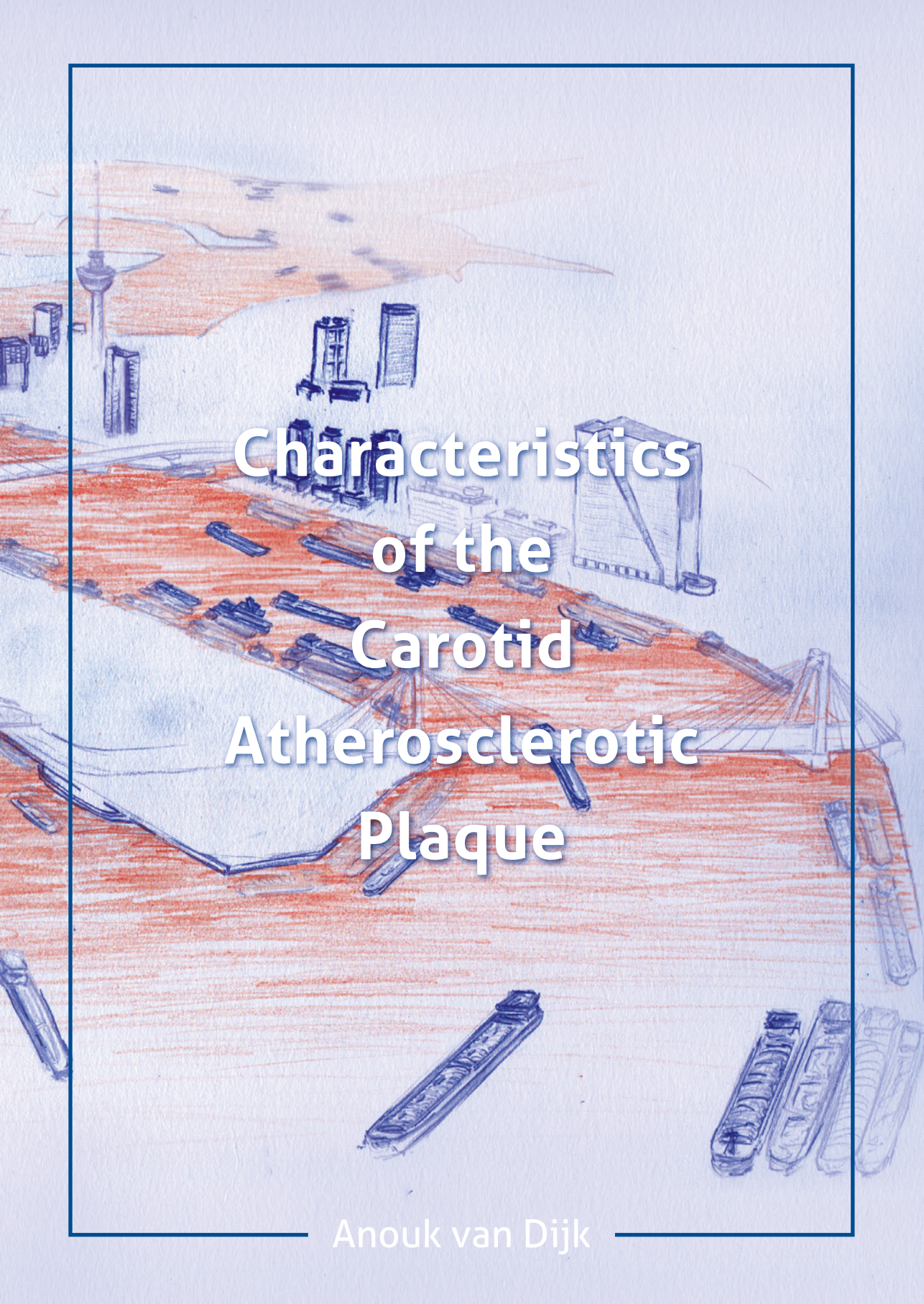

Characteristics of the Carotid Atherosclerotic Plaque

Anouk van Dijk

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Characteristics Of The Carotid Atherosclerotic Plaque

