
no plaque	smooth	4	1	2	1	0
smooth	irregular	4	1	0	3	0
smooth	1 ulceration	1	1	0	0	0
irregular	occlusion	1	0	0	0	1
irregular	1 ulceration	1	1	0	0	0
1 ulceration	2 ulcerations	1	0	1	0	0
	Total progression	**12**	**4**	**3**	**4**	**1**
1 ulceration	irregular	3	2	1	0	0
2 ulcerations	1 ulceration	1	1	0	0	0
irregular	smooth	2	0	1	0	1
	Total regression	**6**	**3**	**2**	**0**	**1**
	Total	**155**	**32**	**66**	**28**	**29**

ditional information that is otherwise difficult to achieve by enabling the investigation of the atherosclerotic plaque in earlier stages, at various time points in the same patient, and in patients in whom no histological material is available.

MRI is potentially suitable for serially studying carotid plaque rupture in more detail. Although the status of the fibrous cap has been studied with MDCTA,[27] no validation with histology is available. MRI can distinguish intact, thick fibrous caps from intact thin and disrupted caps in human atherosclerotic carotid arteries in vivo.[28] By using MRI, Underhill et al. [29] evaluated the predictive value of plaque characteristics for new plaque surface disruption. Thin or ruptured fibrous caps have been associated with subsequent symptoms during follow-up.[30]

Our study has some limitations. First, the relatively small study population and the low overall prevalence of ulcerations meant that we found a small number of ulcerations. Nevertheless, we found a clear trend of mainly unchanged ulcerated plaque morphology. The second limitation was the study design and our selection of patients with follow-up scans that were performed for clinical reasons. These provided a relatively small and heterogeneous study population that was unsuitable for evaluating the correlation between changes in plaque morphology and symptomatology. Temporal changes in plaque ulceration should thus be correlated with recurrent thromboembolic events by including a larger number of patients in a prospective, long term follow-up study. The small selected study population may also underlie the absence of an association between classical cardiovascular risk factors and plaque morphology. Further, these limitations impeded the structural coevaluation of other important plaque characteristics. The third limitation is that we evaluated temporal changes at the level of the carotid artery. Since atherosclerosis is a systemic disease and atherosclerotic plaques are thought to be liable to systemic influences, we might have expected progression or regression of plaque morphology to be clustered in particular patients. However, we did not find such clustering.

Conclusion

By demonstrating that ulcer cavities usually persist for a long time, the results of this serial MDCTA study contribute to the knowledge of the evolution of ulcerated plaques. Our findings suggest that the detection of plaque ulceration on MDCTA images may not have a direct diagnostic value as it may not represent a recent plaque rupture. However, persisting carotid ulcerations may remain a potential source of embolism. Further studies should reveal whether persisting plaque ulcerations constitute an important source of recurrent thromboembolism and whether they may aid future stroke-risk prediction and clinical decision-making in individual patients.

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Chapter 3.3

Association between carotid artery plaque ulceration and plaque composition evaluated with multidetector CT angiography

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Abstract

Background and Purpose Symptomatic carotid artery plaque ulceration is associated with distinct plaque components such as a large lipid-rich necrotic core (LR-NC) in ischemic stroke patients with a ≥50% carotid stenosis. We evaluated the associations between carotid artery plaque ulceration and plaque characteristics in ischemic stroke patients with ≥50% stenosis as well as in those with a low degree stenosis (0% to 49%).

Methods Consecutive patients (n=346) with symptoms in the anterior circulation were evaluated with multidetector CT angiography (MDCTA) for the presence of atherosclerotic plaque, degree of stenosis, and plaque ulceration in the symptomatic carotid artery. Plaque volume and plaque component proportions of LR-NC, fibrous tissue, and calcification were measured. The associations between plaque ulceration and plaque characteristics were analyzed using logistic regression.

Results Atherosclerotic plaque was present in 185 patients. Plaque ulcerations were present in 38 (21%) patients, of which half had a low degree stenosis (0% to 49%). Plaque volume was significantly larger in ulcerated plaques. After adjustment for age, sex, and degree of stenosis, LR-NC proportion was strongly associated with plaque ulceration (odds ratio, 2.21; 95% CI, 1.49 to 3.27), whereas calcification proportion was inversely associated with plaque ulceration (odds ratio, 0.60; 95% CI, 0.40 to 0.89). These associations remained significant in patients with a low degree stenosis (0% to 49%).

Conclusion Plaque volume, degree of stenosis, and LR-NC proportion evaluated non-invasively with MDCTA are associated with carotid artery plaque ulceration, even in patients with a low degree stenosis (0% to 49%). Plaque volume and composition analysis with MDCTA may identify rupture prone plaques and improve risk stratification in ischemic stroke patients.

Introduction

Atherosclerotic carotid plaque ulceration is an independent marker of previous plaque rupture and an influential predictor of ischemic stroke.[1,2] Thus far, histological and non-invasive imaging assessment of the relationship of carotid plaque characteristics with plaque surface disruption has been limited to patients with a ≥50% carotid stenosis.[3,4] In patients with severe symptomatic stenosis, carotid plaque ulceration has been associated with the presence of fibrous cap rupture and distinct plaque components such as intraplaque hemorrhage, large lipid core, and less fibrous tissue.[3] However, a ≥50% carotid stenosis is present in only ≈10% of patients with amaurosis fugax, transient ischemic attack or minor ischemic stroke.[5] Whereas two-third of carotid plaque ulcerations is observed in carotid arteries with a low degree stenosis (0% to 49%),[6] little is known about the relation between carotid plaque characteristics with plaque ulceration in these patients. Also, limited data are available on the association between plaque volume and carotid plaque surface disruption.[4]

Analysis of atherosclerotic plaque volume and plaque composition using non-invasive imaging could be useful to identify rupture prone plaques. However, concomitant assessment of carotid plaque characteristics associated with plaque rupture cannot be advocated in the general population of ischemic stroke patients without knowledge of the relation between plaque characteristics and plaque surface disruption.

In the present study, we analyzed the relationship between the symptomatic carotid plaque characteristics, comprising of plaque component proportions and plaque volume, with plaque ulceration in consecutive patients with amaurosis fugax, transient ischemic attack or ischemic stroke using multidetector CT angiography (MDCTA). The analysis included and compared the associations of plaque characteristics with plaque ulceration in symptomatic carotid arteries with significant stenosis (≥50%), as well as in those with a low degree stenosis (0% to 49%).

Materials and Methods

Study Population

From a prospective registry of 911 consenting patients with amaurosis fugax, transient ischemic attack or ischemic stroke (Rankin score <4) who underwent MDCTA of the carotid arteries, we selected a 2-year cohort of consecutive patients (n=346) with symptoms in the anterior circulation. Patients were enrolled from a specialized transient ischemic attack/stroke outpatient clinic or the neurology ward. All patients underwent an interview, neurological examination, electrocardiography, laboratory analysis, and MDCTA on admission. Medical history and cardiovascular risk factors as defined previously[7] were recorded. Patients without atherosclerotic plaque (n=137), with carotid occlusion (n=20), and patients with an MDCTA of insufficient quality (n=4) were excluded from the analysis.

MDCTA Data Acquisition and Data Analysis

Imaging was performed with a 16-slice MDCT scanner (Sensation 16, Siemens, Erlangen, Germany) or a 64-slice MDCT scanner (Sensation 64, Siemens, Erlangen, Germany) with a standardized optimized contrast-enhanced protocol (120 kVp, 180 mAs, collimation 16x0.75 mm or 64x0.6 mm, pitch≤1).[8] Details of the MDCTA scan protocol have been described previously.[7,9]

MDCTA images were sent to a stand-alone workstation (Leonardo – Siemens Medical Solutions, Forchheim, Germany) with dedicated 3D-analysis software. The symptomatic carotid bifurcation was evaluated by 2 experienced investigators blinded to clinical data with multiplanar reformatting software, which allows reconstruction of sagittal, coronal, and oblique views from axial sections. Discrepancies were solved by consensus.

Symptomatic carotid arteries were evaluated for the presence of atherosclerotic plaque, defined as thickening of the vessel wall or the presence of calcification. Plaque ulceration was defined as extension of contrast media beyond the vascular lumen into the surrounding plaque. Degree of stenosis in the symptomatic carotid artery was determined according to the NASCET criteria[10] on multi-planar reformatting images perpendicular to the central lumen line.

Plaque volume and plaque component proportions were measured with custom-made software, programmed in MeVisLab (MeVis Research, Bremen, Germany). Using this software, the components of the atherosclerotic plaque within regions of interest drawn on axial MDCTA images can be determined from their corresponding Hounsfield values using thresholds determined previously.[11] The threshold for the distinction between fibrous tissue and lipid-rich necrotic core (LR-NC) was set at 60 Hounsfield units. The threshold for distinguishing calcifications from fibrous tissue was set at 130 Hounsfield units; the value currently used for calcium scoring. Based on previous studies it may be assumed that intraplaque hemorrhage, if present, would be classified as LR-NC[11] or fibrous tissue.[12]

Plaque volume and plaque component volumes were automatically calculated from the number and dimensions of voxels for different ranges of Hounsfield unit values within the regions of interest (Figure 1). Plaque component proportions were calculated from plaque component volumes as a percentage of the plaque volume.

Statistical Analysis

Baseline population and plaque characteristics are presented as mean±SD or number of patients (%). Differences were tested with χ^2 tests, Fisher's Exact tests or Mann-Whitney tests when appropriate. For logistic regression analysis continuous data were divided by 10 or 100, as indicated in the relevant tables.

The correlation between degree of stenosis and plaque volume was evaluated by calculation of the Spearman rank correlation coefficient. The associations between carotid plaque ulceration and degree of stenosis, plaque volume, and plaque component proportions were evaluated using logistic regression analysis. Two models were constructed. In model I, plaque characteristics were adjusted for age and sex. In model II, adjustments were made for age, sex, and degree of stenosis. Finally, in a stratified analysis the associations between carotid plaque ulceration and plaque characteristics were evaluated in

patients with low (0% to 49%), and with significant (≥50%) carotid stenosis, with adjustment for age, sex, and degree of stenosis. P-values ≤0.05 were considered statistically significant. All analyses were performed using SPSS 15.0 statistical package for Windows (SPSS Inc, Chicago, IL).

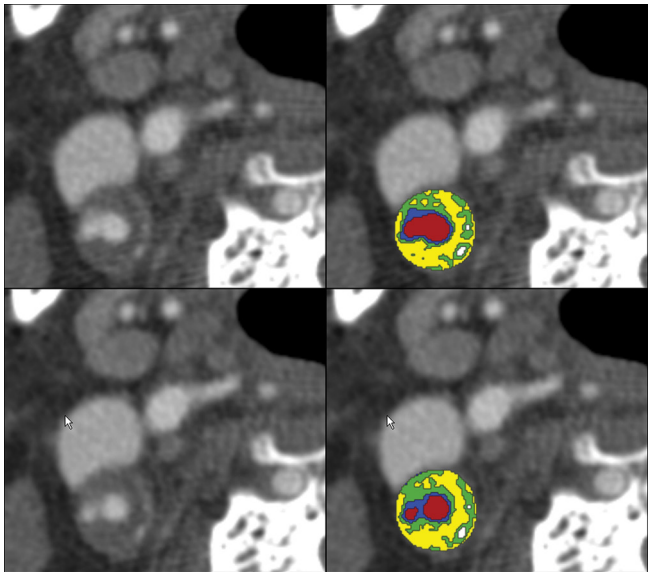

Figure 1. Two succeeding axial MDCTA images illustrating an ulcerated plaque in the internal carotid artery (left). The plaque composition is visible in the image overlay (right), with LR-NC (yellow), fibrous tissue (green), calcifications (white), and the contrast-filled lumen and plaque ulceration (red with blue border-zone).

Results

Patients Characteristics

From the 346 evaluated patients, 185 patients with atherosclerotic plaque were included in all further analyses. Baseline characteristics of patients with and without atherosclerotic plaque ulceration in the symptomatic carotid artery are illustrated in Table 1. Atherosclerotic plaque ulceration in the symptomatic carotid artery was present in 38 (21%) patients. The prevalence of cardiovascular risk factors was not significantly different between the 2 groups.

Plaque Characteristics on MDCTA

Atherosclerotic plaque characteristics of patients with and without atherosclerotic plaque ulceration in the symptomatic carotid artery are illustrated in Table 2. Degree of stenosis was significantly higher in patients with plaque ulceration. In patients with carotid artery ulcerations, 19 had 0% to 49% stenosis, whereas the remaining 19 patients had ≥50% stenosis (Figure 2).

Plaque volume of ulcerated plaques was significantly larger as compared with nonulcerated plaques. A moderate correlation was observed between degree of stenosis and

Table 1. Characteristics of patients with and without symptomatic carotid artery plaque ulceration

	Patients with Plaque Ulceration (n=38; 21%)	Patients without Plaque Ulceration (n=147; 79%)	P-value
Age (yr)	67±10	67±11	0.74
Male	28 (74%)	93 (63%)	0.26
Hypercholesterolemia	28 (74%)	124 (84%)	0.15
Hypertension	27 (71%)	120 (82%)	0.15
Diabetes mellitus	4 (11%)	30 (20%)	0.24
Smoking	17 (45%)	45 (31%)	0.10
Peripheral arterial disease	4 (11%)	15 (10%)	1.00
Previous ischemic stroke	7 (18%)	19 (13%)	0.43
Previous TIA	8 (21%)	27 (18%)	0.82
Previous intracerebral hematoma	2 (5%)	2 (1%)	0.19
History of ischemic heart disease	7 (18%)	42 (29%)	0.30

Data are number (percentage), or mean±SD.

plaque volume (r_s=0.57; P=0.01). Ulcerated plaques contained a significantly larger LR-NC volume, fibrous tissue volume, and LR-NC proportion. Fibrous tissue proportion was significantly lower in ulcerated plaques as compared with non-ulcerated plaques.

Table 2. Plaque characteristics of patients with and without symptomatic carotid artery plaque ulceration

	Patients with Plaque Ulceration (n=38; 21%)	Patients without Plaque Ulceration (n=147; 79%)	P-value
Degree of Stenosis	44±29%	18±27%	<0.001
Plaque volume	1320±708 mm^3	765±588 mm^3	<0.001
LR-NC volume	416±283 mm^3	168±197 mm^3	<0.001
Fibrous volume	736±333 mm^3	468±306 mm^3	<0.001
Calcification volume	163±178 mm^3	129±180 mm^3	0.196
LR-NC proportion	29±10%	18±10%	<0.001
Fibrous proportion	60±11%	67±13%	0.001
Calcification proportion	10±9%	15±14%	0.152

Data are means±SD.

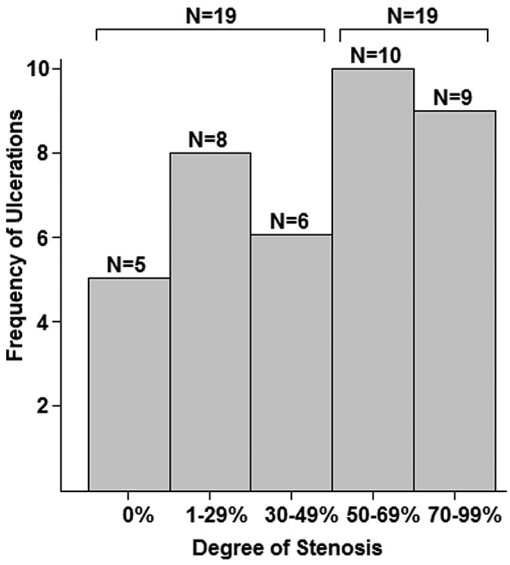

Figure 2. Bar graph illustrating the degree of stenosis of the symptomatic carotid artery in patients with atherosclerotic plaque ulcerations (n=38).

Plaque Characteristics associated with Plaque Ulceration on MDCTA

Results of multivariable analyses relating plaque characteristics and plaque ulcerations are provided in Table 3 and 4. After adjustment for age and sex (model I), degree of stenosis, plaque volume, and the LR-NC proportion were associated with plaque ulceration, whereas fibrous proportion was inversely associated with plaque ulceration. After adjustment for age, sex, and degree of stenosis (model II), plaque volume and the LR-NC proportion remained significantly associated with plaque ulceration, whereas the calcification proportion was inversely associated with plaque ulceration.

In a stratified analysis of patients with a low degree stenosis of 0% to 49% (n=144), the LR-NC proportion remained strongly associated with plaque ulceration, whereas the calcification proportion remained inversely associated with plaque ulceration. In patients with significant stenosis of ≥50% (n=41), plaque volume was associated with plaque ulceration, whereas a trend towards a significant association between the LR-NC proportion and plaque ulceration was observed.

Table 3. Multivariable analysis for the associations between symptomatic carotid artery plaque ulceration and plaque characteristics

	Model I		Model II	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Degree of Stenosis (/10%)	1.33 (1.18-1.50)	<0.001	n.a.	n.a.
Plaque volume (/100mm^3)	1.14 (1.07-1.21)	<0.001	1.09 (1.02-1.16)	0.01
LR-NC proportion (/10%)	2.58 (1.77-3.78)	<0.001	2.21 (1.49-3.27)	<0.001
Fibrous proportion (/10%)	0.64 (0.48-0.87)	0.004	0.85 (0.60-1.20)	0.35
Calcification proportion (/10%)	0.75 (0.54-1.04)	0.08	0.60 (0.40-0.89)	0.01

NA indicates not available; OR, odds ratio

Table 4. Stratified multivariable analysis for the associations between symptomatic carotid artery plaque ulceration and plaque characteristics in patients with low (0-49%) and with significant (≥50) carotid stenosis

	Patients with 0-49% Stenosis (n=144)		Patients with ≥50% Stenosis (n=41)	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**
Plaque volume (/100mm^3)	1.06 (0.98-1.14)	0.15	1.23 (1.04-1.46)	0.02
LR-NC proportion (/10%)	3.04 (1.70-5.45)	<0.001	1.82 (0.98-3.40)	0.06
Fibrous proportion (/10%)	0.88 (0.57-1.36)	0.56	1.05 (0.53-2.08)	0.89
Calcification proportion (/10%)	0.34 (0.16-0.69)	0.003	0.68 (0.40-1.15)	0.15

OR indicates odds ratio

Discussion

In the present study of patients with amaurosis fugax, transient ischemic attack or ischemic stroke, half of the plaque ulcerations were identified in symptomatic carotid arteries with a low degree stenosis of 0% to 49%. Non-invasive carotid artery plaque analysis with MDCTA revealed that degree of stenosis, plaque volume, and the LR-NC proportion were associated with plaque ulceration in the symptomatic carotid artery. Of these plaque characteristics, the LR-NC proportion was most strongly associated with plaque ulceration. In contrast, the calcification proportion was inversely associated with plaque ulceration. The observed associations remained significant in patients with a low degree carotid stenosis of 0% to 49%. The present study is the first to evaluate the associations between plaque ulceration and plaque characteristics irrespective of the degree of stenosis.