Kenmerken van de atherosclerotische plaque in de arterie carotis

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
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Promotoren: Prof.dr. A. van der Lugt
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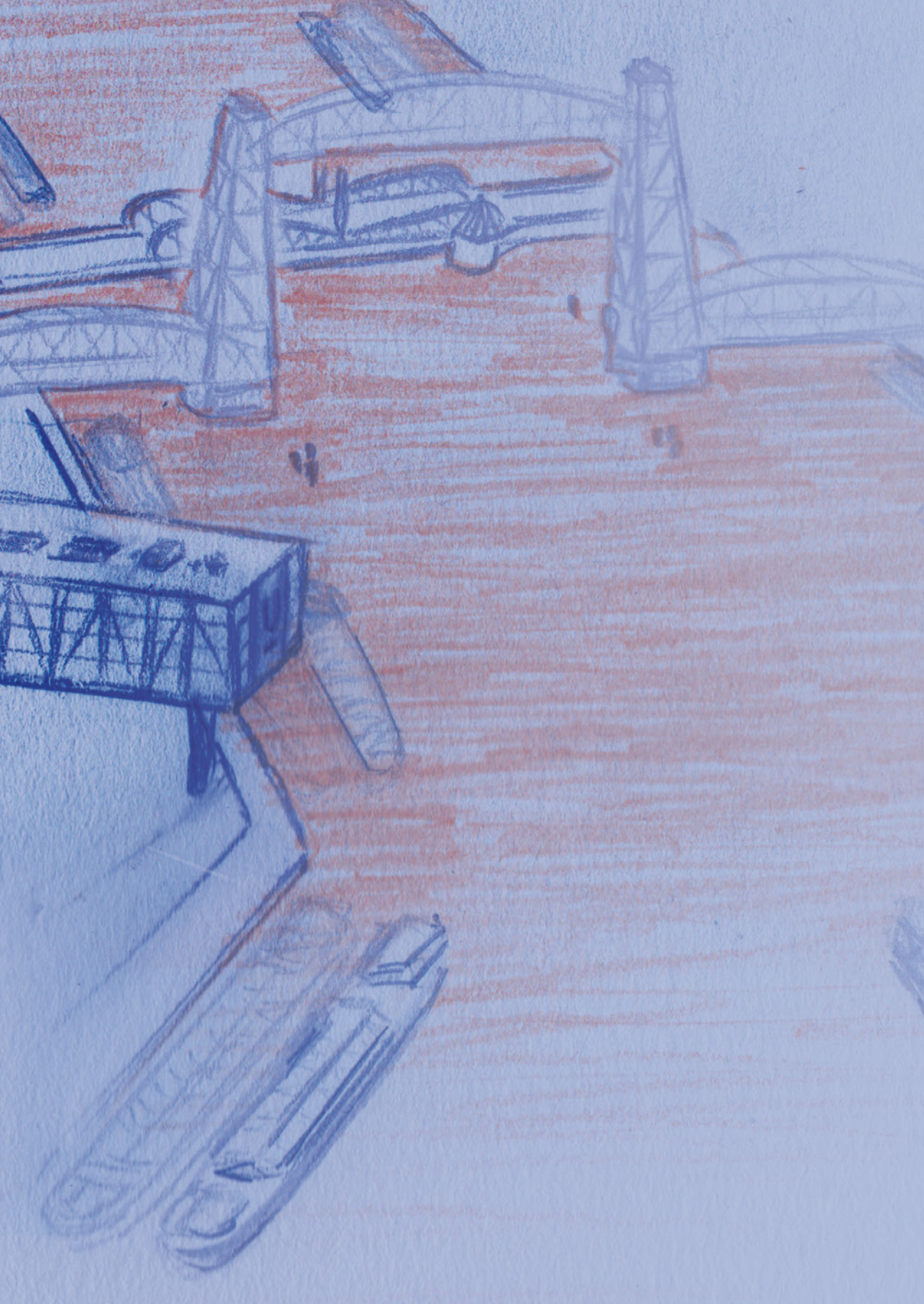