Association of Atherosclerotic Plaque Characteristics with Ischemic Stroke

Several studies have evaluated the relationship of carotid artery plaque characteristics with ischemic stroke.[13-19] In general, imaging studies with magnetic resonance and CT have identified positive associations between fibrous cap thickness, the size of the LR-NC, intraplaque hemorrhage and the presence of carotid plaque surface disruption with ischemic stroke in cross-sectional and follow-up studies.[13-16] Accordingly, in ultrasound studies echolucent carotid plaques were associated with increased risk of cerebrovascular events.[17,18] Echolucent plaques are known to have higher levels of lipid and hemorrhage compared with echogenic plaques, which contain more calcification and fibrous tissue. On the contrary, proportion of carotid plaque calcification is shown to be inversely associated with the occurrence of ischemic stroke.[16,19] However, results of histological analyses have been less consistent.[20] A review by Golledge et al. demonstrated the lack of an association between histologically defined LR-NC and intraplaque hemorrhage with ischemic stroke.[20]

The observed discrepancy may be a consequence of disparate etiology of ischemic stroke. Nevertheless, plaque rupture and subsequent thromboembolism are considered crucial

elements in the pathophysiological cascade between the development of a heterogeneous plaque and thromboembolic stroke.[21] As a result, in the present study we evaluated the direct associations between plaque characteristics comprising of plaque stenosis, plaque volume and composition with plaque ulceration in patients with ischemic stroke.

Association of Atherosclerotic Plaque Characteristics with Carotid Plaque Surface Disruption

Previous research relating atherosclerotic carotid plaque characteristics with plaque surface disruption has focused on stenotic plaques corresponding with luminal narrowing of ≥50%.[3,4] In a magnetic resonance study, the LR-NC proportion of carotid plaques of ≥50% stenosis was the strongest predictor of new surface disruption, in form of an ulceration or a fibrous cap rupture.[4] In that particular study, the calcification proportion was inversely related with plaque surface disruption. In addition, the presence of intraplaque hemorrhage as assessed with magnetic resonance is significantly associated with the presence of plaque ulceration on MDCTA.[22] Plaque ulceration on conventional angiography in symptomatic carotid arteries with ≥50% stenosis was associated with the presence of intraplaque hemorrhage, large lipid core and less fibrous tissue in carotid endarterectomy specimens.[3] Similarly, ultrasonographic examination of carotid arteries demonstrated a relation between echolucency of stenotic plaques and plaque ulceration.[23] However, conventional angiography and ultrasound provide no quantitative information on plaque volume. Therefore, only limited data are available on the relation of plaque volume with plaque surface disruption as assessed using magnetic resonance.[4]

MDCTA allows for fast and reliable evaluation of steno-occlusive disease in extracranial[24] and intracranial arteries[25] and is widely available.[26] The technique is effective in the detection of carotid plaque ulceration with a sensitivity and specificity of 94% and 99% respectively.[27] Furthermore, distinct plaque components as well as plaque volume can be quantified in good correlation with histology.[11,12] In the present study, using MDCTA, the relation between plaque composition and plaque volume with plaque ulceration was evaluated in patients with a symptomatic carotid stenosis of ≥50% as well as in patients with a low degree of stenosis (0% to 49%). Interestingly, in line with previous reports,[5,6] a substantial proportion of the plaque ulcerations were located in symptomatic carotid arteries with a low degree of stenosis. The association between the LR-NC proportion with plaque ulceration was significant in ischemic stroke patients with a low degree of stenosis (0% to 49%), whereas a trend toward significance was observed in patients with a stenosis of ≥50%. The inverse association observed between the calcification proportion and plaque ulceration was significant in patients with a low degree stenosis. Furthermore, only a weak correlation was observed between the degree of carotid artery stenosis and plaque volume on MDCTA. Importantly, plaque volume was associated with plaque ulceration, even after adjustment for the severity of stenosis. Overall, these findings demonstrate that the associations between plaque composition and volume with plaque ulcerations are present in ischemic stroke patients irrespective of the degree of the carotid plaque stenosis. In addition, an etiological explanation is provided for the previously observed correlation of plaque characteristics with ischemic stroke events. Herein, a key role is suggested for plaque ulceration in the pathophysiological cascade between the development of a heterogeneous plaque and thromboembolic stroke. In consequence, apart from degree of stenosis, assessment of carotid plaque composition and volume that predispose ulceration could contribute to risk stratification for plaque instability or stroke recurrence.

Study Limitations

First, the study has a cross-sectional design. Indeed, the prognostic value of plaque composition analysis with MDCTA, and more specifically of the LR-NC proportion for the development of plaque ulceration and subsequent thromboembolic ischemic stroke should be confirmed in longitudinal serial imaging studies. Second, in the present study, the presence of intraplaque hemorrhage was not evaluated as plaque composition analysis software used in the current study has not been validated for differentiation of intraplaque hemorrhage. As a result, both LR-NC and fibrous tissue assessed with MDCTA may contain intraplaque hemorrhage if present in the plaque. Finally, plaque composition analysis can be performed on routine MDCTA scans used for carotid stenosis evaluation. Nevertheless, every MDCTA leads to ionizing radiation exposure. Therefore, repeated examinations should not be advocated.

Clinical and Research Implications

To our knowledge, the present study is the first to examine the associations between carotid plaque characteristics and carotid plaque ulceration in ischemic stroke patients with a ≥50% stenosis, as well as in those with a low degree stenosis of 0% to 49%. The LR-NC proportion was identified as the strongest determinant for plaque ulceration. The association between the LR-NC proportion and carotid plaque ulceration was independent of the degree of stenosis. Plaque composition analysis with MDCTA may prove useful for detection of rupture-prone plaques and could potentially improve risk stratification in ischemic stroke patients.

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Chapter 4

Carotid atherosclerotic plaque development; serial imaging studies

Chapter 4.1

Determinants of calcification growth in atherosclerotic carotid arteries; a serial multi-detector CT angiography study

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Abstract

Background Little is known about the natural course of atherosclerotic plaque in the carotid artery bifurcation. This study investigated the growth pattern of calcifications in atherosclerotic carotid arteries and its determinants using serial multi-detector CT angiography (MDCTA).

Methods From a cohort of consecutive patients with TIA or ischemic stroke and a baseline MCDTA scan of the carotid arteries, subjects were invited for a follow-up scan after 4-6 years. Calcification volumes were scored semi-automatically on baseline and follow-up scans. Progression of calcification and its determinants were analyzed in two ways: 1. as incidence of newly detectable calcification in patients free of calcification at baseline, using logistic regression analysis; 2. as annual change in calcification volume in all patients, using linear regression analysis.

Results Two-hundred-twenty-two patients (aged 61.0±9.6 years, follow-up time 4.7±0.8 years) were included. Calcification volumes increased significantly (median 2.9 mm^3 at baseline versus 9.4 mm^3 at follow-up, $p<0.001$). Newly detectable calcification during follow-up was found in 27 out of 67 patients without baseline calcification (40.3%) and was independently associated with age (OR 4.6 per 10 years increase in age, $p<0.001$) and hypertension (OR 8.2, $p=0.008$). Annual calcification growth was independently associated with age, calcification load, glucose, hypertension, and smoking. Baseline calcification load was the most important risk factor for calcification growth in multivariable analysis.

Conclusion Several modifiable cardiovascular risk factors are associated with carotid calcification growth, however, time and baseline calcification load remain the most important determinants of calcification development.

Introduction

Carotid artery atherosclerosis is one of the major causes of ischemic stroke. Atherosclerotic plaque may rupture, leading to thrombus formation and embolization of atherosclerotic debris or thrombus material into distally located arteries.

Carotid calcification has been used as a surrogate marker for carotid atherosclerosis in studies on stroke risk prediction.[1-3] Little data is published on the determinants of carotid calcification. A few cross-sectional studies on asymptomatic subjects demonstrated the relation between classical cardiovascular risk factors and the presence or volume of calcification.[4-6] However, especially in symptomatic patients, who have a high risk of recurrent events, it might be of clinical importance to predict and influence further plaque development. Although currently available non-invasive imaging techniques enable the monitoring of carotid calcification growth in vivo, calcification development in the carotid arteries has never been studied longitudinally.

In this study, we therefore investigated the growth pattern of calcifications in atherosclerotic carotid arteries and its determinants using serial multi-detector CT angiography (MDCTA) imaging in patients with recent ischemic stroke or TIA.

Methods

Study population

Patients were recruited from a prospective registry of patients with amaurosis fugax, transient ischemic attack or minor ischemic stroke (Rankin score <4) who underwent an MDCTA of the carotid arteries as part of their clinical work-up.[7] Follow-up scans were performed after 4-6 years in a subgroup. Inclusion criterion for this serial study was the presence of atherosclerotic plaque in one or both carotid arteries, defined as thickening and/or calcification of the vessel wall. Exclusion criteria were: bilateral occlusion and/or invasive treatment of the carotid artery, no informed consent, renal insufficiency, hyperthyroidism and poor image quality of the baseline scan. The flow chart in Figure 1 shows the selection process of patients for this serial study. Clinical measures and information on risk factors and medication use were obtained from first admission to the hospital and information on recurrent ischemic cerebrovascular events and medication use was obtained at the time of the follow-up scan. This study was approved by the Institutional Ethics Review Board.

Scan protocol

Baseline scans were performed on a 16- or 64-slice MDCT scanner (Siemens, Sensation16/64, Erlangen, Germany) with a standardized optimized contrast-enhanced protocol (collimation 16 x 0,75mm or 2 x 32 x 0.6 mm, pitch 1, 120 kV). The scan ranged from the ascending aorta to the intracranial circulation. The follow-up scans were performed on a 128-slice MDCT scanner (Siemens, Flash, Erlangen, Germany) with a comparable protocol (collimation 2 x 64 x 0.6 mm, pitch 0.7, 120 kV) and a scan range of 6 cm around the bifurcation. To test for differences in in-plane resolution between similar kernels on

different scanners, we performed a point-spread-function analysis using a standard thin wire phantom.[8] No differences were found. Eighty mL of contrast material was used, with a 40 mL saline bolus chaser and real time bolus tracking in the ascending aorta, using a threshold of 120 HU.

Image reconstructions were made with an FOV of 120 mm, matrix size of 512 x 512 mm, a slice thickness of 1.0 mm, an increment of 0.6 and a smooth (B30) as well as an intermediate (B46) reconstruction algorithm.

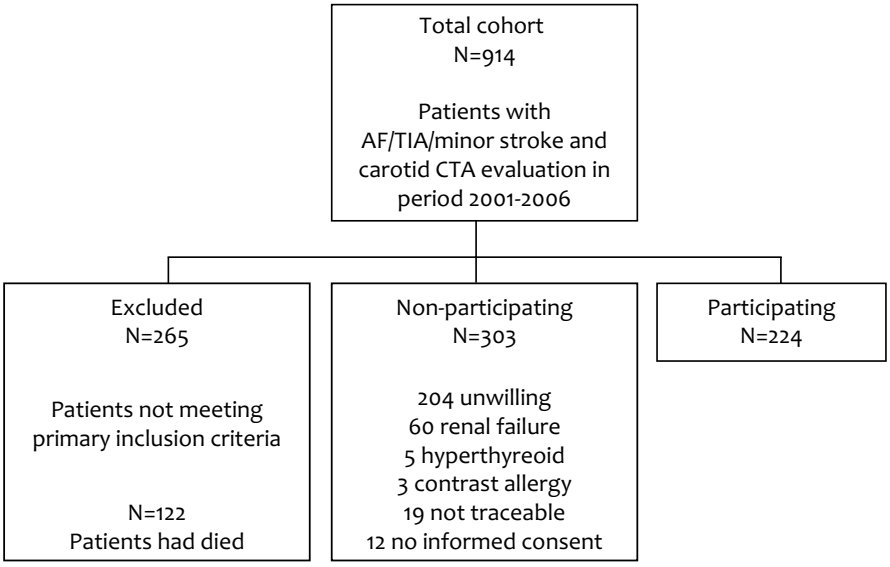

Figure 1. Flow chart depicting patient selection for this follow-up study.

Analysis of calcification

Calcification measurements were done with a commercially available software package on a standalone workstation (Leonardo-Siemens Medical Solutions, Forchheim, Germany). To determine the presence and quantity of carotid calcification, one observer blinded to the clinical history evaluated the axial images of the MDCT data sets. Calcification was semi-automatically scored within a range of 3 cm above to 3 cm under the carotid bifurcation or a range as long as possible given the scan range at follow-up. The analyzed range on baseline and follow-up was fixed within one patient. A threshold of 600 HU was chosen to enable an automatic differentiation between contrast medium in the lumen and calcifications in the vessel wall.[9] Volume scoring results in low rescan variability[10] and a volume score using a threshold of 600 HU has a high intra- and inter-observer repeatability.[9] As a default, an intermediate convolution kernel (B46) was used for reconstruction of the baseline and follow-up MDCTA.[11] In cases no B46 kernel was available either at baseline or follow-up, the smooth B30 kernel was used to compare calcification volumes on both MDCTA scans.

Intra-observer and inter-observer reproducibility of this method were defined in a subset of 40 consecutive data sets and were very good (intra-class correlation coefficients of 1.0 and coefficients of variation < 3%).

Cardiovascular risk factors

Diabetes was defined as fasting serum glucose levels over 7.9 mmol/l, nonfasting serum glucose levels over 11.0 mmol/l, or use of anti-diabetic medication. Hypertension was defined as a mean systolic blood pressure over 140mmHg and/or a mean diastolic blood pressure over 90 mmHg during 2 episodes of at least 15 minutes of continuous non-invasive blood pressure measurement, or on treatment with anti-hypertensive medication. Hypercholesterolemia was defined as fasting cholesterol over 5.0 mmol/l or on treatment with cholesterol lowering drugs. Subjects were categorized as current smoking versus non- and ever smoking. Besides these dichotomized risk factor definitions, the continuous risk factors glucose (in mmol/l), systolic blood pressure and diastolic blood pressure (per 10 mmHg), and cholesterol level (in mmol/l) at baseline were used as determinants in the analyses. Information on medication use was obtained at baseline and at follow-up. Information on a history of coronary artery disease (defined as a history of myocardial infarction, angina pectoris or coronary artery bypass grafting) and on a history of ischemic cerebrovascular disease (defined as a clinical diagnosis of ischemic stroke or transient ischemic attack) was collected.

Statistical analysis

Data are presented as mean +/- SD, median +/- interquartile ranges (IQR) and percentages where appropriate. Differences between patient groups were analyzed using a c^2-test, a *t*-test or a Mann-Whitney U test where appropriate.

The Wilcoxon-Signed Rank test was performed to test for differences in baseline calcification volume and growth rate between calcifications in the left and right carotid arteries within patients. No significant differences in baseline calcification volume (median 0.4 (IQR 0.0-13.6) versus 1.2 mm^3 (IQR 0.0-14.2), p=0.89) or in calcification growth (median 0.4 (IQR 0.0-3.0) versus 0.6 mm^3/year (IQR 0.0-3.5), p=0.62) between left and right carotid arteries were found. We therefore averaged calcification volumes across both carotid arteries within a patient. The calcification volumes of one side were taken in patients treated unilaterally with carotid endarterectomy or stent placement (n=21) or in patients with unilateral focal image artefacts (n=1).

Because calcification volumes had a highly positively skewed distribution, we used the natural log transformed values and added 1 mm^3 to deal with patients who had an initial calcification volume of zero.[12] The associations between the classical cardiovascular risk factors and baseline calcification volumes (ln (calcification volume + 1)) were studied using linear regression analysis.

The changes in the amount of carotid calcification were assessed by subtracting the calcification volumes at baseline from those at follow-up. To test for absolute progression in calcification volume from baseline in the whole group, a Wilcoxon-Signed Rank test was used. The annualized calcification growth rate was calculated by dividing the change in calcification volume by the actual number of months that passed between the two scans,

multiplied by 12. Two endpoints of progression of calcification were analysed separately and were defined as: 1. Incidence of detectable calcification in patients free of detectable calcification at baseline and 2. Annualized change in calcification volume in all patients. Associations between risk factors and incidence of newly detectable calcification at follow-up were assessed with logistic regression analysis in a model adjusted for gender, age and scan interval (model I) and in a multivariable model (II) including all variables from model I with a P-value ≤0.05 and the strongest variable from two risk factors definitions. Linear regression analysis was used to study determinants of absolute annual calcification growth (ln (calcification growth +1)). Age, gender and variables with a P-value ≤0.05 (and the strongest one from two risk factor definitions) from the age and gender adjusted model (model I) were fitted into the multivariable regression model (model II).

To avoid an induced (spurious) correlation between change in calcification and its baseline value, the mean of calcification volume at baseline and follow-up was used as the determinant "calcification load" for the regression analyses.[13]

Statistical significance was assumed at a p-value of less than 0.05. All analyses were performed using SPSS version 20.0 for Windows.

Table 1. Baseline patient characteristics

	All patients (n=222)	**Men (n=141, 63.5%)**	**Women (n=81, 36.5%)**
Age (years, mean ± SD)	**61.0 ± 9.6**	**60.9 ± 9.1**	**61.0 ± 10.3**
Diabetes Mellitus	**26 (11.7%)**	**21 (14.9%)**	**5 (6.2%)**
Hypertension	**152 (68.5%)**	**98 (69.5%)**	**54 (66.7%)**
Hypercholesterolemia	**175 (78.8%)**	**105 (74.5%)**	**70 (86.4%) #**
Smoking (current)	**90 (40.5%)**	**54 (38.3%)**	**36 (44.4%)**
History of CAD	**30 (13.5%)**	**25 (17.7%)**	**5 (6.2%) #**
History of CVD	**43 (19.4%)**	**36 (25.5%)**	**7 (8.6%) #**
Scan interval (years, mean ± SD)	**4.7 ± 0.8**	**4.8 ± 0.9**	**4.4 ± 0.7 ***
BL Ca volume (mm^3, median, IQR)	**2.9 (0.0-19.7)**	**3.7 (0.0-25.9)**	**0.8 (0.0-13.6)**
FU Ca volume (mm^3, median, IQR)	**9.4 (0.7-41.9)**	**11.5 (1.1-58.5)**	**6.5 (0.2-32.3)**
Annual Ca growth (mm^3, median, IQR)	**1.1 (0.1-4.2)**	**1.2 (0.1-5.5)**	**0.8 (0.0-3.4)**

Values are means ± standard deviation or median with interquartile range for continuous variables and numbers (percentages) for dichotomous variables. CAD = coronary artery disease, CVD = cerebrovascular disease, BL = baseline, FU = follow-up, Ca = calcification

Significant difference between men and women at the level p£0.05, analysed using a c^2-test

***** Significant difference between men and women at the level of p=0.001, analysed using a *t*-test

Results

Of the 527 potential candidates, 224 patients were included in this study. The participating patients had a significantly lower prevalence of hypertension (68.8% versus 78.5%, p<0.05) and diabetes mellitus (11.6% versus 22.8%, p<0.001), but were more often smokers (40.6% versus 31.7%, p<0.05) as compared to the non-participating patients. In two cases, poor image quality due to streak artefacts hampered an accurate calcification scoring of both carotid arteries and these patients were therefore excluded.