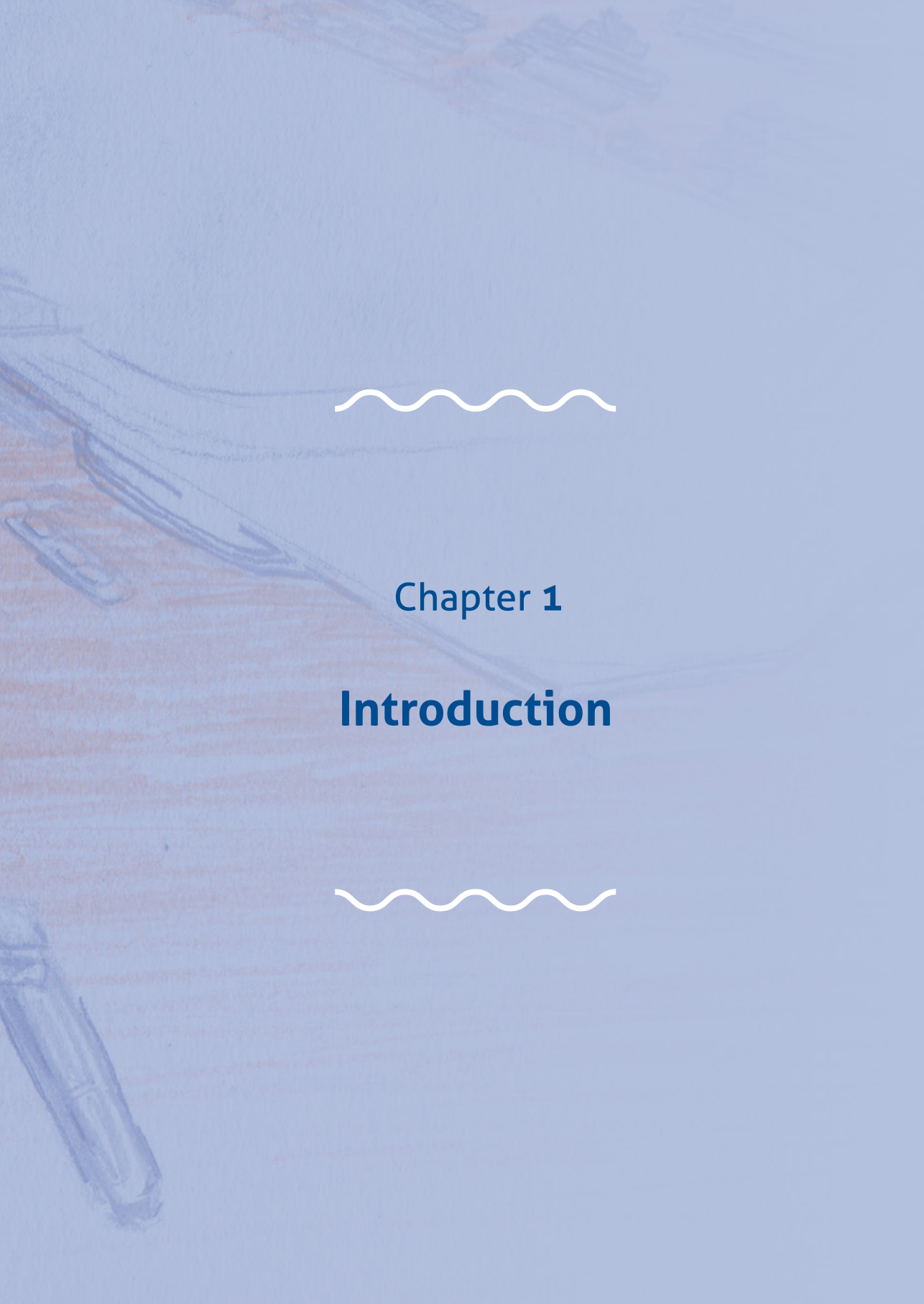

Chapter **1**

Introduction

Introduction

Cardiovascular disease is the second-leading cause of death in the Netherlands with approximately 38.000 deaths a year.[1] In addition, stroke is the leading cause of disability. A stroke is a sudden loss of neurological function due to an interruption of blood flow to the brain or extravasation of blood in the brain. The majority of strokes is of ischemic origin (87%)[2] in which a blood clot occludes one of the arteries of the brain. In a transient ischemic attack (TIA), this interruption and the subsequent symptoms are only temporary, mostly hours, and do not lead to a permanent deficit.[3] Ischemic stroke and TIA have similar causes and their clinical management is identical as atherosclerosis plays an important causal role in both.[2]

Atherosclerosis is a chronic inflammatory disease of especially large arteries with thickening of the arterial vessel wall which progresses into plaque development and stenosis of the vessel lumen. There are different stages of atherosclerotic plaque development[4, 5]; first deposition of lipids in the artery wall, followed by the influx of different cells in the lesion like macrophages, inflammatory cells and foam cells. Eventually this process can lead to a complicated plaque with a lipid-rich necrotic core, intraplaque hemorrhage, calcifications, inflammatory cells and an overlying fibrous cap. One of the major hazards of the atherosclerotic plaque is that it can rupture. Thrombogenic components of the plaque then come into contact with blood and a thrombus develops. This thrombus may generate distal emboli that occlude an artery of the brain, leading to an ischemic stroke. However, not all plaque ruptures lead to a clinical event.[4, 6, 7]

Blood coagulation plays a role in atherosclerosis and the development of ischemic events. Previous studies have shown an association between plasma levels of important coagulation factors (Von Willebrand Factor (VWF), A Disintegrin And Metalloprotease with Thrombo Spondin motif repeats 13 (ADAMTS13)) and cardiovascular disease risk.[8, 9] However, the exact interaction between these coagulation factors and ischemic events is unclear; one of the candidate mechanism underlying this association may be atherosclerosis. Previous studies suggest that atherosclerosis (and not only thrombus formation) is a determinant of VWF levels.[10] On the other hand, in vitro and in vivo studies suggest that VWF might contribute to the pathogenesis of atherosclerosis.[11] In addition, studies on animals with a VWF deficiency suggest that VWF deficiency has a protective effect against atherosclerosis and VWF may be a determinant of atherosclerosis.[11-13] When taking all these studies together, the data on this topic are inconclusive.[11, 14, 15] Detailed analyses of the atherosclerotic plaque in vivo by new imaging biomarkers may help unravel the association between these coagulation factors and atherosclerosis.

The last few decades have witnessed a rapidly growing evaluation of imaging techniques for the assessment of imaging biomarkers of atherosclerosis. Carotid intima-media thickness (CIMT), the combined thickness of the intimal and medial layers of the carotid artery, assessed by means of ultrasound (US), was the first imaging biomarker widely used in research on atherosclerosis. A CIMT of $\geq$ 1.5 mm or >50% of the surrounding IMT is indicative for an atherosclerotic plaque.[16, 17] Advantages

of US are its noninvasive nature, low costs and wide availability. Drawbacks are its high operator variability, loss of signal behind calcifications, and limited information on plaque composition. Furthermore, the development of other image modalities like multidetector-row computed tomography angiography (MDCTA) and magnetic resonance imaging (MRI), have shifted interest from US to these image techniques.[17, 18] MDCTA is a fast, widely available and minimally invasive imaging modality. However, disadvantages of MDCTA are radiation exposure and limited soft-tissue contrast.[17, 18] Histological studies have shown that imaging biomarkers like degree of carotid artery stenosis, carotid plaque burden, plaque component volumes (especially calcifications), and plaque ulcerations, can be evaluated well with MDCTA.[19-21] MRI is currently one of the most promising imaging modalities for plaque imaging due to its excellent soft-tissue contrast, the use of multiple contrast weightings each providing additional information, minimally invasive nature and no ionizing radiation.[17, 18] Histological studies have shown that imaging biomarkers like carotid plaque burden, plaque component volumes (especially intraplaque hemorrhage) and fibrous cap status, can be correctly evaluated with MRI.[6, 22, 23] However, MRI is less available, contra-indicated in patients with claustrophobia, pacemakers, defibrillators or other implanted electronic devices and has a relative long scan time.[17, 18]