The clinical indication for the baseline MDCTA of the included patients was amaurosis fugax in 27 patients (12.2%), transient ischemic attack in 92 patients (41.4%) and ischemic stroke in 103 patients (46.4%). Mean age of the patients at inclusion was 61.0 ± 9.6 years and 64% of participants were male. No significant differences were found between men and woman in calcification volumes at baseline and follow-up, or in annual calcification growth. Average time between the two scans was 4.7 ± 0.8 years (Table 1). The majority of patients used from baseline or earlier: cholesterol lowering drugs (92.8%), anti-hypertensive medication (69.4%), anti-diabetic therapy (11.3%) and anti-platelets or anti-coagulation (99%) (at follow-up 84.7%, 73.4%, 16.2% and 96%, respectively).

Table 2 displays the associations between risk factors and baseline calcification volume. Gender, age, diabetes mellitus, glucose, and current smoking were significant predictors in model I and all variables put into model II remained significantly associated with baseline calcification volume in model II.

Table 2. Cardiovascular risk factors and baseline calcification volume in the carotid artery bifurcation (n = 222)

	difference (95%-CI) [I]	**difference (95%-CI) [II]**
Male gender	0.48 (0.06-0.89)*	0.43 (0.02-0.84)*
Age (per 10 years)	0.64 (0.43-0.84)*	0.74 (0.52-0.96)*
Diabetes Mellitus	0.66 (0.03-1.29)*	
Hypertension	0.25 (-0.18-0.68)	
Hypercholesterolemia	0.45 (-0.05-0.95)	
Smoking (current)	0.53 (0.10-0.96)*	0.58 (0.16-1.01)*
Glucose	0.22 (0.05-0.38)*	0.23 (0.07-0.40)*
SBP (per 10 mmHg)	0.07 (-0.02-0.16)	
DBP (per 10 mmHg)	-0.02 (-0.19-0.14)	
Cholesterol	0.11 (-0.07-0.29)	

Values represent differences in baseline calcification volume (Ln (calcification volume + 1)) (mm^3) per unit increase of the cardiovascular risk factors with 95% confidence intervals (CI).

Model I: Adjusted for gender and age

Model II: Additionally adjusted for all significant risk factors from model I with a p-value £0.05

*P<0.05

The calcification volumes in the whole group were significantly higher at follow-up (median 9.4 mm³, IQR 0.7-41.9) than at baseline (median 2.9 mm³, IQR 0.0-19.7; Z =-11.5, p<0.001). Median annual growth rate was 1.1 mm³ (IQR 0.1-4.2 mm³).

Sixty-seven patients had no detectable calcification at baseline. Twenty-seven of these patients had newly detectable calcifications at follow-up (after 4.7±0.8 years), a cumulative incidence of 40.3%. Median annual growth was 0.16 mm³ (IQR 0.06-0.66). Table 3 displays the associations between the presence of newly detectable calcification at follow-up and cardiovascular risk factors. Age, scan interval, and hypertension were significantly associated with the occurrence of newly detectable calcification in model I. In model II, the same determinants remained significantly associated.

Patients with detectable calcification at baseline (n=155) showed a median annual growth rate of 2.2 mm³ (IQR 0.8-6.4). Four patients demonstrated regression of calcifications, one patient did not show any progression at all and the other 150 patients all showed some progression (min 0.03, max 37.4 mm³ per year).

The results of the linear regression analyses for calcification growth in all patients (n=222) are displayed in Table 4. Age, calcification load, diabetes mellitus, glucose, hypertension, and current smoking were significant predictors for calcification growth in model I. In model II, these same risk factors remained significantly associated.

Table 3. Cardiovascular risk factors and newly detectable calcification at follow-up among patients free of calcification at baseline (n = 67)

	OR (95%-CI) [I]	**OR (95%-CI) [II]**
Male Gender	0.43 (0.12-1.54)	0.34 (0.08-1.43)
Age(per 10 years)	4.01 (1.90-8.42)*	4.61 (1.85-11.49)*
Scan interval (per year)	3.16 (1.20-8.35)*	3.06 (1.04-9.02)*
Diabetes Mellitus	0.97 (0.11-8.71)	
Hypertension	7.95 (1.69-37.29)*	8.15 (1.73-38.34)*
Hypercholesterolemia	0.50 (0.12-2.05)	
Smoking (current)	2.15 (0.49-9.41)	
Glucose (mmol/L)	1.37 (0.74-2.52)	
SBP (per 10 mmHg)	1.24 (0.93-1.67)	
DBP (per 10 mmHg)	1.25 (0.75-2.11)	
Cholesterol (mmol/L)	0.74 (0.43-1.28)	

Values represent odds ratios (newly detected calcification versus no calcification at follow-up) with 95% confidence intervals (CI).

Model I: Adjusted for gender, age and scan interval

Model II: Additionally adjusted for all significant risk factors from model I with a p-value £0.05

*P<0.05

Table 4. Cardiovascular risk factors and annual calcification growth among all patients (n = 222)

	difference (95%-CI) I	**difference (95%-CI) II**
Male gender	0.18 (-0.06-0.42)	0.04 (-0.15-0.22)
Age (per 10 years)	0.32 (0.20-0.44)*	0.24 (0.13-0.34)*
Ca load (per 10mm³)* *	0.10 (0.08-0.11)*	0.09 (0.07-0.11)*
Diabetes Mellitus	0.54 (0.18-0.90)*	
Hypertension	0.33 (0.08-0.57)*	0.27 (0.08-0.46)*
Hypercholesterolemia	0.20 (-0.09-0.49)	-
Smoking (current)	0.38 (0.14-0.63)*	0.30 (0.11-0.50)*
Glucose	0.14 (0.05-0.24)*	0.10 (0.02-0.17)*
SBP (per 10 mmHg)	0.05 (-0.001-0.10)	
DBP (per 10 mmHg)	0.07 (-0.09-0.10)	
Cholesterol	0.05 (-0.06-0.15)	

Values represent differences in annual calcification growth (Ln (calcification growth + 1)) (mm³) per unit increase of the cardiovascular risk factors with 95% confidence intervals (CI).

** Ca load = calcification load, defined as the mean of baseline and follow-up calcification volume within patients

Model I: Adjusted for gender and age

Model II: Additionally adjusted for Ca load and all significant risk factors from model I with a p-value £0.05

*P<0.05

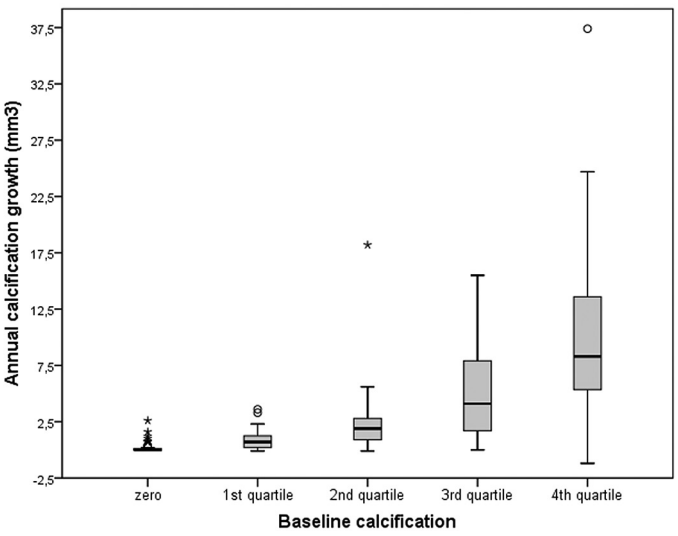

Figure 2. Graph shows the mean annual calcification growth in accordance with baseline calcification volume categories. The different bars-and-whiskers represent: subjects without calcification at baseline (n=67); subjects with calcification present at baseline were divided into quartiles: first quartile (n=39); second quartile (n=39); third quartile (n=38) and fourth quartile (n=39).

Age and gender accounted for only 12% of the variability in calcification growth. R^2 significantly increased by adding the risk factors glucose, hypertension and smoking (R^2 = 0.22) or by adding calcification load (R^2 = 0.44). R^2 of the final model II was 0.48. Calcification load was therewith the most important determinant. Its influence is also depicted in Figure 2.

During follow-up, 37 patients experienced one or more recurrent ischemic events from the territories of the carotid arteries. No differences in calcification volume at baseline or in calcification growth was found between the patients with and those without recurrent disease.

Discussion

Summary

In this serial MDCTA study, we investigated the in vivo growth pattern of calcification and its determinants in atherosclerotic carotid arteries of symptomatic patients. Calcification volume significantly increased compared to the baseline volume. We found the cardiovascular risk factors age, serum glucose level, hypertension, and smoking to be associated with a fast annual calcification growth. Furthermore, calcification load was the most important predictor for absolute calcification growth. The development of newly detectable calcification was associated with age and hypertension.

Literature context

Large cross-sectional studies in carotid arteries have shown that classical cardiovascular risk factors are associated with the presence and amount of carotid calcification.[4-6] We found similar risk factors associated with calcification volume and calcification growth. No prospective studies investigating calcification growth in the carotid arteries are available for comparison. In contrast to calcification measures in the carotid arteries, coronary artery calcium score (CAC) has already proven to be a marker of risk of cardiovascular events and to have incremental prognostic value beyond traditional risk factors.[14] Several large prospective serial coronary calcification studies have been performed.[15-21] Our longitudinal study shows comparable findings with those from prospective coronary artery studies. First, in prospective coronary studies, comparable incidence rates, varying from 5 to 12% per year, were found.[16-18,22] In agreement with those studies [17,18] we found age and hypertension as risk factors for incidence of detectable calcification. Secondly, in this study calcification load was the dominant predictor of fast calcification growth. Baseline calcification load was found to be the only independent risk factor for calcification growth in some coronary studies,[20] and its importance was confirmed in several others. [18,20-23] This finding can be explained by the fact that the chronic influence of risk factors is already reflected in the baseline calcification volume, since baseline calcium itself is part of the pathophysiological process under investigation. When treated as a determinant and corrected for in a multivariable model, the effects of the other risk factors will diminish. Furthermore, in a large community-based cohort (n=2807) of subjects having coronary calcification at baseline, body mass index, a family history of heart attack, diabetes mellitus, and glucose remained significantly associated with coronary calcification

progression after adjustment for baseline calcification burden.[18] Another study found hypertension and diabetes mellitus to remain significant beside baseline calcification.[21] In addition to hypertension and diabetes, we also found age and smoking to be related to fast annual calcification growth.

This study fills a gap in the literature, since no prospective data on calcification of carotid atherosclerotic plaques is available. However, in carotid arteries, the clinical significance of calcification of plaques is not as clear as in the coronary arteries. Whereas some studies suggest that degree of carotid calcification is associated with increased stroke risk,[1,3,24] others found that a relatively high calcification content of carotid plaques is associated with plaque stabilization.[25-29] The relative proportion of calcification within plaques seems to be of significant importance. Future work should therefore focus on the determinants of relative calcification contribution within carotid plaques and on the relation between (relative) calcification burden and recurrent stroke.

Limitations

Advantages of this study are its longitudinal and in vivo nature, which increases the sensitivity for finding predictors of carotid calcification progression.

Whereas most longitudinal studies are performed on asymptomatic subjects, a symptomatic stroke population was subject of this study. Once having experienced an ischemic cerebrovascular event, patients are prone to recurrent events. It is therefore important to perform longitudinal atherosclerotic studies on symptomatic patients. However, methodological obstacles are introduced, since most patients already use one or several drugs for secondary prevention. We have to be aware of an obvious mingling of possible influences on plaque calcification growth: the ominous effect of the risk factors and in addition the, possibly reverse, influence of the drugs. For example in hypercholesterolemic patients, statin use may reduce the progression of calcification.[15] In our prospective, observational study it is impossible to unravel these different influences. To completely separate possible drug effects from risk factor influences, a randomized clinical trial should be performed.

Furthermore, in contrast to the 130 HU threshold used in non-contrast enhanced scans, we used a threshold of 600 HU to be able to automatically separate calcifications from the dens contrast material in the lumen. It can be questioned whether changes in calcification burden are optimally caught by using this 600 HU threshold. Both an increasing density of calcifications as well as circumferential expanding of the calcification dots might be partly underscored, therewith underestimating calcification growth and its variability. This may reduce the sensitivity for finding significant associations with determinants. However, the same would hold for the standard quantification methods using a threshold of 130 HU. Moreover, Glodny et al. found a strong, linear correlation between the calcification volumes derived using a threshold of 600 HU on coronary CT angiographies and the calcification volumes derived using 130 HU as a threshold or the Agatston score on non-contrast enhanced scans.[9]

Conclusion

In this study, we presented the determinants of calcification growth in the carotid arteries, filling a gap in the literature. Although several modifiable classical cardiovascular risk factors are associated, age and especially calcification load are the most important predictors for calcification progression. These findings should be taken into account in the design of future studies that investigate the determinants of calcification proportion within carotid plaques and the relation between calcification changes and recurrent ischemic cerebrovascular events.

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Chapter 4.2

Carotid atherosclerotic plaque progression and change in plaque composition over time; a 5-year follow-up study using serial CT angiography

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Abstract

Background and Purpose Serial in vivo imaging of atherosclerosis is important for understanding plaque progression and is potentially useful in predicting cardiovascular events and monitoring treatment efficacy. This prospective study aims to quantify temporal changes in carotid atherosclerotic plaque volume and plaque composition using MDCTA.

Materials and Methods In 109 patients with TIA or ischemic stroke, serial MDCTA of the carotid arteries was performed after 5.3±0.7 years. The carotid bifurcation was semiautomatically registered for paired baseline-follow-up datasets. Outer vessel wall and lumen boundaries were defined using semi-automated segmentation tools. Plaque component volumes were measured using HU thresholds. Annual changes in plaque volume and plaque component proportions were calculated.

Results One-hundred-ninety-three carotid arteries were analyzed. Plaque volume decreased in 31% and increased in 69% of vessels (range -5.6-10.1%/year). Overall, plaque volume increased 1.2% per year (95% CI: 0.8-1.6, p£0.001). Plaque composition changed significantly from baseline (fibrous 66.4%, lipid 28.8%, calcifications 4.8%): fibrous tissue decreased by 1.5%, lipid decreased by 1.8%; calcification increased by 3.3% ($p<0.001$). Intraobserver reproducibility of all volume and proportion measurements was good (ICC: 0.78-1.00), interobserver reproducibility moderate (ICC: 0.76-0.99).

Conclusions Changes in carotid plaque burden and plaque composition can be quantified using serial MDCTA. Plaque burden development is a heterogeneous and slow process.

Introduction

Atherosclerosis is a slowly progressing disease, subclinical for decades before suddenly causing clinical manifestations like coronary artery disease, ischemic cerebrovascular disease and peripheral artery disease. Increased atherosclerotic burden first causes outward remodeling of the artery, with compensatory expansion of the outer vessel wall boundary without narrowing of the lumen.[1] In later stages of plaque progression the compensatory mechanism of outward remodeling no longer suffices, and vessel lumen becomes compromised, eventually causing stenosis or occlusion. Beside the hemodynamic effects on blood flow and blood pressure, atherosclerotic disease is thought to induce ischemic events by plaque rupture, causing thrombosis and obstruction or thromboembolization into distal arteries. Increased lipid content, large lipid-richnecrotic cores, intraplaque hemorrhage, inflammation, and thin fibrous caps are the hallmarks of plaques vulnerable to rupture.[2-3]

Knowledge of atherosclerotic plaque development has predominantly been derived from animal and histopathological studies.[4] Still, little is known about progression of atherosclerosis in humans. Serial in vivo imaging of both vessel lumen and plaque is important for understanding the development of atherosclerotic plaque and its progression from subclinical lesions into rupture-prone plaques. Accurate monitoring of changes in atherosclerotic plaque burden and composition can potentially be used in risk prediction and in assessing efficacy of pharmaceutical treatment.

MDCTA has been validated for imaging atherosclerotic plaque in the carotid arteries in vivo.[5-6] Plaque volume and several plaque components like calcifications, lipid and fibrous tissues can be accurately quantified.[5, 7] MDCTA therefore provides a minimally invasive tool to investigate carotid plaque progression in humans.

In this prospective study, serial CT angiography of the carotid arteries was performed in patients with TIA or ischemic stroke and changes in plaque burden and plaque composition were quantified using a semi-automated custom-made plaque segmentation tool.

Methods

Study population

Patients were recruited from a cohort of 914 consecutive patients with TIA or ischemic stroke, who had undergone a standard clinical work-up, including multidetector CT angiography of the carotid arteries.[8] All patients with atherosclerotic plaque (i.e. thickening and/or calcification of the vessel wall) in one or both carotid arteries were invited to participate in this serial imaging study. Exclusion criteria were: no atherosclerotic plaque(s) present at the level of the carotid bifurcation, bilateral occlusion and/or treatment of the carotid arteries, no informed consent, renal insufficiency or hyperthyroidism and bad image quality of the baseline scan. Blood samples to determine renal function were taken if necessary. The study was approved by the Institutional Review Board. All patients gave written informed consent.

The serial MDCTA datasets of the first 113 patients included in this study were used for analysis. The MDCTA data from 4 patients were excluded because of motion artifacts or

low lumen contrast density. In addition, 6 arteries were excluded from the analysis because of occlusion on baseline and/or follow-up scan, 14 because of local treatment with carotid endarterectomy or stent placement and 5 because of (focal) poor image quality (perivenous artifacts or streak artifacts due to dental material) impeding correct plaque segmentation. In total, 193 carotid arteries from 109 patients were included in this study.

Clinical data

As a part of routine clinical work-up all patients underwent a detailed health questionnaire, a physical examination, laboratory measurements, a CT scan of the brain and multidetector CT angiography of the carotid arteries. Information was collected on age, gender, medication use, hypertension, hypercholesterolemia, diabetes mellitus, smoking, and history of cerebrovascular or cardiovascular disease as previously described [8]. During the visit for the follow-up MDCTA scan, a detailed health questionnaire was taken.

Multidetector CT angiography protocol

Baseline scans were performed on a 16- or 64-slice multidetector CT scanner (Sensation 16/64, Siemens Medical Solutions, Erlangen, Germany), with a standardized optimized contrast-enhanced protocol (collimation 16 x 0.75 mm (n=106) or 2 x 32 x 0.6 mm (n=3), pitch 1) and a scan range ranging from the ascending aorta to the intracranial arteries. The follow-up scans were performed on a 128-slice multidetector CT scanner (Somatom Definition, Siemens Medical Solutions), with a comparable protocol (collimation 2 x 64 x 0.6 mm, pitch 0.7) and a scan range of 6 cm around the carotid bifurcation. Eighty mL of contrast material was used, with a saline bolus chaser of 40 mL and real-time bolus tracking in the ascending aorta, using a threshold of 120 HU.

Image reconstructions for all scans were made with a FOV of 120 mm, matrix size 512 x 512, slice thickness of 1.0 mm, increment of 0.6 mm and with both a smooth (B30/B31) and an intermediate (B46) filter kernel, of which the last one has been demonstrated to give the best soft tissue contrast.[9] We performed a point-spread-function analysis using a standard thin wire phantom[10] to test for differences in in-plane resolution between similar kernels on different scanners, but we did not find any differences.