Currently, patients with acute ischemic stroke are treated with intravenous thrombolytic drugs if possible or intra-arterial thrombectomy in case of an intracranial arterial occlusion. Following the acute phase, patients with ischemic stroke or TIA are treated with antiplatelet therapy and statins, antihypertensive and/or antidiabetic drugs if indicated, resulting in a significant reduction of recurrent stroke risk.[24] In addition, carotid endarterectomy (CEA) is indicated in men with a stenosis of 50-99% and in women with a 70-99% carotid artery stenosis, preferably performed within two weeks after the stroke or TIA.[25, 26] However, selection for CEA based primarily on the degree of carotid stenosis is suboptimal in light of the current knowledge of the pathophysiology of atherosclerosis. Histological studies have identified several plaque characteristics associated with an increased risk of plaque rupture; the so-called 'vulnerable plaque' characteristics. Examples of vulnerable plaque characteristics are a large necrotic core, a thin overlying fibrous cap, many inflammatory cells, neovascularization and intraplaque hemorrhage.[27] Visualizing and measuring these vulnerable plaque characteristics could help identify patients with an increased risk for recurrent stroke who may also benefit from a CEA or reduce the number of unnecessary interventions.[28] To investigate if plaque imaging indeed enables us to better identify patients in the 30-69% carotid stenosis group with an increased stroke risk, a large multicenter cohort study was started in 2010 by the multicenter Plaque At RISK (PARISK) consortium.[29]

Outline of this thesis

The focus of my thesis is the imaging of the atherosclerotic plaque in the carotid artery using US, MDCTA and MRI and how we can use imaging biomarkers to better understand atherosclerotic plaque pathophysiology and the role of blood coagulation. In addition, I aimed to evaluate which image modality and which imaging biomarkers can be valuable in the clinical setting.

In **Chapter 2**, I investigate the relationship between two important coagulation factors (VWF, ADAMTS13) and imaging biomarkers to try and unravel the underlying mechanisms of the association between the blood biomarkers and cardiovascular disease risk.

All imaging modalities have different advantages and limitations and it is not clear yet how the different modalities are interrelated. In **Chapter 3**, I assess the accuracy of contrast-enhanced US to detect plaque ulcerations compared to MDCTA, investigate the association between intraplaque hemorrhage on MRI and plaque surface on MDCTA, and finally, compare the performance of MRI and MDCTA in the detection and quantification of plaque calcifications.

In **Chapter 4**, I present examples of the use of imaging biomarkers in the clinical setting. First, I evaluate the calcifications in the aortic arch and carotid artery in a large cohort of patients with ischemic stroke and assess the differences between nonlacunar and lacunar ischemic strokes. In addition, I describe the study design of the PARISK study, a multicenter cohort study focusing on the identification of patients with an increased stroke risk in a group of patients with a recent stroke or TIA and a non-significant carotid stenosis, using plaque imaging.

Finally, in **Chapter 5**, I discuss all findings, methodological aspects, implications and future directions.