Registration of baseline and follow-up scan

In order to accurately compare the baseline plaque measurements with those at follow-up within each patient, the carotid bifurcation on both scans were registered using a custom-made semi-automated registration tool (Figure 1). First lumen segmentation was generated by a level-set based method initialized with three seed points in the CCA, ICA and ECA. [11] From this lumen segmentation a central lumen line was extracted. Then 3 new observer-defined initialization points in, respectively, the CCA, the ICA and the ECA on the follow-up MDCTA marked the range to be segmented for plaque analysis. Using the bifurcation point (the level at which CCA lumen separates into 2 separate lumens of ICA and ECA) as a landmark, the absolute distances along the central lumen line from this point to the initialization points were calculated and copied to the central lumen lines of the baseline MDCTA to define the corresponding vessel part of interest on the baseline scan (on axial sections). In this way, adjustments were made for possible differences between the

baseline and the follow-up scan in the curvature of the carotid arteries due to elongation or head position. Scan data reconstructed with a B46 kernel were used.

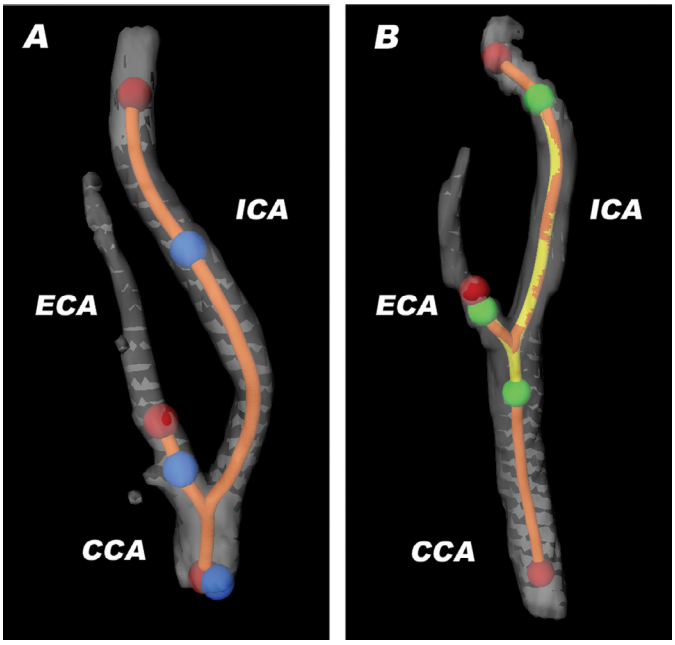

Figure 1. Explanation of the method of semiautomated registration of vessel wall range of interest. 3D reconstructions of the lumen segmentations of a carotid bifurcation at FU (A) and at BL (B). Lumen segmentation is semiautomatically generated after clicking 3 initialization points in the CCA, the ICA and the ECA, respectively (*red dots*), separately in both FU and BL data sets. From this lumen segmentation, CLLs are extracted (*orange line*). On the FU CTA the vessel range containing atherosclerotic plaque is defined by clicking another set of initialization points (*blue dots*). The absolute distances along the CLL between the 3 blue points are measured and copied to the CLL of the BL CTA (*yellow line*), which define the range on axial images to be analyzed by the automated plaque segmentation tool (*green dots*).

Plaque analysis

Volume measurements of lumen, vessel wall and vessel wall components (further defined as plaque and plaque components, respectively) were derived using a custom-made semi-automated plaque analysis method. The lumen segmentation used in registering the vessel range of interest between baseline and follow-up scan was also used here. The outer vessel wall was segmented based on a method using a Gentle Boost classifier which had been trained by manually annotated data.[12-13] The lumen segmentations and outer vessel wall segmentations were checked and adjusted manually where appropriate. The plaque was derived by subtracting the lumen segmentation from the outer vessel wall segmentation.

After segmenting the carotid artery outer vessel wall and lumen, plaque components were differentiated based on Hounsfield Unit thresholds. The cut-off value between calcifications and fibrous tissue was set at 130 HU, as used for calcium scoring. The threshold used to differentiate lipid from fibrous tissue was 60 HU, a setting that was previously validated with histology.[5] The volumes of the plaque and plaque components were calculated as the product of the number of pixels in the segmented areas, the pixel size and the slice increment. The proportion of plaque components was calculated as the ratio of the volume of the component to the total plaque volume, multiplied by 100. Figure 2 shows an example of axial slices through a carotid bifurcation at baseline and at follow-up and their corresponding plaque segmentations.

Stenosis measurements were performed according to the NASCET criteria. Using 3D software, MPRs were made and the smallest diameter was measured on axial images perpendicular to the vessel axis.

Reproducibility of semiautomated plaque measurements

Although the procedure was automated for a large part, there were some observer interventions: clicking initialization points for lumen segmentation, clicking initialization points for defining the range for plaque segmentation, and manually adjusting the lumen and outer vessel wall segmentations, if necessary. To assess the intraobserver and interobserver variability of the complete method, a subset of 30 carotid arteries was analyzed 2 times by the same observer with a delay of 3 months, and once by a second observer. This subset was randomly taken from the group of patients without a treated artery, an occluded carotid artery, or a poor image quality. The intraobserver and interobserver variability are presented as ICC and CoV.

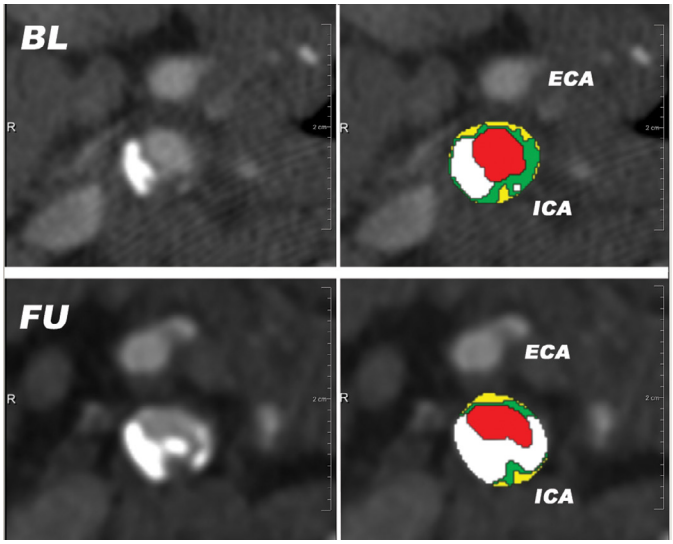

Figure 2. Semiautomatically generated plaque segmentations on matched BL and FU axial MDCTA images just above the level of the carotid bifurcation. The time interval between the scans is 5.8 years; a slight plaque progression with an increase in calcifications is visible. *Red = lumen, green = fibrous tissue, yellow = lipid, white = calcification.*

Data-analysis

Plaque volume and plaque component volumes and proportions were measured on both sides in each individual at baseline and follow-up, within the registered range of interest. Within each carotid artery, the changes over time of the different plaque measures were derived.

The data are presented as mean ± SD. Total vessel, lumen, plaque and plaque components are presented as absolute volumes (in mm^3) on baseline and follow-up. Plaque components are also expressed as proportions. A paired *t*-test was used for the analysis of absolute progression from baseline to follow-up.

Temporal changes are expressed as absolute annual change in volume or proportion ((change in volume or proportion / FU time in months)*12), and as a percentage ('relative') annual volume change from baseline (absolute annual change in volume / BL volume). A one-sample *t* test was used for comparison of "annual change" to 0.

Correlations between percentage annual growth of plaque volume and baseline plaque characteristics were described by Pearson's correlation coefficient. Correlations between absolute change in plaque component proportion and baseline characteristics were described by Pearson's correlation or Spearman's correlation coefficient when appropriate. To avoid an induced (spurious) correlation between absolute or percentage annual change in a plaque feature and its baseline value, the mean of the measure at baseline and follow-up was used.[14]

Table 1. Baseline patient characteristics

Baseline patient characteristics (n=109)	**Value**
Age (years; mean ± SD)	62.8 ± 8.5
Male sex (n [%])	70 (64%)
Hypertension (n [%])	76 (70%)
Hypercholesterolemia (n [%])	90 (83%)
Diabetes mellitus (n [%])	11 (10%)
Smoking (current or past; n [%])	87 (80%)
Previous cardiovascular disease (n [%])	19 (17%)
Previous cerebrovascular disease (n [%])	25 (23)%
Symptoms (n [%])	
Amaurosis fugax	18 (17%)
Transient ischemic attack	50 (46%)
Minor stroke	41 (38%)
Use of secondary preventive medication during FU (n [%])	
Cholesterol lowering	98 (90%)
Anti-hypertensive	80 (73%)
Anti-diabetic	17 (16%)
Time delay BL – FU MDCTA (years; mean ± SD)	5.3 ± 0.7

Results

Baseline characteristics

The clinical information on the 109 included patients is summarized in Table 1. The median time delay between the event and the baseline scan was 11 (IQR 5-21) days and the mean interval between the scans was 5.3 ± 0.7 years. In just a few cases, the degree of stenosis of the artery changed into a higher degree over time (Table 2). Baseline plaque volume was 1100 ± 464 mm^3. The contributions of the different components at baseline were 66.4 ± 7.8%, 28.8 ± 5.4% and 4.8 ± 6.9% for fibrous tissue, lipid and calcifications, respectively (Table 3). Calcifications were present in 132 carotid arteries (68.4%).

Table 2. Stenosis measurements at baseline and at follow-up as assessed according to the NASCET criteria

	Follow-up				
Baseline	**0-29%**	**30-49%**	**50-69%**	**70-99%**	**Total**
0-29%	160	7	0	1	168
30-49%	1	13	4	0	18
50-69%	0	0	5	1	6
70-99%	0	0	0	1	1
Total	161	20	9	3	193

Table 3. Baseline and follow-up measurements of plaque volume and plaque components

	BL (± SD)	**FU (± SD)**	**Mean paired diff (95%-CI)**	**P-value***
Vessel volume (mm^3)	2575.4 ± 993.6	2657.6 ± 1022.5	82.2 (55.9 - 108.5)	<0.001
Lumen volume (mm^3)	1475.2 ± 620.2	1506.5 ± 621.9	31.3 (9.8 - 52.8)	0.005
Plaque volume (mm^3)	1100.2 ± 464.0	1151.1 ± 466.7	50.9 (28.1 - 73.7)	<0.001
Fibrous volume (mm^3)	712.2 ± 260.7	726.4 ± 262.9	14.2 (-2.4 - 30.8)	0.094
Lipid volume (mm^3)	318.3 ± 154.1	309.0 ± 134.1	-9.3 (-21.1 - 2.6)	0.124
Calcium volume(mm^3)	69.7 ± 124.6	115.7 ± 152.9	46.0 (37.6 - 54.4)	<0.001
Fibrous proportion (%)	66.4 ± 7.8	64.9 ± 9.4	-1.5 (-2.3 - -0.7)	<0.001
Lipid proportion (%)	28.8 ± 5.4	27.0 ± 5.9	-1.8 (-2.5 - -1.1)	<0.001
Calcium proportion (%)	4.8 ± 6.9	8.1 ± 8.6	3.3 (2.8 - 3.9)	<0.001

***** As evaluated with a paired *t* test

Atherosclerotic plaque growth

Of 193 arteries, 60 (31%) showed decrease in plaque volume (mean difference between BL and FU -119 mm^3, 95% CI, -115 - -86 mm^3), while in 133 (69%), plaque volume increased (mean difference: 127 mm^3, 95% CI, 109 - 146 mm^3). The mean volumes and plaque component proportions at baseline and follow-up for the entire group are summarized in Table 3. There was a significant overall growth in plaque volume of 51 mm^3 (95% CI, 28-74 mm^3). Lumen and total vessel volume also increased significantly over time. Whereas the absolute values of fibrous tissue and lipid did not change significantly, their relative contribution to the plaque volume decreased (-1.5% and -1.8%, respectively, p<.001). The proportion of calcification within the plaque increased with 3.3% (p<.001).

Annualized changes in plaque measures

Overall, the percentages annual growth of total vessel volume, lumen volume, and plaque volume were 0.63% per year (95% CI, 0.44-0.83), 0.55% per year (95% CI, 0.25-0.85) and 1.16% per year (95% CI, 0.76-1.55), respectively. Absolute annual changes in the proportions of the components were a decrease of 0.26% per year for fibrous tissue (95% CI, -0.41% - -0.10%), a decrease of 0.37% per year for lipid (95% CI, -0.51% - -0.24%) and an increase of 0.64% per year for calcifications (95% CI, 0.54-0.74) (all p≤.001).

Relation between plaque changes and baseline plaque characteristics

Regression analysis showed no significant correlation between percentage annual plaque growth and plaque volume (p=0.196). No correlations were found between percentage annual plaque growth and plaque component proportions at baseline.

The change in calcium proportion was significantly correlated with baseline plaque volume (r_s=0.45, p<.001) and calcium proportion (r_s=0.68, p<.001). Annual increase in calcium proportion was larger in arteries with baseline plaque volume larger than 975 mm^3 (i.e. larger than median of baseline plaque volume, n=96) compared to arteries with a baseline plaque volume smaller than 975 mm^3 (n=97; 0.88% versus 0.39% per year; p<.001). Annual increase in calcium proportion was larger in plaques with a high baseline calcium proportion (>3.9%, median of average calcium proportion on baseline and follow-up, n=96) compared with those with a low baseline calcium proportion (<3.9%, n=97; 1.00% versus 0.26% per year; p<.001).

Reproducibility of the method

The ICC for all volume and proportion measures varied between 0.92 and 1.00 for intra-observer reproducibility, except for lipid proportion and lipid volume, for which the ICC was 0.78 and 0.86, respectively. The ICC for interobserver reproducibility varied between 0.76 and 0.99. The CoVs were good for intraobserver variability, varying between 4.6% and 11.4%, except for lipid volume, for which the CoV was 19.0%. For interobserver variability the CoVs were moderate and varied between 4.5% and 22.0% (Table 4).

Table 4. Intra-observer and inter-observer variability (n=30 carotid arteries) of the semi-automatic segmentation method

	Intraobserver				Interobserver			
	Mean ± SD	Diff obs	ICC	CoV (%)	Mean ± SD	Diff obs	ICC	CoV (%)
Vessel volume (mm^3)	2606 ± 830	50 ± 244	0.96	9.4	2743 ± 848	323 ± 407	0.84	14.9
Lumen volume (mm^3)	1527 ± 535	-5 ± 152	0.96	10.0	1630 ± 567	201 ± 266	0.85	16.3
Plaque volume (mm^3)	1079 ± 350	55 ± 120	0.93	11.1	1112 ± 334	123 ± 164	0.84	14.7
Fibrous volume (mm^3)	649 ± 178	33 ± 64	0.92	9.9	675 ± 185	86 ± 115	0.76	17.0
Lipid volume (mm^3)	316 ± 114	19 ± 60	0.86	19.0	326 ± 110	39 ± 70	0.78	21.5
Calcium volume(mm^3)	114 ± 120	3 ± 7	1.00	6.5	112 ± 115	2 ± 13	0.99	12.0
Fibrous proportion (%)	61 ± 8	0.5 ± 2.8	0.94	4.6	62 ± 8	0.8 ± 2.7	0.94	4.5
Lipid proportion (%)	29 ± 4	0.1 ± 3.0	0.78	10.4	29 ± 5	0.4 ± 3.0	0.80	10.2
Calcium proportion (%)	9 ± 8	-0.6 ± 1.1	0.99	11.4	9 ± 8	1.2 ± 2.0	0.96	22.0

Note: –Diff Obs indicates difference between observers

Discussion

To our knowledge, this is the first in vivo serial CTA study to investigate atherosclerotic carotid plaque progression in humans. This study demonstrates that multidetector CT angiography can be used to quantify atherosclerotic plaque measures in vivo and track changes over time in large study populations.

In 193 carotid arteries, the mean percentage annual growth in plaque volume was 1.2% per year, with a wide range of -5.6% to 10.1% per year. MR imaging has been proven a reproducible method for plaque quantification,[15-18] and several serial MR imaging studies have been performed for tracking changes in carotid atherosclerosis. In a prospective MRI study, Saam et al. described a yearly increase of 2.2% in wall area in >50% stenosed carotid arteries.[19] Boussel et al. found an annual rate of progression of carotid vessel wall volume of 2.3% per year in arteries with >50% stenosis.[20] In these studies, however, the included arteries had a more severe stenosis compared to the arteries included in our study. In another study, a carotid total wall volume increase of 1.2% and 1.8% was found after 16 and 24 months respectively, in patients who were on statin treatment.[15] These results were comparable with ours. MR imaging studies often use different measures (area or eccentric wall volume) for assessing plaque growth, which leads to another difficulty in comparing our results to those from previous serial in vivo studies. Nevertheless, the growth rate of carotid plaque turns out to be remarkably small and highly variable, a comparable finding across studies. Statin treatment has been shown to induce reduced progression or even regression of plaque burden.[19-25] Approximately 90% of all patients in our study were using statins during the follow-up period. Thirty-one percent of our patients showed plaque regression, a number similar to that found in other plaque progression studies in which the majority of patients were on statin therapy.[19-20] Statin treatment might therefore be a reason for the small mean growth in plaque volume.

We did not find any local baseline plaque characteristics significantly associated with plaque volume growth. In contrast, Saam et al. found that a normalized wall index >0.64, as a measure of large plaque burden, was associated with a reduced rate of progression in mean wall area.[19]

In this study we found a change in plaque composition over time: calcification proportion increased, which coincides with a decrease in fibrous and lipid proportion. Larger plaques had a faster increase in calcium proportion compared to smaller plaques and the more calcified plaques showed a significantly larger annual increase in calcium proportion than the less calcified plaques. Calcified carotid plaques are thought to be more stable compared with noncalcified plaques.[26-28] The results of this longitudinal study therefore suggest that when plaques progress, they finally get a more stable profile. This might also be an effect of the secondary preventive medication that is used by the majority of the patients. A few studies reported on the change in composition towards a more stable plaque phenotype in statin-treated patients, by demonstrating a decrease in lipid content[29-30] and a trend towards more calcium.[30] However, these results should be confirmed in larger randomized trials investigating the influence of statin use on plaque composition.

Glagov et al. were the first to report on outward vascular remodeling, showing an increase in total vessel area as an adaptive mechanism to preserve lumen area in response to increasing atherosclerotic disease burden. Luminal narrowing does not occur until the atherosclerotic lesion occupied >40% of the internal elastic lamina area.[1] In our study, in-

cluding only arteries with mild atherosclerotic disease, we also found an overall increase in total vessel volume following an increase in plaque volume. The changes in plaque burden over time appeared to be too small for lumen to become compromised. This might also explain why a change in degree of stenosis was demonstrated in only a minority of vessels.

One of the major challenges in serial imaging studies on atherosclerotic plaques is the reproducibility of quantitative measures. In our study, we used volume measurements, which have been shown to be more precise than area measurements.[17] Several studies have investigated factors influencing measurement errors. Differences in head positioning is one such factor that can cause the 3D position of the carotid bifurcation to change significantly,[31] thereby causing difficulties in matching corresponding axial cross-sectional images across different time points. So far, registration of carotid arteries has been performed manually by using fiducial landmarks, such as the carotid bifurcation point. In this study, we matched the range of interest in the carotid artery semi-automatically using the distance along the central vessel lumen line, accounting for possible variation in carotid position over time. Further improvement would be achieved if analyses could be performed in slices perpendicular to the central line of the artery.