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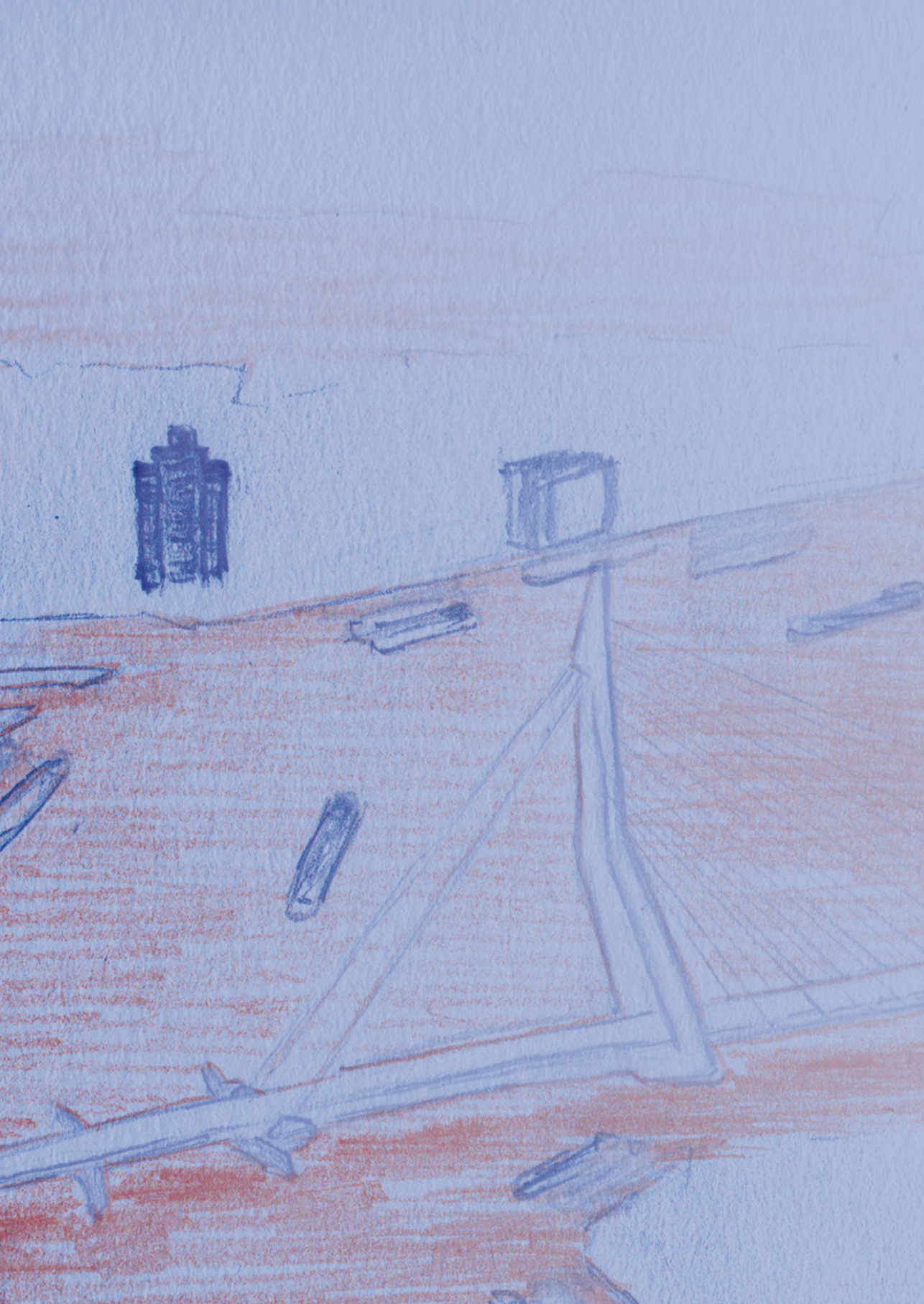
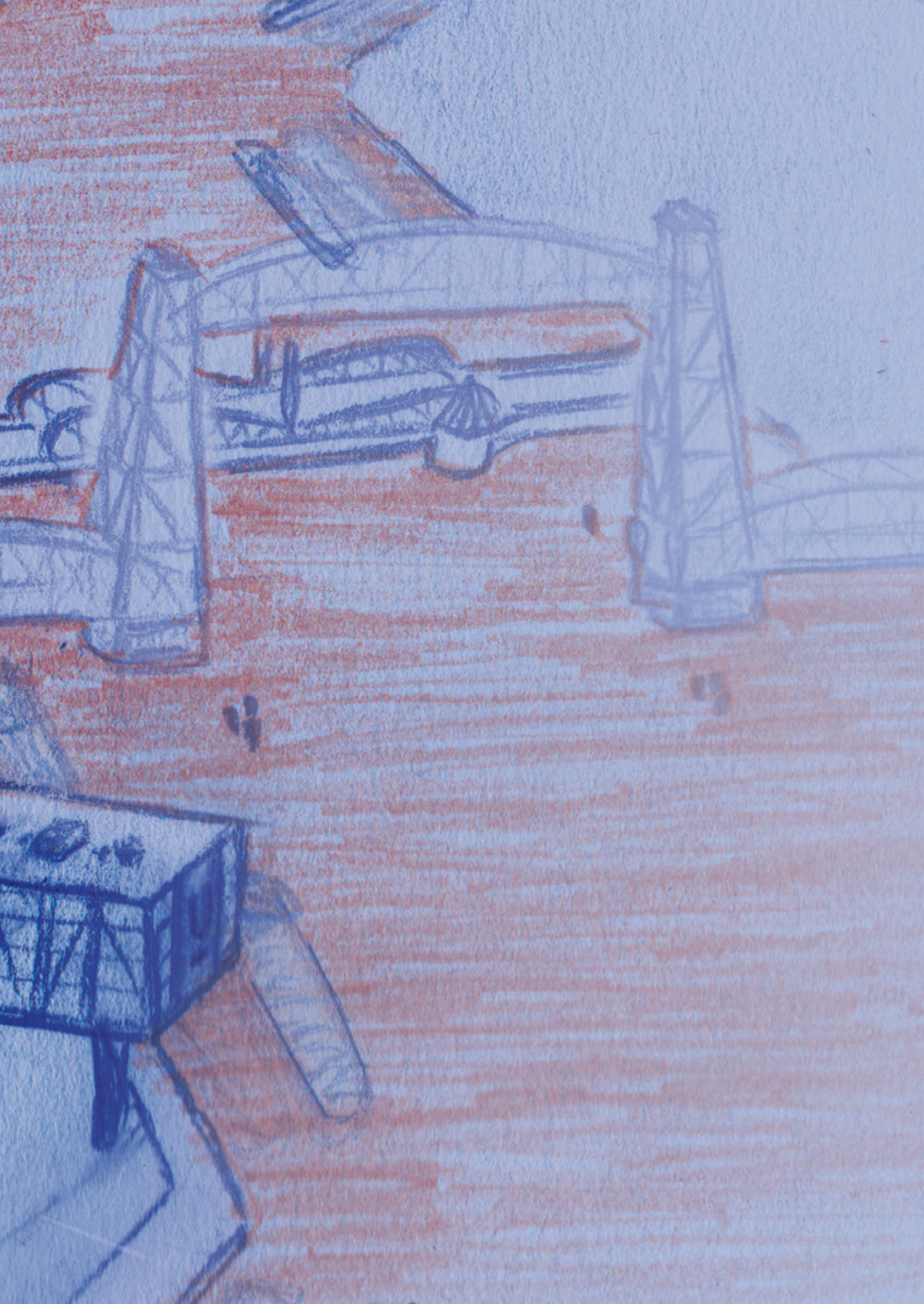