(Semi-) automated measurement of plaque volumes might improve the reproducibility. When we compare our results, using a semiautomated plaque segmentation method, with the inter-observer reproducibility found in a previous study in which plaque segmentation was done manually, interobserver reproducibility improved; CoV for plaque volume was 15% compared to 23-34% found with the manual segmentation method. De Weert et al. demonstrated that their moderate interobserver reproducibility was partly due to the difficulty of defining the exact plaque range; consensus about the segmentation range improved the reproducibility.[7] Therefore, taking a fixed range around the bifurcation to be segmented may further improve interobserver and intraobserver reproducibility of the semiautomated method. Nevertheless, the annual changes in plaque measures are relatively small compared to the current intraobserver variability and, although significant for the whole group, these would not be meaningful for the individual patient.

Our study has some limitations. First, in contrast to MR imaging, MDCTA is not capable of visualizing fibrous cap rupture or intraplaque hemorrhage. Both plaque features are highly associated with plaque rupture and ischemic events.[32-36] Intraplaque hemorrhage has also been associated with an accelerated plaque growth.[35] We have not been able to analyze the influence of this important plaque characteristic on plaque development. On the other hand, HU-based differentiation of plaque components enable serial and automated plaque composition measurements in MDCTA data. Calcifications and changes herein can be accurately measured using MDCTA. Compared with MR imaging datasets, in which multiple sequences are necessary to obtain all plaque information, MDCTA data are more robust and allow minimal observer intervention in the steps towards registration and lumen and vessel wall segmentation. The high availability and the quick procedure are advances of MDCTA that make it a promising tool for serial plaque imaging. With the present CT scanners and precautionary measures, the risks of the potential harmful radiation exposure and intravenous contrast material can be reduced to a minimum.

Secondly, we reported on plaque volume, whereas with MDCTA, we are not able to distinguish atherosclerotic plaque itself from the underlying media. In serial studies this is of minor importance, because the outcome measure of interest -the difference over time- will merely consist of changes in the plaque itself as the range of interest is registered across BL and FU scans.

No evidence exists for a linear pattern of atherosclerotic plaque growth, and the annual change rate is therefore an artificial measure. However, this study corrects for differences in follow up time and it creates a measure that can be compared across groups and studies.

Further, this study is performed in TIA and stroke patients who were all on secondary preventive medication. Given the known effects of statins on plaque progression, our results can not be extrapolated to the asymptomatic population.

Conclusions

The present study shows that MDCTA enables quantification of carotid plaque progression and plaque composition changes in vivo in large patient groups. Carotid atherosclerotic plaque progression seems to be a slow and heterogeneous process. It is unknown yet whether monitoring plaque progression might have a future clinical value in risk prediction. Further research should therefore focus on the associations between temporal plaque changes found with serial imaging and (recurrent) ischemic cerebrovascular events.

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Chapter 4.3

Carotid atherosclerotic plaque development in optimally treated patients; a serial CT angiography study

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Submitted

Abstract

Background and purpose Serial in vivo plaque imaging helps our understanding of atherosclerotic plaque progression. This study investigates the determinants of changes in atherosclerotic carotid plaque volume and composition by means of serial CT angiography (CTA) imaging, in patients who initially presented with TIA or ischemic stroke.

Methods Patients (n=224) underwent serial CTA of the carotid arteries with an interval of 4.7 ± 0.8 years. After the baseline CTA patients were treated with standard preventive medication in accordance with national guidelines. A semi-automated plaque segmentation analysis tool was used to quantify plaque and plaque component volumes. The association of risk factors with plaque growth and change in plaque composition was analysed using multivariable regression analyses.

Results Overall, no significant increase in plaque volume was found, however plaque composition changed: lipid proportion decreased and calcification proportion increased significantly (-3.1 ± 0.4% and 3.1 ± 0.3% respectively, both $p<0.0001$). Lipid volume and to a lesser degree fibrous volume at baseline were associated with less plaque growth (β=-0.625 ± 0.148, $p<0.0001$ and -0.356 ± 0.068, $p<0.0001$). Hypertension was associated with a decrease in lipid proportion (β=-0.08, CI:-0.15 - -0.01, $p=0.02$). Smoking and hypertension were associated with an increase in calcification proportion (β=0.33, CI:0.15-0.49, $p<0.0001$ and β=0.17, CI:0.01-0.33, $p=0.037$).

Conclusion In symptomatic patients, optimally treated with standard secondary preventive medication, carotid atherosclerotic plaque remains stable in volume but plaque composition changes towards a more stable plaque profile over the years following their primary event.

Introduction

Severe atherosclerotic carotid stenosis is associated with an increased risk of recurrent brain infarction. Plaque ulceration and rupture have been linked to the occurrence of ischemic cerebrovascular events. Certain plaque features have been found to increase the risk of atherosclerotic plaque rupture.[1] It is therefore of interest to study the size and composition of carotid plaques as a function of time. Knowledge of atherosclerotic plaque development, assessed by serial plaque imaging, could improve risk prediction and create opportunities to prevent the progression of silent plaques to more vulnerable atherosclerotic plaques.

In a previous serial MDCTA study we examined calcification growth in carotid plaques, using calcification volume as a surrogate marker for the amount of atherosclerotic disease.[2]

MDCTA has also been validated for in vivo atherosclerotic plaque imaging in the carotid arteries, enabling accurate quantification of plaque volume and plaque components like calcifications, lipid and fibrous tissues.[3] In a pilot study we showed that serial MDCTA enables monitoring of changes in carotid atherosclerotic plaques.[4] It therefore provides a minimally invasive tool to investigate carotid plaque progression in humans. Moreover, it creates the possibility to study the relative contribution of plaque components. In carotid arteries, a high relative calcification content has been associated with plaque stabilization.[5-8]

In this prospective serial CT angiography study of the carotid arteries we describe the changes in carotid plaque volume and composition and its determinants in a group of 224 patients who presented with TIA or ischemic stroke.

Methods

Study population

Patients were recruited from a cohort of 914 consecutive patients with TIA or ischemic stroke, who had undergone a standard clinical work-up, including MDCT angiography of the carotid arteries. All patients (n=649) with atherosclerotic plaque (i.e. thickening and/or calcification of the vessel wall) in one or both carotid arteries were invited to participate in this serial imaging study. Exclusion criteria were: no atherosclerotic plaque(s) present at the level of the carotid bifurcation, bilateral occlusion and/or treatment of the carotid arteries, renal insufficiency or hyperthyroidism and bad image quality of the baseline scan. Blood samples to determine renal function were taken if necessary. The Institutional Review Board of Erasmus MC approved this study. All patients gave written informed consent.

Clinical data

As a part of routine clinical work-up all patients filled out a detailed health questionnaire, had a physical examination, laboratory assessments, a CT scan of the brain and MDCT angiography of the carotid arteries at baseline. Information was collected on classical cardiovascular risk factors, history of cardiovascular disease and medication use. During the

follow-up period, patients were treated with standard secondary preventive medication where necessary, in accordance with national guidelines (antithrombotic or anticoagulant agents, cholesterol lowering drugs, antihypertensive medication and antidiabetic medication).

Diabetes was defined as fasting serum glucose levels over 7.9 mmol/L, non-fasting serum glucose levels over 11.0 mmol/L, or use of anti-diabetic medication. Hypertension was defined as a mean systolic blood pressure over 140 mmHg and/or a mean diastolic blood pressure over 90 mmHg during 2 episodes of at least 15 minutes of continuous non-invasive blood pressure measurement, or on treatment with anti-hypertensive medication before admission. Hypercholesterolemia was defined as fasting cholesterol over 5.0 mmol/L or being on treatment with cholesterol lowering drugs. Subjects were categorized as currently smoking versus non- or ever smoking. During the visit for the follow-up MDCTA scan, a detailed health questionnaire was taken.

MDCT angiography protocol

Baseline scans were performed on a 16- or 64-slice MDCT scanner (Sensation 16/64, Siemens Medical Solutions, Erlangen, Germany) with a standardized optimized contrast-enhanced protocol (collimation 16 x 0.75 mm (n=106) or 2 x 32 x 0.6 mm (n=3), pitch 1) and a scan range ranging from the ascending aorta to the intracranial arteries. The follow-up scans were performed on a 128-slice MDCT scanner (Somatom Definition, Siemens Medical Solutions) with a comparable protocol (collimation 2 x 64 x 0.6 mm, pitch 0.7) and a scan range of 6 cm around the carotid bifurcation. Eighty mL of contrast material was used, with a saline bolus chaser of 40 mL and real-time bolus tracking in the ascending aorta, using a threshold of 120 HU.

Image reconstructions for all scans were made with a FOV of 120 mm, matrix size 512 x 512, slice thickness of 1.0 mm, increment of 0.6 mm and with both a smooth (B30/B31) and an intermediate (B46) filter kernel, of which the last one has been demonstrated to provide the best soft tissue contrast.

Plaque analysis

The carotid arteries were analyzed over a range of 3 cm around the carotid bifurcation, by manually locating the carotid artery bifurcation (first slice where the lumens of the internal and the external carotid artery could be assigned as separate) and analyzing 25 slices above and under this bifurcation point or a range as long as possible given the scan range at follow-up. Lumen, vessel wall and vessel wall components volumes (further defined as plaque and plaque components, respectively) were derived using a custom-made semi-automated plaque analysis method.[9, 10] Following lumen segmentation, the outer vessel wall was segmented based on a method using a Gentle Boost classifier, which had been trained by manually annotated data. The lumen segmentations and outer vessel wall segmentations were checked and were manually adjusted deemed necessary. The plaque was derived by subtracting the lumen segmentation from the outer vessel wall segmentation.

After segmenting the carotid artery outer vessel wall and lumen, plaque components were classified based on Hounsfield Unit thresholds. The threshold to distinguish be-

tween calcifications and fibrous tissue was set at 130 HU, as used for calcium scoring. The threshold used to differentiate lipid from fibrous tissue was 60 HU. This threshold was previously validated with histology.[3] The volumes of the plaque and plaque components were calculated as the product of the number of pixels in the segmented areas, the pixel size and the slice increment. The proportion of plaque components was calculated as the ratio of the volume of the component to the total plaque volume, multiplied by 100.

Data-analysis

Plaque volume and plaque component volumes and proportions were measured on both sides in each individual at baseline and follow-up. Continuous variables are reported as mean values and standard deviations.

Plaque and plaque components are presented as absolute volumes (in mm^3) on baseline and follow-up. Plaque components are also expressed as proportions of total plaque volume. Because we have a measurement on both sides for most patients we use Generalized Estimating Equations (GEE) to account for the within patient correlation. A compound symmetry working correlation structure is used.

The relations between the outcomes growth of plaque volume (value at follow-up minus value at baseline) and plaque composition (logit of fraction of the component in the total plaque volume; log[P/(100-P)]) and covariates age, gender, scan delay, hypertension, hypercholesterolemia, diabetes mellitus, smoking, and baseline plaque characteristics, were analyzed using multivariable regression analysis. A second model tested the influence of the baseline plaque components on plaque volume growth.

Since not all scans had a scan range of exactly 25 slices above and below the carotid bifurcation (n=26), all analyses were repeated excluding the arteries without a scan range of 50 slices.

All calculations were made using SAS 9.3. Results are considered to be statistically significant when $p<0.05$.

Results

Study subjects

Two-hundred-and-twenty-four patients underwent serial MDCT angiography of the carotid arteries. In total, 67 carotid arteries were excluded from the analysis for the following reasons: occlusion either at baseline and/or follow up (n=14); treatment with carotid endarterectomy or stent placement before a follow-up scan was made (n=20); poor image quality at the carotid bifurcation (low lumen contrast, streak artifacts, perivenous artifacts or swallowing artifacts) (n=29) or technical problems with plaque segmentation based on image data properties (n=4). In total, 210 patients were included, 39 with only one carotid artery that could be assessed. Table 1 provides demographic information and baseline characteristics of the study patients. The vast majority of patients used secondary preventive medication from baseline and during the follow-up period; for example:

93.3% of patients used statins or other lipid lowering drugs, of whom 84.8% continued to use it until the time of the second scan, which is supposed to be a good compliance.

Table 1. Patient characteristics at baseline (BL) and medication use at baseline and follow-up (FU) (N=210)

Baseline patient characteristics	**Value**	
Sex (male, %)	136 (65%)	
Age (years ± SD)	61 ± 9.3	
FU time (years ± SD)	4.7 ± 0.8	
Risk factors (%):		
Hypercholesterolemia	167 (80%)	
Hypertension	147 (70%)	
Diabetes mellitus	25 (12%)	
Smoking (current)	86 (41%)	
Medication use (%):	***From BL on:***	***FU:***
Anticoagulant agents:		
Cumarin	18 (8.6%)	37 (17.6)%
Heparin	3 (1.4%)	1 (0.5 %)
Antiplatelet agents:		
Acetylsalicyl Acid	150 (71.4%)	180 (85.7%)
Acetylsalicyl Acid & Persantin	30 (14.3%)	39 (18.6%)
Persantin	0	2 (1.0%)
Clopidogrel	4 (1.9%)	3 (1.4%)
Clopidogrel & Acetylsalicyl Acid	9 (4.3%)	3 (1.4%)
Lipid-lowering drugs:	196 (93.3%)	178 (84.8%)
Statins	192 (91.4%)	170 (81.0%)
Others	2 (1.0%)	5 (2.4%)
Statin + other	2 (1.0%)	3 (1.4%)
Anti-hypertensives	151 (71.9%)	158 (75.2%)
Anti-diabetics	24 (11.4%)	34 (16.2%)

Plaque progression and changes in plaque composition

A statistically significant increase in mean plaque volume was not observed (Table 2; mean change 16.8 ± 9.4 mm^3, p=0.075). The change in plaque volume varied from – 998 to +804 mm^3. Smaller plaques (< median, 944 mm^3) showed an increase of 59.9 ± 106.4 mm^3, and larger plaques (> median, 947 mm^3) a decrease of 26.7 ± 175.1 mm^3. There was a significant decrease in lipid volume (mean -27.6 ± 5.1 mm^3, p<0.0001) and proportion, and a significant increase in calcification volume (mean 34.8 ± 2.9 mm^3, p<0.0001) and proportion.

Cardiovascular risk factors and plaque changes

Age was significantly associated with plaque volume increase (p<0.0001) (Table 3). The larger the baseline plaque volume, the smaller the plaque growth (β=-0.35 ± 0.06, p>0.0001). When we substituted baseline plaque volume by the baseline volumes of the different components in the model for plaque growth, lipid volume and to a lesser degree fibrous volume were associated with a lower plaque growth (β=-0.625 ± 0.148, p<0.0001 and -0.356 ± 0.068, p<0.0001), whereas calcification volume was not significantly associated

Table 2. Baseline (BL) and follow-up (FU) measurements of plaque volume and plaque components (n=381)

	BL (± SD)	FU (± SD)	Δ (FU-BL±SD)	P-value*
Plaque volume (mm^3)	965.9 ± 220.8	982.6 ± 207.2	16.7 ± 9.4	0.07
Fibrous volume (mm^3)	619.0 ± 132.9	628.5 ± 135.9	9.5 ± 7.3	0.19
Lipid volume (mm^3)	292.3 ± 88.2	264.7 ± 70.2	-27.6 ± 5.1	<0.0001
Calcium volume (mm^3)	54.7 ± 100.6	89.5 ± 129.3	34.8 ± 2.9	<0.0001
Fibrous proportion (%)	64.7 ± 8.2	64.6 ± 9.6	-0.01 ± 0.4	0.83
Lipid proportion (%)	30.4 ± 5.7	27.3 ± 6.2	-3.1 ± 0.4	<0.0001
Calcium proportion (%)	4.9 ± 7.7	8.0 ± 10.1	3.1 ± 0.3	<0.0001

*Derived from a GEE model that adjusts for clustered data within patients

Table 3. Determinants of plaque growth (value at FU minus value at BL)

	β	95% CI	p-value
Baseline plaquevolume	-0.35	-0.47 - -0.23	<0.0001
Gender	-26.08	-61.37 - 9.21	0.15
Age	0.31	0.10 - 0.51	0.003
Time interval	-0.39	-1.94 - 1.17	0.63
Hypercholesterolemia	2.30	-37.37 - 41.97	0.91
Hypertension	-38.24	-77.98 - 1.50	0.059
Diabetes Mellitus	26.93	-18.43 - 72.29	0.24
Current smoking	38.76	-0.59 - 78.10	0.054

Table 4a. Determinants of plaque composition: lipid proportion (FU minus BL)

	β	95% CI	p-value
BL plaquevolume	-0.0004	-0.0005 - -0.0002	<0.0001
BL lipid proportion	0.57	0.43 - 0.71	<0.0001
Gender	-0.02	-0.09 - 0.06	0.68
Age	0.0001	-0.0002 - 0.0004	0.52
Time interval	0.006	0.003 - 0.009	<0.0001
Hypercholesterolemia	-0.03	-0.13 - 0.07	0.53
Hypertension	-0.08	-0.15 - -0.01	0.02
Diabetes Mellitus	-0.05	-0.15 - 0.06	0.39
Current smoking	0.025	-0.05 - 0.10	0.51

Table 4b. Determinants of plaque composition: fibrous proportion (FU minus BL)

	β	95% CI	p-value
BL plaquevolume	0.0001	-0.0001 - 0.0002	0.52
BL fibrous proportion	0.85	0.72 - 0.98	<0.0001
Gender	0.002	-0.07 - 0.08	0.96
Age	-0.0003	-0.0006 - -0.000	0.047
Time interval	-0.009	-0.012 - -0.005	<0.0001
Hypercholesterolemia	-0.01	-0.11 - 0.09	0.84
Hypertension	-0.001	-0.08 - 0.07	0.97
Diabetes Mellitus	-0.04	-0.13 - 0.06	0.48
Current smoking	-0.12	-0.20- -0.04	0.002

Table 4c. Determinant of plaque composition: calcification proportion (FU minus BL)

	β	95% CI	p-value
BL plaquevolume	0.0002	-0.0002 - 0.0005	0.29
BL calcification proportion	0.87	0.82 - 0.91	<0.0001
Gender	0.11	-0.05 - 0.28	0.18
Age	0.0005	-0.0003 - 0.0013	0.24
Time interval	0.012	0.005 - 0.019	0.0004
Hypercholesterolemia	0.09	-0.08 - 0.27	0.30
Hypertension	0.33	0.17 - 0.49	<0.0001
Diabetes Mellitus	0.09	-0.12 - 0.30	0.40
Current smoking	0.17	0.01 - 0.33	0.037

with plaque growth (β= 0.025 ± 0.092, p=0.782). Smoking and hypertension showed a trend towards respectively an increase and a decrease in plaque growth (β=38.8, p=0.054 and β=-38.2, p=0.059, respectively).

Table 4a-c show the association between risk factors and the changes in different plaque components. A larger baseline lipid proportion was associated with an increase in lipid proportion, whereas a larger baseline plaque volume and hypertension were associated with a decrease in lipid proportion (Table 4a). A larger baseline fibrous proportion, younger age, and no current smoking were associated with an increase in fibrous proportion (Table 4b). A larger baseline calcification proportion as well as hypertension and smoking were significantly associated with an increase in calcification proportion (Table 4c).