Chapter **2**

Atherosclerosis and coagulation



Chapter **2.1**

Relationship of Von Willebrand Factor with carotid artery and aortic arch calcification in ischemic stroke patients

M.A.H. Sonneveld, A.C. van Dijk, E.G. van den Herik, J.E. van Loon, L.M.L. de Lau, A. van der Lugt, P.J. Koudstaal, M.P.M. de Maat, F.W.G. Leebeek

Atherosclerosis. 2013;230:210-215

Abstract

Background: Large population studies have revealed that increased von Willebrand Factor (VWF) levels are associated with an increased risk of ischemic stroke. In previous studies VWF was associated with atherosclerosis in healthy individuals. However, it is yet unknown what the association is between atherosclerosis and VWF levels in patients with ischemic stroke.

Objectives: The aim of our study was to determine the association of atherosclerosis, measured with recent developed techniques, and VWF levels in a large, well characterized, cohort of ischemic stroke patients and to determine the prognostic value.

Methods: We included 925 consecutive patients with transient ischemic attack (TIA) or ischemic stroke. Calcification volumes (mm3) were scored in the aortic arch and both carotid arteries using multidetector computed tomography (CT) angiography. VWF antigen (VWF:Ag) levels were measured using ELISA.

Results: Mean VWF:Ag levels were significantly higher in the presence of calcification in either the aortic arch (1.47 vs. 1.37 IU/ml [$P = 0.039$]) or the carotid arteries (1.49 vs. 1.34 IU/ml [$P = 0.001$]). Patients with a large artery atherosclerosis ischemic stroke had significantly higher VWF:Ag levels then the other TOAST subtypes ($P < 0.0001$). High VWF:Ag levels were associated with an unfavorable outcome (modified Rankin Scale >2 vs. ≤ 2; 1.64 vs. 1.41 IU/ml, [$P < 0.0001$]).

Conclusion: Our study showed a strong association between the extent of atherosclerosis in both the aortic arch and the carotid arteries and VWF levels in patients with TIA or ischemic stroke. Higher VWF levels are found in large artery atherosclerosis and are associated with a poor outcome.

Introduction

Von Willebrand Factor (VWF) plays a crucial role in platelet adhesion and aggregation, the initial steps in thrombus formation. VWF is a multimeric plasma protein that is produced by endothelial cells and megakaryocytes.[1] Since VWF plasma levels increase as the result of endothelial damage, VWF levels can be used as a marker of endothelial dysfunction.[2]

Previous studies have shown a positive association between levels of VWF and risk of coronary heart disease and stroke.[3-6] In ischemic stroke, a particular association of VWF levels with etiologic subtypes, such as large artery atherosclerosis and cardioembolic stroke, was found.[7] Despite the fact that prospective studies have identified VWF levels as a predictor of ischemic stroke, the mechanism by which increased VWF levels are related to stroke is still unclear.[8-10]

We have previously shown that genetic variation strongly determines VWF levels, however these genetic variations are not or minimally associated with ischemic stroke risk.[11,12] Therefore it has been suggested that the VWF levels may predominantly be determined by endothelial dysfunction and atherosclerosis.[13]

Endothelial dysfunction is the first phase in the development of atherosclerotic plaques. Because endothelial activation is related to atherosclerosis and an association of VWF with ischemic stroke has been found, atherosclerosis may be a determinant of VWF levels.[14] Previous studies have shown a significant association between atherosclerosis, measured by the ankle-brachial index and intima- media thickness, and increased VWF levels in healthy individuals.[15,16]

In recent years, new techniques have been developed to study the extent of atherosclerosis more precisely. Calcification volume is an important indicator of atherosclerosis severity and may have a strong association with VWF levels.[17-19]

We hypothesized that a higher degree of calcification volume, measured both in the aortic arch and carotid arteries, is associated with higher levels of VWF in patients with ischemic stroke, which may provide more insight in the relationship between VWF levels and ischemic stroke risk and the prognostic value of VWF.

Materials and methods

Study population

We studied 925 consecutive patients with TIA or ischemic stroke from the Erasmus Stroke Study, an ongoing registry of patients with cerebrovascular diseases treated at our hospital, from December 2005 until December 2010 of whom plasma, DNA and a CT angiography was available.[20] TIA was defined as a focal neurological deficit of presumed vascular origin lasting less than 24 h, with imaging studies showing no abnormalities. Ischemic stroke was defined as a focal neurological deficit of presumed vascular origin lasting ≥24 h, with brain imaging studies showing no abnormalities or typical signs of infarction. Patients were classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria and additionally to a phenotypic classification which is a variant of the A-S-C-O score.[21,22] This score is characterized

by 9 categories: definite lacunar stroke, definite atherothrombotic stroke, probable atherothrombotic stroke, definite cardiac cause, possible cardiac cause, definite hematologic cause, possible hematologic cause, other cause of stroke and unknown cause of stroke. Definitions are described in the supplementary information. Patients who have multiple causes of stroke did not fit into one of the categories and are therefore excluded from the analysis (N = 125). Hypertension and hypercholesterolemia were defined as the use of antihypertensive or cholesterol lowering drugs, respectively, before the inclusion event. Diabetes mellitus was defined as the use of oral and/or parenteral antidiabetic drugs before the event. Smoking status (smoking versus non-smoking) was assessed at the time of the event. Patients were considered to have a history of ischemic heart disease when they had a documented myocardial infarction, angina pectoris or cardiac revascularization therapy. Peripheral arterial disease was defined as a history of intermittent claudication or peripheral vascular surgery or amputation due to lower limb ischemia. A history of cardiovascular disease indicates a history of ischemic heart disease and/or peripheral arterial disease and/or atrial fibrillation and/or TIA or ischemic stroke. The National Institutes of Health Stroke Scale (NIHSS) was assessed at admission from the stroke unit or outpatient clinic.[23] The functional outcome was assessed using the modified Rankin scale (mRS) at discharge and was dichotomized as favorable (≤ 2) or unfavorable (>2) outcome.[24]

All participants provided written informed consent. The study was approved by the Medical Ethics Committee of the Erasmus University Medical Center.

MDCTA angiography

A multidetector CT angiography (MDCTA) was performed routinely according to a standard protocol. MDCTA was performed at a median of 5 days (interquartile range 2-14 days) after onset of symptoms. Image acquisition was performed using a 16, 64 or 128 slice multidetector CT system (Sensation 16, Sensation 64, Definition, Definition AS+ or Definition flash, Siemens Medical Solutions, Erlangen, Germany) using a standardized optimized contrast- enhanced protocol (120 kVp, 180-200 mAs, collimation 16 x 0.75 mm; 32 x 2 x 0.6 mm; 64 x 2 x 0.6 mm, pitch <1). The scan range extended from the ascending aorta to the intracranial circulation. All patients received 80 ml of contrast agent (320 mg/mL iodixanol, Visipaque, Amersham Health, Little Chalfont, UK), followed by 45 ml saline bolus chaser, both at an injection rate of 4 or 5 ml/s. Real-time bolus tracking at the level of the ascending aorta was used to synchronize passage of contrast agent and data acquisition. Image reconstructions were made with field of view of 120 mm, matrix size 512 x 512, slice thickness 0.75 or 1.0 mm, increment 0.4-0.6 mm and with an intermediate reconstruction algorithm. All MDCTA studies were evaluated by trained readers blinded for clinical data. Dedicated commercially available software (Syngo Calcium Scoring, Siemens) was used to quantify calcifications at the aortic arch and the carotid arteries; expressed as calcification volume in mm^3. The aortic arch was defined as the origin of the aortic arch to the first 1 cm of the common carotid arteries, the vertebral arteries and the subclavian arteries beyond the origin of the vertebral arteries. Both carotid arteries were scored within 3 cm proximal and distal of the bifurcation and calcification volume of both

carotid arteries was added. A threshold of 600 Hounsfield units (HU) was used to differentiate calcifications from contrast material in the lumen. A detailed description of the measurement is provided elsewhere.[17,25]

Reasons for not performing MDCTA were poor renal function, significant comorbidity with resultant very short life expectancy and very severe stroke with likely fatal outcome. Patients with a time interval between event and MDCTA of more than 180 days were excluded (n = 20). Because of poor image quality of both the aortic arch and the carotid arteries caused by artifacts, 17 scans were not gradable. In 7 patients the aortic arch and in 10 patients the carotid arteries could not be analyzed due to dissection of the carotid artery, presence of artifacts or stents.

Blood samples, VWF measurement and blood group assessment

Blood sampling was performed at a median of six days (interquartile range 3-14 days) after onset of symptoms. There was no significant correlation between VWF levels and time from event till blood sampling (ß -0,0001 IU/ml per day; P = 0.33).

Citrated blood was centrifuged at 1700 xg for 15 min at room temperature, and stored at -80 °C within 2 h from collection. DNA was isolated from blood using MagNA Pure (Roche Diagnostics) and stored at -80 °C. VWF antigen (VWF:Ag) levels were determined with an in-house ELISA, using rabbit anti-human VWF antibodies (DakoCytomation, Glostrop, Denmark) for catching and tagging. Reference standard plasma, calibrated against the international standard (Cryocheck Reference, Kordia, Leiden, the Netherlands), was used as a calibrator. The intra-assay coefficient of variation was 3.2%. Blood groups were assessed with a standard test of blood group antibodies in 572 patients. In the remaining 353 patients, we genotyped rs687289, which can be used to discriminate blood group O from non-O status, using custom TaqMan Genotyping Assays (Applied Biosystems, Foster City, CA, USA)[26] Genotyping was successful in 99% of all patients.