Repeating above analyses after excluding arteries not meeting the criteria of 25 slices above and below the bifurcation point yielded similar results.

Discussion

Except for a pilot study, this is the first serial imaging study with CT angiography on carotid atherosclerotic plaque using HU-based plaque composition analysis. After a mean follow-up time of 4.7 years, we observed a non-significant increase in plaque volume. Nevertheless, we found a significant change in the composition of the plaques, which was associated with age and the cardiovascular risk factors hypertension and smoking.

Plaque growth

Plaque progression is a slow and heterogeneous process. In contrast to our previous pilot study[4] we did not observe significant plaque growth and we found volume change to be variable. In the current study we restricted the range of analysis to a region of 3 cm centered around the bifurcation in order to capture the changes in the region with atherosclerotic plaque. However, the small percentage plaque growth over years is comparable to outcomes of prospective MR imaging studies of the carotid plaque.[11-13]

One explanation for the small mean growth in plaque volume in this study is probably the fact that included patients were on standard secondary preventive medication. Statin treatment has been shown to reduce plaque growth in multiple MRI studies. Saam et al demonstrated statin therapy to significantly reduce the rate of progression of mean wall area after 18 months.[13] Zhao et al showed a significant decrease in percentage wall volume after three years of statin therapy, an effect seen especially in lipid-rich-necrotic-core (LRNC)-containing slices.[14] In a randomized trial in which patients received either low or high dose rosuvastatin, both groups did not show an increase in overall plaque burden after 24 months.[15] Other serial MRI studies also found statin therapy to slow down or halt lesion progression.[12, 16, 17]

It is assumed that plaque growth is not a gradual process, but comes in spurts, which are thought to be triggered by external or internal stimulants, like intraplaque hemorrhage.[18] In our cohort, we might be looking at a group of indolent carotid plaques, probably caused by plaque stabilization that has occurred since the event.

Determinants of plaque growth

This study shows age to be significantly associated with plaque growth. The larger the baseline plaque volume, the lesser its growth, which is partly explained by regression to the mean. Hypertension and smoking showed a trend towards plaque regression and plaque progression respectively.

There only exist a few serial studies on associations between cardiovascular risk factors and change in plaque burden. One long-term follow-up study showed most general cardiovascular risk factors to be associated with total wall volume.[19] However, only one short-term serial imaging study explicitly describes not to have found any associations between general risk factors and change in plaque burden.[13]

All other serial MRI studies focus on plaque characteristics as determinants of growth, instead of general risk factors for atherosclerosis. Especially intraplaque hemorrhage is found to be an important determinant for plaque progression and LRNC volume increase.[17, 18, 20] Xu et al found lipid rich necrotic core to be the most important determinant of plaque burden progression.[21]

In a subanalysis including plaque components in the model for plaque growth, especially lipid proportion, and to a lesser degree fibrous proportion, were associated with a decrease in plaque growth. This effect might be ascribed to the effects of lipid-lowering therapy, which have shown to halt plaque progression, especially by decreasing lipid-rich necrotic cores.[13, 14]

Plaque composition

Besides change in plaque burden, a change in plaque composition is studied in serial plaque imaging studies. In the current study we found a significant decrease in lipid proportion and a significant increase in calcification proportion, with fibrous proportion being stable.

Lipid content is assumed to play an important role in plaque vulnerability. In MRI studies, the LRNC is one of most investigated plaque features. In a subgroup of a multicenter clinical trial, on standard therapy (with 66% of patients on lipid-lowering therapy), LRNC proportion decreased significantly over 6 months in 16-79% stenosed carotid arteries[22]. Another study in asymptomatic patients with 16-49% stenosis showed a significant increase in LRNC volume, but a decrease in LRNC volume in individuals on statin therapy at 18 months follow-up.[17] Statins have been shown to reduce LRNC volume and relative LRNC content.[14, 15]

Calcification content has not been the main focus of study in serial MRI; in cases its development is described, it remained stable.[14, 15, 17] In studies on coronary artery atherosclerosis, in which calcification burden is used as a surrogate marker for atherosclerosis burden, mean calcium volumes are generally found to increase over time.[23, 24] In our previous serial CT study we also observed an increased absolute calcification volume in carotid arteries using a calcification scoring method.[2] The current study however adds information by showing the increase in calcified proportion of the plaque.

Though the amount of carotid calcification has been associated with increased stroke risk in some studies,[25, 26] other studies suggest that a relatively high calcification content

of carotid plaques is associated with plaque stabilization.[5-8] LRNC volume and its increase over time are associated with new plaque surface rupture[1, 21] and LRNC proportion was associated with occurrence of cerebrovascular events.[27] Our findings of increased calcification proportion and decreased lipid proportion therefore suggest plaque stabilization in the carotid plaques of these patients, who were followed over a relative long period after their ischemic cerebrovascular event.

These findings correspond with histological studies, which also show plaque stabilization after events.[28-30]

Determinants of plaque composition

For the composition of the plaque it holds that every component grows more when its baseline value is higher.

Baseline plaque volume is associated with a decrease in lipid content, and moreover, with a reduced plaque growth. This is in correspondence to MRI studies that showed a correlation between change in plaque burden and change in LRNC proportion,[17, 22] although we did not directly correlate both change in plaque volume with change in lipid proportion.

Time interval between the baseline and follow-up scan was significantly associated with a larger change in plaque composition; an increased lipid and calcification proportion and a decreased fibrous proportion. Apparently, time is a factor in plaque composition, not plaque growth.

Hypertension significantly decreased lipid proportion and increased calcification proportion. Hypertension was also near-significantly negatively associated with plaque volume change, pathologically probably via a decrease in lipid. Although a previous serial MRI study described a correlation between hypertension and total vessel wall volume, no association with plaque composition was found.[19] In serial CT studies of coronary artery calcifications (CAC), hypertension has been associated with incidence of calcification and increase of CAC volume.[31-33] Previously, we showed that hypertension is a determinant for occurrence of calcification and increase of calcification volume in carotid arteries.[2]

Smoking was also a significant determinant of calcification proportion. We previously found this association with calcification volume,[2] a relation not described in serial CAC studies.

Limitations and future perspectives

This study was performed in a symptomatic patient cohort. It is important to be able to predict plaque development and risk for cerebrovascular events, especially in these patients prone to recurrent events. However, the study design also introduced some difficulties in the interpretation of the results. Patients were on preventive medications for various time periods either before and/or during the study period. Therefore the ominous effects of risk factors on plaque development and the possible effects of the medication becomes mixed, and it is impossible to unravel these different influences. A controlled study would be necessary to investigate these effects separately. Further, this study had to exclude those arteries that received a carotid endarterectomy or stent placement be-

tween the baseline and follow-up scan. Unfortunately, those vessels might have had the most significant plaque changes and our results might therefore be an underestimation of the real plaque growth. However, the effect would be small, since the number of treated arteries was only 20.

Conclusions

This is the largest-ever serial CT angiography study investigating carotid plaque development in symptomatic patients using secondary preventive medication. Plaque volume does not increase significantly over a mean time period of 4.7 years. Plaque composition shows a significant change towards a more stable plaque profile with a decreased lipid content and increased calcification content. Evidence from the literature subscribes this development of indolent plaques over a long period in patients who experienced an ischemic cerebrovascular event and are on standard secondary preventive therapies. Especially lipid lowering medication seems to have a large effect on the changes on plaque level in this patient group.

Although hypertension and/or its treatment as well as smoking are significant determinants for this change in plaque composition, its pathophysiology remains unclear.

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Chapter 5

General discussion

This thesis focused on the role of multidetector CT angiography (MDCTA) in providing carotid atherosclerotic plaque characteristics that can be used as imaging biomarkers for atherosclerotic disease burden and plaque vulnerability in vivo. The final aim is to contribute to the knowledge of the pathophysiology of atherosclerotic plaque development and its change into a clinical significant lesion, to finally enable interfere with this process.

This thesis had three main objectives:

I. To accurately obtain (semi-)automatically derived quantitative imaging biomarkers of carotid atherosclerotic plaque burden and composition with CTA.

II. To investigate the role of atherosclerotic plaque ulceration as an imaging biomarker for plaque vulnerability.

III. To study carotid atherosclerotic plaque development and its determinants using quantitative plaque imaging in a serial CTA study.

In this part of the thesis, I will describe the main findings of our studies and their interpretation, thereby discussing some methodological considerations and deliberating on the future use of CT derived atherosclerotic imaging biomarkers.

I. Quantitative imaging biomarkers of carotid atherosclerosis using CTA

Quantitative imaging of biomarkers requires validated, highly standardized and reproducible methods, and correlation of the imaging biomarkers to biological effects and clinical endpoints.[1]

Technical aspects

Technical aspects, as contrast density, kVp setting, window-level setting and convolution kernel, highly influence quantitative measurements on MDCTA scans. Our research group previously optimized the MDCTA scan parameters and contrast injection protocol in order to reduce artefacts, optimally distinguish lumen from arterial wall/atherosclerotic plaque and differentiate calcifications and the non-calcified components of the plaque.[2-4] In ***Chapter 2.1*** I reflect on all these issues concerning the use of MDCTA-based imaging biomarkers for atherosclerotic disease in the carotid artery. Based on these issues an optimized, standardized scan protocol was developed for comparable CT scanners with similar post-processing techniques, which is a principal requirement for the implementation of quantitative imaging biomarkers.

Validation and reproducibility of measures of plaque burden and composition

In **Chapter 2.1** we reviewed on previous work that investigated CTA derived biomarkers of atherosclerotic disease in the carotid arteries.

The assessment of different plaque components with CTA relies upon their differences in linear attenuation coefficient, expressed as Hounsfield Units (HU). This differentiation highly depends on CT scan parameters. Reconstruction algorithms and kVp have been optimized in a previous study, that showed that an intermediate reconstruction kernel allows optimal plaque interpretation.[4]

Using this optimized scan acquisition protocol ex vivo and in vivo validation studies were performed with carotid endarterectomy (CEA) specimens as histological standard. From these studies, 60 HU was derived as the optimal cut-off point for differentiation between lipid and fibrous tissue within the plaque,[4] with a sensitivity and specificity of 100%.[5] Calcifications are detected on CT images as high density structures, with HU>130, equivalent to coronary calcium scoring in electron beam CT.

The next step was the validation of the quantitative measurements of the different plaque components derived using the optimal threshold values found. Area measurements between ex vivo MDCTA images and histology correlated well for total plaque area, calcified areas and lipid areas.[4] For the in vivo validation study manually annotated outer vessel wall segmentations were needed. MDCTA overestimates area measurements of calcifications, however the correlation with histology was good. Correlation between MDCTA and histology for fibrous area measurements was good, but poor for lipid, which is probably due to blooming artefacts from calcification, since this correlation improved in mildly calcified plaques and non-calcified plaques.[5]

Interobserver variability of area measurements was moderate for calcifications and fibrous tissue, but poor for lipid, whereas intraobserver variabilities were good.[5] The poor observer variability of lipid area is possibly due to inclusion of peri-articular fat in segmentation of the outer vessel wall contours. Quantitative measurements could be improved by volumetric measurements of plaque components. In vivo manual assessment of atherosclerotic plaque and plaque component volumes in carotid arteries with MDCTA is feasible with a moderate interobserver reproducibility and a moderate to good intraobserver reproducibility.[6]

Automated quantitative plaque measurements

A further improvement in the reproducible assessment of plaque volume and plaque volume components could be expected from semi-automated or automated analysis. Also, as manual annotation of vessel lumen and outer wall boundaries is very labour-intensive, (semi-)automated plaque segmentation is required to be able to perform larger and longitudinal studies. In order to automate plaque quantification, a post-processing algorithm to automatically segment the lumen and outer vessel wall, was developed, enabling the assessment of wall (plaque) volume. This semiautomatic plaque segmentation algorithm has been trained and tested on manually annotated MDCTA data.[7] Considering the scarce availability of CEA specimens, automated segmentations have not been validated against histology, but were compared to manually annotated segmentations, which have been histologically validated, as described above.[8]

In the study described in ***Chapter 2.2*** we investigated the performance of this semi-automated plaque segmentation method in quantifying plaque volume and plaque component volumes by comparing measurement error of the automated method using manual contours as a reference standard with the interobserver variability in manual annotations. In this study, manual segmentation comprised manual drawing of the outer vessel boundary, whereas the boundaries between lumen and plaque were automatically calculated and were only corrected manually in cases were calcifications bordered the lumen. We found the differences between the automated method and the manual observers to be comparable to the interobserver differences. It is good to realize that the performance of an automated tool cannot be better than that of the manual method on which it is trained. A certain difference will always exist between manual and automated segmentation and one should therefore be careful to directly compare results from studies using manual segmentations with those using automated segmentation. The performance of automated segmentation is highly influenced by the training set and agreement could be optimized by training the algorithm with the study population on which it will be applied. Nevertheless, an automated tool will always be more robust and reproducible than a human being, since its results are based on statistics. We found this semi-automated method to have good intraobserver and interobserver reproducibility for plaque volume and plaque composition measurements, except for lipid, for which reproducibility was moderate. In quantitative plaque analysis using MRI, measurement errors for plaque burden measures are excellent, the most reliable measure being percentage wall volume (CV 3%). Measures of plaque composition in MRI studies have also been less reproducible, for example CV of 11.1 and 31.7 % for LRNC volume.[9]

One could argue whether to investigate the performance of a semi-automated segmentation method for assessing temporal changes in plaque volume and plaque composition. Good agreement with manual segmentation on baseline does not necessarily translate

into a good agreement in assessing changes during follow-up, as was suggested in a serial MRI plaque component quantification study.[10] They argued that the (blinded) visual side-by-side comparison, done by human reviewers to eliminate artefacts and discern real changes, could improve plaque delineation as information from both scans is used. This was not taken into account by the computer algorithm, which could have resulted in a lower agreement between the manual and automated results in assessing temporal changes. When the segmentation on baseline and follow-up scans are performed completely independent of each other by an semi-automated algorithm, variation in temporal changes not representing true plaque changes, are merely due to inter-scan variability, an issue discussed in the third part 'Serial carotid plaque imaging and determinants of atherosclerotic plaque changes'.

Apart from plaque analysis based on plaque segmentation, we also performed a serial study on the development of calcifications in the carotid arteries, using calcification as a surrogate marker of atherosclerosis, equivalent to calcium scoring in coronary arteries. Calcification measurements can be automatically performed with commercially available software packages. Calcium volume scoring has a low rescan variability and a high intra- and interobserver repeatability.[11, 12] When using 600 HU as a threshold, we also found very good intra- and interobserver reproducibility for the assessment of calcification volumes in the carotid arteries on MDCTA.

Semi-automated quantitative plaque imaging for serial studies

In conclusion, quantitative measurements of carotid plaque burden and plaque components assessed with MDCTA are validated against histology and can be derived in a reproducible way. Though not perfect, we thought the segmentation results from our automated method to be a good starting point for additional manual editing if necessary. We used this strategy in the serial studies investigating temporal changes in plaque and plaque composition in a larger patient cohort **(Chapters 4.2 and 4.3)**.

II. Carotid atherosclerotic plaque ulceration

Atherosclerotic carotid plaque ulceration is considered to be a marker of previous plaque rupture.[13, 14] Carotid atherosclerotic plaque ulceration has primarily been described on angiography and is defined as extension of contrast material beyond the vascular lumen into the surrounding plaque.[13] MDCTA has been found to be effective in plaque ulceration detection, with a high sensitivity and specificity,[15] and is able to identify and classify ulcerations with a good interobserver agreement.[16] The work in this thesis focussed on the potential of MDCTA assessed plaque ulceration as a (qualitative) imaging biomarker of plaque rupture in clinical studies.

Plaque ulceration and non-lacunar ischemic stroke

Large deep and non-lacunar ischemic strokes are thought to be caused by thrombo-embolism from extracranial arteries or the heart.[17] Atherosclerotic carotid plaque rupture might be the trigger for thrombus formation on the plaque surface and embolization of

plaque material and thrombus into the intracranial circulation. We tested the hypothesis that if non-lacunar stroke is associated with thromboembolism, it may also be associated with atherosclerotic plaque ulceration in the symptomatic arteries, since plaque ulcerations have been associated with plaque rupture ***(Chapter 3.1)***. Patients with ischemic cerebrovascular events were classified according to presumed stroke etiology. Plaque ulceration in the symptomatic artery was indeed associated with clinically defined non-lacunar stroke, independent of degree of stenosis. This relation was confirmed by an independent association between the presence of atherosclerotic carotid plaque ulceration and non-lacunar infarction on MDCT of the brain. These results support the underlying mechanism of plaque rupture and subsequent thrombo-embolism to the brain. It further subscribes different pathophysiologic mechanisms between lacunar and non-lacunar stroke and the importance of etiologic stroke subtype assessment in stroke patients in studies evaluating the relation between atherosclerotic carotid disease and clinical events or brain tissue damage. Further, it suggests that plaque ulceration can be an important imaging biomarker for plaque rupture in future diagnostic and therapeutic studies of patients with atherosclerotic carotid disease. However, as this was a cross-sectional study, additional longitudinal studies should assess whether plaque ulcerations increase the risk of (recurrent) stroke and whether CEA will reduce this risk.

Serial imaging of plaque ulcerations

As ulcerations are found in symptomatic carotid arteries as well as in asymptomatic carotid plaques, questions are raised as whether plaque ulcerations are indeed the consequence of plaque rupture, whether ulcerations must always be associated with a recent ischemic event and whether they might form a cause of future thrombo-embolization. In general, little is known about the natural history of plaque ulcerations in carotid atherosclerotic plaques. Therefore, I performed a study in which plaque surface morphology was investigated on serial CTA of TIA and stroke patients ***(Chapter 3.2)***. Plaque surface morphology was classified as smooth, irregular or ulcerated on both baseline and follow-up MDCTA. Progression (i.e. development of irregularities or ulceration) and regression (i.e. disappearance of irregularities or ulceration) in morphology were evaluated. At follow-up, plaque morphology remained unchanged in most ulcerated plaques. These results show that carotid ulcerations persist for a long time, and that finding an ulcerated plaque might therefore not have a direct clinical meaning, i.e. does not necessarily correlate to a recent plaque rupture. Theoretically, ulcerations may, due to disturbance of blood flow, remain a potential source of thromboembolism.[18, 19] Its implication for (recurrent) cerebrovascular disease therefore remains elusive.

Plaque composition and ulcerations

According to the vulnerable plaque theory, specific plaque features, like large lipid-rich necrotic cores, predispose an atherosclerotic plaque to rupture.[20, 21] In order to study the association between plaque burden and plaque composition and plaque ulceration on CT, we evaluated the symptomatic arteries of ischemic stroke patients ***(Chapter 3.3)***. Plaque volume and the proportions of LRNC, fibrous tissue and calcifications were measured semi-automatically based on HU-cut off values, after manual annotation of the plaque. Plaque ulcerations were also present in plaques in arteries with a low degree of stenosis. We found plaque volume to be significantly larger in ulcerated plaques. In addition, LRNC proportion was strongly associated with plaque ulceration, whereas calcification

proportion was inversely associated with plaque ulceration. These associations remained significant in patients with a low degree stenosis. These results indicate that plaque volume and composition assessed with MDCTA may identify rupture prone plaques, therewith possibly improving risk stratification in ischemic stroke patients. Comparable results are found in cross-sectional MRI studies, that showed that intraplaque haemorrhage and large LRNC are associated with thin and ruptured fibrous caps.[21, 22]

Plaque ulcerations assessed with CTA

In summary, we showed that ulcerated atherosclerotic plaque in the carotid artery is associated with a thrombo-embolic clinical endpoint, i.e. ischemic non-lacunar stroke, as compared to lacunar stroke. It furthermore correlates with plaque volume and plaque composition, indepent of degree of stenosis. Plaque ulceration assessed with MDCTA could therefore be useful as a marker of plaque rupture, and plaque burden and plaque composition could be used to identify plaques prone to rupture. These studies, however, had a cross-sectional design and the findings should be confirmed in large longitudinal studies. Our serial imaging study showed that the clinical importance of finding an ulcerated plaque remains elusive. This study comprised a small cohort, which, in combination with the relative low incidence of ulcerations and the study design, hindered us to investigate the association between plaque ulceration and recurrent ischemic cerebrovascular events (i.e. non-lacunar stroke). Large clinical trials should be performed to answer the question whether plaque ulceration as an MDCTA biomarker of advanced carotid atherosclerotic plaque can improve risk prediction and the decision model for surgical intervention.

MRI is capable of imaging smaller, disrupted fibrous caps and also thin fibrous cap.[23, 24] The ulcerations as seen on CTA are thought to represent larger ruptures, in which part of the LRNC has been embolized into the intracranial circulation. CTA therefore probably underscores the entity of plaque rupture and MRI has the advantage to identify the plaques with a thin fibrous cap, with the accepted view that these plaques will progress to rupture, therewith enabling prevention of thromboembolism. However, CT angiography nowadays is – or can be- part of clinical work-up of acute stroke patients in many institutions. Designating plaque ulceration on CTA as a risk factor for recurrent stroke would therefore provide an easily applicable adjustment of current treatment decision protocols.

III. Serial carotid plaque imaging and determinants of atherosclerotic plaque changes

Knowledge of atherosclerotic plaque development has primarily been derived from animal and histopathological studies. Risk factors for plaque development[25, 26] and associations between vulnerable plaque characteristics and clinical endpoints have been suggested from cross-sectional studies.[27-29] Last decades, temporal changes in atherosclerosis in human beings has been subject of prospective longitudinal studies which are supposed to provide the definite prove of evidence. Quantitative measures of atherosclerotic carotid plaque generated by non-invasive imaging techniques are important tools in serial in vivo imaging studies and can help us in understanding atherosclerotic disease mechanisms, therewith improving (recurrent) risk prediction and monitoring response to pharmaceutical interventions.

Serial imaging – a real challenge

In addition to the technical challenges in quantitative imaging, certain issues in serial quantitative imaging require attention, since they influence inter-observer variability as well as inter-scan variability. Ongoing technical developments during the course of a serial study can either create complications or opportunities.

Over time new scanners were introduced, which use different image reconstruction techniques. In our studies we adjusted the scan protocols in order to enable a secure comparison of follow-up and baseline scans. Additionally, we performed a phantom study using the point spread function test to assess differences between different reconstruction kernels on CT scanners used. Due to radiation exposure it was not possible to scan patients twice on the same or on different scanners within a short time frame in order to perform inter-scan variability studies.

Further, there are potential variations that are more difficult to correct for. Variation in imaging quality can occur due to motion artefacts or differences in contrast availability. Also possible variations in the geometry of the carotid arteries, caused by patient reposition, hamper the one-by-one comparison. Optimization and standardization of the acquisition protocol is important, nevertheless, a certain in-patient variability will always be present.

In order to reduce the impact of possible changed vessel geometry, there is the need to register the images of serial CT scans. In our pilot study **(Chapter 4.2)** in carotid arteries containing atherosclerotic plaque at baseline, we used a custom-made semi-automated registration tool. With this tool, lumen segmentations were generated by a level-set-based method after initialization with three seed points in de common carotid, internal carotid and external carotid artery, followed by extraction of a center lumen line. Three new observer-defined initialization points on the follow-up scan defined the craniocaudal plaque range to be analysed. Using the carotid bifurcation point as a landmark, the absolute distances along the center lumen line to the initialization points were calculated and copied to the center lumen line on the baseline scan, which determined the range to be segmented. Lumen and outer vessel wall segmentations were manually adjusted deemed necessary.

In the second serial study **(Chapter 4.3)**, we chose to analyze plaque in a fixed range around the carotid bifurcation, a method that had been used in previous published serial MRI studies.[30-33] This enabled better comparison of our results on plaque changes with those described in the existing literature. Further, as the carotid artery is relatively fixed around its bifurcation, we assumed that the least variation in vessel geometry occurs in this segment, which is also the part that contains most atherosclerotic plaque and will change most during follow-up. A drawback of this method compared to the one above is that changes in plaque at other levels and the plaque growth in craniocaudal direction are not taken into account. Manual interventions were restricted to pointing the carotid bifurcation slice (which has a high intra- and inter-observer reproducibility) and manual adjustment of the annotations deemed necessary. The interobserver reproducibility would increase compared to that of the registration method described above, since one does not have to decide on the plaque length, as is described in a previous study on different manual plaque segmentation methods.[6] Automated measurements are thought to be robust compared to manual measurements and will consistently create the same segmentation given the same image data, since they are based on statistical models. Since in

our second serial study, the automated plaque segmentations were corrected by a single reader for all scans, blinded for patient number and time sequence, observer variability was reduced to a minimum.

During the course of the study there was an ongoing development and improvement of the (semi)-automated tool for the carotid atherosclerotic plaque segmentation. Nevertheless, a significant manual intervention was needed, especially at the level of the bifurcation, since ellipses were automatically fitted to segment the outer vessel wall. Especially at the division into the intracranial and extracranial artery, the contour does not fit an ellipse, but more or less a number 8 figure. Therewith the method is really a semi-automated one and further efforts should be made in creating a fully automated tool to ensure availability in large-scale serial studies. These issues also still exist in automated quantitative plaque imaging in MRI,[10] in which automated quantification of certain plaque components is feasible, but lumen and outer vessel contours still have to be drawn manually.

Atherosclerotic plaque development

Increased atherosclerotic burden first causes outward remodelling of the artery, implying a compensatory expansion of the outer vessel wall delaying lumen narrowing.[34] Yet, little is known about the progression of a subclinical atherosclerotic plaque into a vulnerable one in human beings. I performed the first serial CTA study on carotid plaque progression in humans in vivo. Plaque progression is a slow and heterogeneous process. Our studies confirmed this by demonstrating small annual growth rates and large variations in changes, ranging from plaque regression to plaque progression in a study population that was optimally treated for their ischemic cerebral event. Though difficult to compare to the findings from serial studies using other imaging modalities and techniques, our findings seem comparable to results described in the MRI literature, especially when comparing progression rates expressed as percentage annual growth.[30, 32, 35]

In the serial study in which I used a variant of the CAC scoring method **(Chapter 4.1)**, I found calcification volume to increase in the carotid arteries of TIA and stroke patients over time. In our serial studies on plaque development using the (semi-)automated quantitative plaque segmentation method, I found lipid volume and proportion to decrease, whereas calcification volume and proportion increased. The two methods to investigate calcification changes were not directly compared to each other in one study using the exact same region of interest. Nevertheless, our study results suggest that the increase in calcification volume is merely based on significant changes in plaque composition, rather than on an increase in atherosclerotic plaque burden. Existing cross-sectional literature suggest calcification of plaque to be associated with stabilization of the plaque.[36-39] MRI studies found proportion LRNC and its increase to be associated with plaque surface rupture[21, 40] and LRNC proportion to be associated with occurrence of cerebrovascular events.[41] Decrease in lipid content therefore also suggest plaque stabilization. Recurrent cerebrovascular event rate in our study population was too small to investigate the clinical impact of these temporal plaque changes. Future, larger longitudinal studies should confirm the relation between temporal changes in plaque composition and recurrent stroke.

CTA plaque segmentation enables complete (semi-)automated plaque segmentation because the outer vessel wall can be separated from the environment. This differentiation seems to be harder to automatically perform in MRI plaque analysis. Serial MRI studies

on plaque development merely focus on certain plaque components known to characterize the vulnerable plaque. Lipid-rich necrotic core (LRCN) for example has been found to be the most important determinant of plaque burden progression and plaque surface disruption in asymptomatic patients with 50-79% carotid stenosis.[40] Intraplaque hemorrhage (IPH) has also been shown to contribute to plaque progression and destabilization.[42-45] An important shortcoming of CTA is that it does not enable the characterization of IPH, as this seems the most important and promising plaque imaging biomarker.

The changes found in our serial studies and those of others are very small and often within the measurement variability. This is a common problem in longitudinal studies, independent of the imaging technique used. Temporal changes and their determinants can therefore only be evaluated in large study populations.

Further, I investigated plaque changes in a symptomatic population, in which patients got secondary preventive therapies according to national guidelines. Therefore, these temporal changes do not reflect the 'natural evolution' of carotid atherosclerotic plaques, but could rather be explained by a healing mechanism and possible treatment effects. Studies on CEA specimens from symptomatic arteries showed plaque stabilizing changes on a histological level over time after an ischemic event.[46, 47] Further, the transformation into a more stable plaque phenotype has been proved in serial MR studies in statin-treated patients.[48, 49] From the results of our studies we can conclude that these optimally treated patients have quite indolent plaques. Therewith there remains a need for serial population-based studies, to enable the investigation of the real development of subclinical, but vulnerable atherosclerotic plaques.

Risk factors for atherosclerotic plaque changes

In the study described in **Chapter 4.3**, I investigated the determinants of plaque growth and changes in plaque composition. Age and baseline plaque volume were the only (respectively positive and negative) determinants for plaque growth. Especially lipid proportion was associated with a decrease in plaque growth, an effect that might be ascribed to the effect of lipid-lowering therapy, which has been demonstrated to halt plaque progression, especially by decreasing the LRNC.[32, 50]

In all our serial studies, and therefore independent of the assessment method used, hypertension was an independent determinant for calcification growth, measured in volumes as well as in proportions, and for newly developed calcifications. This finding is similar to that found in serial coronary calcification studies[51-53] and cross-sectional studies in carotid arteries[54]. Hypertension was also associated with a decrease in lipid proportion and in plaque volume. The pathophysiologic mechanism behind this association is not yet elucidated.

Serial CTA plaque imaging in symptomatic patients

In summary, we showed the feasibility of CTA to investigate temporal changes in plaque burden and plaque composition. In our symptomatic cohort, plaque changes were small, but a significant composition change into a more stable plaque profile could be demonstrated. These effects may probably largely be ascribed to the pharmaceutical influences of the preventive treatment these patients are on. As this study was not a controlled, randomized trial, it provides a suboptimal setting to unravel the determinants of plaque development.

Methodological considerations

The ultimate goal of research in the field of atherosclerosis would be to decrease stroke incidence by preventive measures in the general population. Longitudinal population-based imaging studies would increase our knowledge about atherosclerotic plaque progression and help improve risk assessment. However, since CTA is a semi-invasive technique - requiring intravenous contrast material and radiation exposure - it is not suitable for a screening setting in healthy individuals. On the other hand, especially symptomatic persons are at increased risk for recurrent ischemic events and it is therefore also important to investigate carotid plaque development in this group. In most of the studies in this thesis we therefore focused on a cohort of TIA and stroke patients. We choose patients who already had undergone a CTA in the setting of a clinical work-up because of their ischemic cerebrovascular event.

A drawback of this study design however is the fact that all patients received secondary preventive therapies and measures. Although this situation does reflect the real situation of stroke patients from the time they first present with a TIA or stroke, it makes it impossible to unravel the effects of risk factors and the potential opposite effects of the treatment. A randomized clinical trial would help to increase insight into the role of different risk factors and the effect of certain therapies. To investigate the natural evolution of atherosclerosis, which appears to be very slow, a long-lasting, longitudinal population-based cohort remains preferred.

A very difficult issue in performing serial imaging in order to measure changes in plaque burden and composition, is the variation in imaging quality caused by patient reposition, movement artefacts, and changes in contrast availability in the carotid artery. This introduces measurement errors that might be larger than the temporal changes we are looking for. We dealed with this issue by optimizing and standardizing the acquisition protocol, improving registration of serial scans and the use of automated plaque segmentation tools. Nevertheless, the semi-automated plaque segmentation tools still need significant manual interventions and inter/intra-observer variabilities are considerable, especially seen the small temporal changes. Therefore serial plaque imaging is only useful in large patient groups in a research setting and should not be used on an individual basis. This simultaneously hampers the introduction of current quantitative plaque imaging tools in clinical practice.

Future directions

CTA as imaging technique to provide imaging biomarkers of atherosclerosis has advantages, but also some shortcomings. Although we showed that it enables quantification of the plaque components lipid, calcifications as well as fibrous tissue, it seems unable to assess other potential plaque features like intraplaque hemorrhage and fibrous cap thickness and rupture. Further, with the need of intravenous contrast material and radiation, it is not completely non-invasive and harmless. Moreover, even with semi-automated plaque segmentation tools, the CTA based plaque imaging remained very laborious. For these reasons it is less suitable for prospective serial imaging studies in asymptomatic patients. Serial MRI studies are probably more promising in studying the transformation of subclinical plaques into rupture prone plaques in asymptomatic patient populations.

Further effort should be put in investigating the potential role of other plaque characteristics, like intraplaque hemorrhage, in this process.

Nonetheless, CTA does have potential in further optimizing clinical decision making strategies. In comparison to MRI, it is a quick, largely available method and radiation dose can be reduced to a minimum nowadays. In the last years, amongst others, the MR CLEAN trial provided evidence for the usefulness of intraarterial thrombectomy in acute ischemic stroke patients with thrombo-embolic occlusions of the proximal intracranial arteries.[55] These studies strongly contributed to the general acceptance of CTA as first diagnostic tool in ischemic stroke patients. This strategy provides CT-based imaging data, including CTA of extracranial and intracranial arteries, CT of the brain and CT perfusion. This setting could be used to further investigate the role of CT imaging biomarkers, like plaque ulcerations, in the pathophysiology of (recurrent) thrombo-embolic stroke.

Plaque ulceration is a potential, clinically useful imaging biomarker, as it is highly feasible to assess plaque ulceration on clinical available CTA of the carotid arteries with a high reproducibility and little extra effort. It seems to have a correlation with plaque rupture and cerebrovascular events as found in cross-sectional studies. This should be confirmed in larger, longitudinal studies and its performance in identifying patients at risk for recurrent stroke should be further investigated.

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Chapter 6

Summary and Conclusions / Samenvatting en Conclusies

Summary and Conclusions

Chapter 1 introduces the background and purpose of this thesis. Atherosclerotic disease of the carotid arteries is an important cause of ischemic stroke. Current clinical practice provides diagnostic and treatment options for individuals presenting with symptoms. The currently used treatment decision model is based on the severity of atherosclerotic disease expressed as degree of carotid artery stenosis. As the incidence of ischemic cerebrovascular events (i.e. cerebral infarction, TIA, amourosis fugax or retinal infarction) remains high in the aging population, research in the field of atherosclerosis concentrates on improving risk prediction and stroke prevention. Common purpose of atherosclerosis research is to increase our knowledge of the transformation of subclinical, early stage atherosclerotic disease into advanced atherosclerotic plaques that are vulnerable to rupture and may cause subsequent thrombo-embolization. Non-invasive imaging tools create a window to the carotid artery and allow the evaluation of the pathophysiology of atherosclerosis in vivo. Imaging biomarkers of early and advanced atherosclerotic disease can be assessed and serial assessment of quantitative imaging biomarkers enables the study of plaque development and its determinants and could be used to monitor treatment effects.

The work in this thesis focuses on quantitative imaging biomarkers of carotid atherosclerotic plaque using CTA, and the investigation of plaque development and its determinants based on these CTA-based biomarkers in a serial imaging study in a symptomatic patient population. Furthermore, the role of ulcerated plaque surface, evaluated with CTA, as a qualitative imaging biomarker in clinical studies is explored.

In ***Chapter 2***, the focus is on quantification of CTA derived biomarkers of plaque burden and plaque components. ***Chapter 2.1*** reviews the CTA technique used to evaluate carotid atherosclerotic disease in a quantitative way. Technical aspects and CT scan acquisition parameters are crucial for the accurate assessment of lumen, plaque and plaque components, and scan protocols should be optimized and standardized. Quantitative plaque measurements have been validated against histology in ex vivo and in vivo studies and can be assessed with a moderate to good interobserver and intraobserver reproducibility. (Semi-)automated algorithms to segment carotid atherosclerotic plaque and to quantify plaque volume and plaque component volumes have been developed to improve reproducibility and to enable large-scale application in large serial studies.

Chapter 2.2 presents the results of the performance study of an automated plaque segmentation algorithm in quantifying plaque volume and plaque component volumes. The differences between the automated method and the manual observers, accepted as the reference standard, were comparable to the interobserver differences in manual annotations. Further, comparable risk factor associations were found for plaque burden and plaque composition with the automated method as compared to manual segmentations. These results suggest that the automated method is applicable in large cohort studies.

Chapter 3 is dedicated to plaque ulceration on CTA as a qualitative biomarker of plaque rupture. Atherosclerotic carotid plaque ulceration is considered to be a marker of previous plaque rupture. In ***Chapter 3.1*** we showed that plaque ulceration in the symptomatic

artery was associated with clinically defined non-lacunar stroke as compared to lacunar stroke, independent of degree of stenosis and cardiovascular risk factors. This relation was confirmed by an independent association between the presence of atherosclerotic carotid plaque ulceration and non-lacunar infarction on MDCT of the brain. These results support the underlying hypothesis on the pathophysiologic mechanism of plaque rupture and subsequent thrombo-embolism to the brain in non-lacunar stroke.

In ***Chapter 3.2*** I present a clinical, retrospective serial study on the evolution of atherosclerotic plaque surface morphology. Though histopathological studies suggest healing of plaque ulcerations and ruptures, I showed that plaque ulcerations persist in TIA and stroke patients after their ischemic cerebrovascular event. This implicates that the presence of an ulcerated plaque not necessarily suggests a recent plaque rupture. The clinical significance of plaque ulcerations for (recurrent) thrombo-embolic events remains unresolved. As plaque ulceration could theoretically provoke thrombo-embolization, its risk for recurrent events should be investigated in large, prospective longitudinal studies.

According to the vulnerable plaque concept, certain plaque characteristics are thought to predispose the plaque to rupture. We investigated the relation between MDCT derived plaque volume and plaque component measures and plaque ulceration in a cross-sectional CT study of the symptomatic arteries of TIA and stroke patients ***(Chapter 3.3)***. Plaque volume and LRNC proportion were significantly associated with plaque ulceration, whereas calcification proportion was inversely associated with plaque ulceration. These associations remained significant in patients with a low degree of carotid stenosis. This indicates that MDCTA derived plaque volume and composition may identify rupture prone plaques, and eventually improve stroke risk stratification.

In ***Chapter 4***, I present the results of the first serial CTA study on quantitative plaque imaging. In order to improve our insight in atherosclerotic plaque development in the carotid arteries, we studied plaque changes in a clinical cohort of TIA and ischemic stroke patients, who underwent a follow-up CTA with a mean delay of five years after the baseline scan they initially had undergone in the context of their clinical work-up.

Chapter 4.1 describes the study in which we used carotid calcification as a proxy for atherosclerosis burden and investigated the determinants for calcification development and growth. Besides the modifiable classical cardiovascular risk factors, age and baseline calcification load were important determinants for calcification progression. This can be explained by the fact that chronic influences of risk factors are reflected in the baseline calcification, which is part of the pathophysiologic process under investigation. Although carotid calcification has been related to increased risk for cerebrovascular events, evidence is being accumulated for calcification of plaque, and so relative calcification contribution, to be associated with plaque stabilization.

Being able to quantify the relative contribution of plaque components might therefore be of interest. In ***Chapter 4.2*** we proved the feasibility of assessing temporal changes in plaque burden and plaque components using a semi-automated plaque segmentation algorithm on the serial CTA data. Plaque changes were small and heterogeneous. Nevertheless, we found a significant change in plaque composition towards a more stable plaque profile, i.e. a decreasing lipid proportion and increasing calcification proportion.

In ***Chapter 4.3*** we aimed to investigate the determinants of these temporal plaque changes. Baseline lipid proportion was associated with a decreased plaque progression. This finding is probably caused by lipid-lowering therapy, used by most of the study participants, which has been shown to halt plaque progression by decreasing the LRNC. Another seemingly important determinant for plaque stabilization is hypertension. The pathophysiologic mechanism behind this, however, remains unresolved. As our study was observational instead of experimental, it was impossible to unravel the chronic influences of risk factors from the therapy effects of the secondary preventive pharmaceutical treatment that the patients were on.

In the general discussion, in ***Chapter 5***, the main findings as well as methodological considerations, possible clinical implications and directions for future research are discussed.

In conclusion, we demonstrated the –though still restricted- feasibility of semi-automated quantitative serial carotid plaque imaging using CTA. We studied the plaque development in carotid atherosclerotic plaques in symptomatic patients, treated according to national guidelines with secondary preventive therapies, and found it to be a quite indolent process. In interpreting our findings, we have to be aware that plaque evolution is subject to a very complex combination of biochemical and biomechanical factors during lifetime, which is then also influenced by therapeutic interventions. Therefore, large, prospective longitudinal studies are necessary to study the determinants of plaque development in asymptomatic patients, whereas randomized clinical trials are needed to unravel the therapy effects in high risk patients. Further, this thesis provides additional evidence for the potential of carotid plaque ulceration on CTA as a clinically applicable imaging biomarker. Prospective, longitudinal studies should further investigate its role in (recurrent) stroke risk prediction.

Samenvatting en Conclusies

Hoofdstuk 1 introduceert de achtergrond en het doel van dit proefschrift. Atherosclerose van de halsslagaders vormt een van de belangrijkste oorzaken van herseninfarcten. In de huidige klinische praktijk zijn de diagnostische middelen en behandelingsopties gericht op individuen die zich presenteren met symptomen. Het klinische beslissingsmodel dat thans in gebruik is, is uitsluitend gebaseerd op de mate van vernauwing van de halsslagader. Aangezien de incidentie van herseninfarcten hoog blijft in onze vergrijzende populatie, richt het onderzoek op het gebied van atherosclerose zich op het verbeteren van de risico-inschatting en het voorkomen van beroerte. Het gemeenschappelijk doel in dit onderzoeksveld is het vergroten van onze kennis over de transformatie van subklinische ziekte naar de atherosclerotische plaques die een vergrote kans hebben om te scheuren (plaque ruptuur) en vervolgens voor embolisatie van plaque deeltjes of bloedstolsels kunnen zorgen. Niet-invasieve beeldvormende technieken bieden de mogelijkheid om de pathofysiologie van atherosclerose in vivo te bestuderen en leveren biomarkers van zowel vroege als gevorderde atherosclerotische ziekte. Seriële, kwantitatieve beoordeling van dergelijke biomarkers maakt het mogelijk om de ontwikkeling van atherosclerotische plaques en de determinanten ervan te onderzoeken en kan gebruikt worden om de effecten van behandeling te vervolgen.

Het werk in dit proefschrift richt zich op de kwantitatieve biomarkers van atherosclerotische plaque in de halsslagaders, verkregen met behulp van CT angiografie (CTA), en het onderzoek naar de ontwikkeling van plaque en determinanten daarvan met behulp van deze biomarkers in een seriële CTA studie in een symptomatische patiëntenpopulatie. Verder wordt de rol van op CTA vastgestelde plaque ulceratie als kwalitatieve biomarker onderzocht in klinische studies.

In ***Hoofdstuk 2*** ligt de focus op de kwantificering van middels CTA verkregen biomarkers van plaque hoeveelheid en plaque samenstelling. ***Hoofdstuk 2.1*** betreft een review over de CTA techniek die wordt gebruikt om atherosclerose in de halsslagaders op een kwantitatieve manier te evalueren. Technische aspecten en CT scan acquisitieparameters zijn cruciaal voor de nauwkeurige beoordeling van lumen, plaque en plaquecomponenten en scanprotocollen moeten worden geoptimaliseerd en gestandaardiseerd. Kwantitatieve plaquemetingen werden reeds gevalideerd in ex vivo en in vivo studies en kunnen worden verkregen met een matige tot goede interobserver en intraobserver reproduceerbaarheid. We hebben (semi-)geautomatiseerde algoritmen ontwikkeld voor het segmenteren van atherosclerotische plaque in de halsslagader en het kwantificeren van volumina van plaque en plaquecomponent om zo de reproduceerbaarheid te verbeteren en grootschalige toepassing in seriële, grote cohortstudies mogelijk te maken.

Hoofdstuk 2.2 presenteert de resultaten van het prestatievermogen van een geautomatiseerd plaque segmentatie algoritme om volumina van plaque en plaque componenten te kwantificeren. De verschillen tussen de geautomatiseerde methode en de handmatige segmentatie, welke als referentiestandaard wordt beschouwd, waren vergelijkbaar met de interobserver verschillen tussen handmatig uitgevoerde annotaties. Verder werden vergelijkbare associaties gevonden voor risicofactoren voor plaque hoeveelheid en plaque samenstelling bij gebruik van de geautomatiseerde methode in vergelijking met de handmatige segmentaties. Op deze manier werd de semi-geautomatiseerde methode indirect gevalideerd voor gebruik in grotere studiecohorten.

Hoofdstuk 3 is gewijd aan plaque ulceratie op CTA als een kwalitatieve biomarker van plaque ruptuur. Ulceratie in een atherosclerotische plaque wordt beschouwd als een marker van doorgemaakte plaque ruptuur. ***Hoofdstuk 3.1*** beschrijft dat plaque ulceratie in de symptomatische halsslagader wordt geassocieerd met klinisch gedefinieerde niet-lacunaire beroerte wanneer vergeleken wordt met lacunaire beroerte. Deze associatie bleek onafhankelijk van de mate van stenose en cardiovasculaire risicofactoren. Deze relatie werd bevestigd door een onafhankelijke associatie tussen de aanwezigheid van atherosclerotische plaque ulceratie en niet-lacunair infarct op de CT van de hersenen. Deze resultaten ondersteunen de hypothese over het pathofysiologische mechanisme van ruptureren van plaques en daaropvolgende trombo-embolisatie naar de hersenen bij niet-lacunaire beroerte.

In ***hoofdstuk 3.2*** presenteer ik een klinische retrospectieve seriële studie over de evolutie van de oppervlakte morfologie van atherosclerotische plaques. Hoewel histopathologische studies suggereren dat plaque ulceraties zouden genezen, laat ik zien dat plaque ulceraties na het optreden van een TIA of infarct lang en onveranderd kunnen blijven bestaan. Dit impliceert dat de aanwezigheid van een plaque ulceratie niet noodzakelijkerwijs betekent dat er een recente ruptuur is opgetreden. De klinische betekenis van het bestaan van plaque ulceraties voor het (opnieuw) optreden van trombo-embolieën blijft onopgelost. Aangezien een bestaande plaque ulceratie in theorie ook trombo-embolieën kan veroorzaken, moet het risico op recidiverende herseninfarcten in grote prospectieve longitudinale studies worden onderzocht.

Volgens het 'vulnerable plaque' concept zorgen bepaalde plaquekenmerken ervoor dat een plaque makkelijker scheurt. We onderzochten daarom de relatie tussen plaque volume en plaque componenten en plaque ulceratie in een cross-sectionele CT studie van de symptomatische slagaders in patiënten met een TIA of beroerte ***(hoofdstuk 3.3)***. Plaque volume en de lipide-rijke necrotische kern (LRNC) waren significant geassocieerd met plaque ulceratie, terwijl de proportie kalk omgekeerd geassocieerd was met plaque ulceratie. Deze associaties bleven significant in vaten met een lage stenosegraad. Dit geeft aan dat plaquevolume en -compositie op CT mogelijk plaque ulceraties zouden kunnen voorspellen en daarmee uiteindelijk het risico op een herseninfarct beter kan worden ingeschat.

In ***Hoofdstuk 4*** presenteer ik de resultaten van de eerste seriële CTA-studie over kwantitatieve beeldvorming van plaques in de halsslagaders. Om onze kennis te verbeteren van atherosclerotische plaqueontwikkeling in de halsslagaders, bestudeerden wij veranderingen in atherosclerotische plaque in een klinisch cohort van patiënten die een TIA of een herseninfarct doormaakten. Zij kregen een follow-up CTA gemiddelde vijf jaar na de CTA die zij hadden ondergaan in het kader van de klinische beoordeling ten tijde van hun TIA of herseninfarct.

Hoofdstuk 4.1 beschrijft de studie waarin we verkalkingen in de carotiden hebben gebruikt als indirecte mate voor de hoeveelheid atherosclerotische plaque, en de groei van deze verkalkingen, evenals de determinanten daarvan, hebben onderzocht. Naast de bekende klassieke cardiovasculaire risicofactoren waren met name leeftijd en de baseline kalk load belangrijke determinanten voor toename van de verkalkingen. Dit kan worden verklaard door het feit dat chronische invloeden van risicofactoren weerspiegeld zijn in de baseline kalk load, wat eigenlijk deel uitmaakt van het pathofysiologische proces dat

wordt onderzocht. Hoewel verkalkingen in de halsslagaders worden gerelateerd aan een verhoogd risico op herseninfarcten, is er een toenemende bewijslast dat het proces van verkalking van de plaque, en daarmee de relatieve bijdrage ervan, wordt geassocieerd met stabilisatie van de plaque.

Het meten van de relatieve bijdrage van plaque componenten zou daarom van belang kunnen zijn. In ***hoofdstuk 4.2*** laten we zien dat het haalbaar is om temporele veranderingen in plaque hoeveelheid en plaque componenten te beoordelen met behulp van een semi-geautomatiseerd plaque segmentatie algoritme in seriële CTA data. De geconstateerde plaque veranderingen waren klein en heterogeen. Niettemin vonden we een significante verandering in plaquesamenstelling in de richting van een stabieler plaque profiel, d.w.z. een afnemende vet component en een toenemende kalk component.

In ***hoofdstuk 4.3*** onderzoeken we de determinanten van deze temporale plaqueveranderingen. De baseline vet component was geassocieerd met een verminderde plaque groei. Deze bevinding is waarschijnlijk toe te schrijven aan cholesterol-verlagende therapie, dat werd gebruikt door de meeste deelnemers aan de studie. In andere studies heeft men laten zien dat cholesterol-verlagende middelen een remmende invloed hebben op de plaque door het doen afnemen van de vet component in de plaque. Een andere belangrijke determinant voor plaque stabilisatie bleek hypertensie. Het pathofysiologische mechanisme hierachter blijft echter onduidelijk. Aangezien onze studie niet een gerandomiseerde, gecontroleerde klinische studie betrof, was het onmogelijk om de chronische effecten van risicofactoren te onderscheiden van de potentiële therapie-effecten van de secundaire preventieve behandeling die onze patiënten krijgen.

In de algemene discussie, in ***Hoofdstuk 5***, worden de belangrijkste bevindingen uit onze studies, evenals de methodologische overwegingen, potentiële klinische implicaties en richtingen voor toekomstig onderzoek besproken.

Concluderend, dit proefschrift omvat de eerste studies waarin de bruikbaarheid van CTA en een semi-geautomatiseerd plaque segmentatie algoritme voor kwantitatieve seriële plaque imaging wordt geëvalueerd. We bestudeerden de plaqueontwikkeling in atherosclerotische plaques in halsslagaders van symptomatische patiënten, die worden behandeld volgens nationale richtlijnen met secundaire preventieve therapieën, waarbij bleek dat dit een behoorlijk indolent proces betreft. Bij het interpreteren van dergelijke seriële data, moeten we ons ervan bewust zijn dat plaque evolutie onderworpen is aan een zeer complexe combinatie van biochemische en biomechanische factoren gedurende een geheel mensenleven, waarbij er vervolgens ook therapeutische effecten doorheen spelen. Om die reden zijn grote, prospectieve, longitudinale studies nodig om de determinanten van plaqueontwikkeling bij asymptomatische patiënten te onderzoeken, en zijn gecontroleerde, gerandomiseerde klinische studies nodig om de therapie-effecten bij symptomatische patiënten te ontrafelen. Verder voorziet dit proefschrift in extra bewijslast voor de potentie van op CTA gedetecteerde plaque ulceratie als een klinisch toepasbare imaging biomarker. De rol van plaque ulceraties in het risico op nieuwe herseninfarcten, en daarmee in een eventuele verbetering van risico voorspelling, moet verder worden onderzocht in prospectieve, longitudinale studies.

Chapter 7

Appendices

Dankwoord

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Regelmatig heb ik de afgelopen jaren de vraag gekregen of ik –als ik de tijd zou kunnen terugdraaien- opnieuw mijn opleiding zou onderbreken voor een promotietraject. Bepaalde dingen zou ik zeker anders aanpakken, maar ik kan – nu de proefdruk voor mijn neus ligt – met opgeheven schouders zeggen dat ik op vele fronten een leerzame ervaring rijker ben.

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List of Publications

Journal papers

Van Gils MJ, Bodde MC, Cremers LG, Dippel DW, van der Lugt A. Determinants of calcification growth in atherosclerotic carotid arteries; a serial multi-detector CT angiography study. *Atherosclerosis*. 2013;227(1):95-99. DOI: 10.1016/j.atherosclerosis.2012.12.017

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Book chapter

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In: Saba L, Miguel Sanches J, Mendes Pedro L, Suri JS. Multi-Modality Atherosclerosis Imaging and Diagnosis. Springer Link 2013

PhD Portfolio

Courses	Year	Workload ECTS
• Clinical Epidemiology (NIHES, Rotterdam, The Netherlands)	2008	5.7
• Courses in the Medical Library (Erasmus MC, Rotterdam, The Netherlands)	2008	0.6
• Basiscurses Regelgeving en Organisatie voor Klinisch Onderzoekers (Erasmus MC, Rotterdam, The Netherlands)	2009	0.9
• Peripheral and Intracranial Obstructive Vascular Disease (COEUR, Rotterdam, The Netherlands)	2009	1.5
• Classical Methods for Data-analysis (NIHES, Rotterdam, The Netherlands)	2009	5.7
• Repeated Measurements in Clinical Studies (NIHES, Rotterdam, The Netherlands)	2010	1.4
• Vascular Clinical Epidemiology (COEUR, Rotterdam, The Netherlands)	2010	1.5
• Cardiovascular Imaging and Diagnostics	2010	1.5
• Regression Analysis for Clinicians (NIHES, Rotterdam, The Netherlands)	2010	1.4
• Biomedical English Writing and Communication	2010	3

(Inter)national conferences and presentations	Year	Workload ECTS
• Radiologendagen (NVvR, Amsterdam, The Netherlands); *poster presentation*	2009	1.0
• Radiological Society of North America Annual Meeting (RSNA, Chicago, United States); *poster presentation*	2009	1.8
• European Congress of Radiology (ECR, Vienna, Austria); *oral presentation*	2010	1.5
• Radiologendagen (NVvr; Veldhoven, The Netherlands); *oral presentation*	2010	1.0
• Radiological Society of North America Annual Meeting (RSNA, Chicago, United States); *educational exhibit*	2010	1.8
• Radiologendagen (NVvR, Maastricht, The Netherlands); *oral presentation*	2011	1.0
• Radiological Society of North America Annual Meeting (RSNA, Chicago, United States); *oral presentation*	2011	1.8
• European Congress of Radiology (ECR, Vienna, Austria); *poster*	2012	1.0

Other	Year	Workload ECTS
• Research Seminar: Shear Stress (COEUR, Rotterdam, The Netherlands)	2008	0.4
• Research Seminar: New developments in percutaneous interventions (COEUR, Rotterdam, The Netherlands)	2009	0.4
• Research Seminar: Vascular imaging: Atherosclerosis and Biomechanics (COEUR, Rotterdam, The Netherlands)	2010	0.4

Presentations and lectures	Year	Workload ECTS
• Serial CT angiography of atherosclerotic plaque; determinants and prognosis of changes in volume, composition and morphology (Department of Radiology, Erasmus MC)	2009	0.4
• Serial CT angiography of the atherosclerotic carotid plaque (COEUR seminar, Rotterdam, The Netherlands)	2010	0.4

Teaching activities	Year	Workload ECTS
• Supervising research projects of 2 medical students *(L. Cremers, M. Bodde)*	2010	0.8

Curriculum Vitae

Marjon van Gils was born on the 26th of May 1980 in Ermelo, the Netherlands.

In 1998 she started the study 'Biomedical Health Sciences' at the Radboud University in Nijmegen. For student research project she participated in a pharmacological study on intra-cranial medication for Parkinson disease in rats at the Radboud University, Nijmegen. Her second research project was on Isolated Limb Perfusion in soft tissue tumors, at the Department of Experimental Surgery, Erasmus Medical Center, Rotterdam. During this study, she realized that she preferred to perform more clinically oriented work and research. In the last year, she combined her study with the study of Medicine and she graduated in both studies in 2004 and 2006 at the Radboud University of Nijmegen.

Upon finishing her studies, her medical career started as a resident at the Emergency department, and later at the department of Surgery at the Albert Schweitzer Hospital (Zwijndrecht and Dordrecht respectively). It was in this period that she got interested in Radiology. From December 2007 she worked as a resident in Radiology at Erasmus Medical Center, Rotterdam. In December 2008 she was challenged to interrupt her residency and start the PhD research project 'Serial Atherosclerotic Carotid Plaque Imaging with MDCTA', under supervision of prof. dr. A. van der Lugt (department of Radiology) and prof. dr. D.W.J. Dippel (department of Neurology), the results of which are presented in this thesis. From May 2012 on she continued her Radiology training at Erasmus MC in Rotterdam and partly at Albert Schweitzer Hospital in Dordrecht, with Abdominal Radiology as a subspecialty. In December 2017 she finalizes her residency and will continue working at EMC as a fellow in Abdominal Radiology.

Marjon is married to Patrick. They live with their two children Maeke and Jop in Heinenoord.