Statistical analysis

Levels of VWF:Ag and the calcification volumes in the aortic arch and carotid arteries were normalized by logarithmic trans- formation. The data are presented as geometric means and 95% confidence interval (CI). Correlation between two groups was assessed with Spearman rank correlation. The aortic arch and carotid calcification volume were divided into four subgroups. The first group consists of all patients with a calcification volume of 0 mm3, the remaining patients were divided into tertiles. VWF:Ag levels were divided into two categories: low level below the median (≤1.43 IU/ml) and high level above the median (>1.43 IU/ml). Groups were compared using independent T-tests or ANOVA with Bonferroni correction. Linear regression analysis was used to analyze the relationship between calcification volume with VWF:Ag levels. All analyses were adjusted for potential con- founders: age, sex, ABO blood group, smoking, hypertension, hypercholesterolemia, diabetes mellitus and a history

of cardiovascular disease. Only confounders that were significantly associated with VWF:Ag levels were used in the multivariate analyses. All analyses were performed using SPSS version 20.0 (IBM, Somers, NY, USA). A P value <0.05 was considered to indicate statistical significance.

Results

Baseline characteristics of the 925 patients in this study are shown in Table 1. Mean age was 62 ± 13.6 years and 47% were females. As expected in our cohort of patients with TIA or ischemic stroke, they frequently suffered from hypertension, hypercholesterolemia and the majority of patients smoked. The geometric mean of the VWF:Ag levels in our patients was 1.43 IU/ml (95% CI 0.63-3.24). Patients with ischemic stroke had significantly higher levels of VWF than patients with TIA (1.50 vs. 1.35 IU/ml; P < 0.0001). Patients with blood group O had significantly lower levels of VWF:Ag compared with patients with non-O blood groups (1.24 vs 1.62 IU/ml, p < 0.0001). 113 patients had a time interval between event and MDCTA of more than one month. The results of the study did not change, when excluding those patients.

Calcification volume of the aortic arch and VWF:Ag levels

The geometric mean calcification volume of the aortic arch of the total group was 17.59 mm^3 (95% CI 0-3374.8 mm^3). Patients with calcifications in the aortic arch (n = 593) had significantly higher VWF:Ag levels compared with patients without calcifications in the aortic arch (n = 325; 1.47 vs. 1.37 IU/ml, P = 0.039). VWF:Ag levels increased linearly with increasing groups of the aortic calcification volume (P for trend 0.003; Fig. 1a). After multivariate adjustment, age and blood group were significantly associated with levels of VWF:Ag (P < 0.0001; P < 0.0001; respectively). Patients with blood group O, with mean VWF:Ag levels of 1.24 IU/ml, had similar aortic calcification volume compared with patients with blood group non-O, who had a mean VWF:Ag level of 1.62 IU/ml (17.2 vs. 18.0 mm^3, P = 0.80). The association between the calcification volume and VWF:Ag levels was seen both in blood group O and non-O and this was not statistically significant different (P for indication = 0.49).

Calcification volume of the carotid arteries and VWF:Ag levels

The geometric mean of the carotid calcification volume of the total group was 5.7 mm^3 (95% CI 0.0-396.9 mm^3). Patients with calcifications (n = 529) had significantly higher levels of VWF:Ag compared with those without calcifications (n = 386; 1.49 vs 1.34 IU/ml, P = 0.001).

VWF:Ag levels increased linearly in increasing groups of the calcification volume (P for trend <0.0001; Fig. 1b). Age and blood group were significantly associated

Table 1. Baseline characteristics of the study population.

	Total cohort	Patients in whom both calcification volume were determined (n=908)		
	N = 925	No calcification N = 256	Any calcification N = 652	P value
Age, years	62.0 (13.6)	49.0 (11.5)	67.1 (10.7)	<0.0001
Female sex	443 (46.8)	137 (53.5)	290 (44.5)	0.014
Smoking	283 (30.6)	82 (32.0)	195 (29.9)	0.677
Body mass index (kg/m2)	27.3 (13.5)	27.9 (5.8)	27.1 (15.6)	0.543
Hypertension	488 (52.8)	76 (29.7)	400 (61.3)	<0.0001
Hypercholesterolemia	311 (33.6)	43 (16.8)	258 (39.6)	<0.0001
Diabetes mellitus	128 (13.8)	21 (8.2)	104 (16.0)	0.002
History of CVD	367 (39.7)	54 (21.1)	303 (46.5)	<0.0001
Bloodgroup				
O	416 (45)	115 (44.9)	294 (45.1)	0.888
Non-O	504 (54.5)	141 (55.1)	353 (54.1)	0.888
Diagnosis				
TIA	428 (46.3)	130 (50.8)	289 (44.3)	0.079
Stroke	497 (53.7)	126 (49.2)	363 (55.7)	0.079
TOAST classification				
Large artery atherosclerosis	154 (16.6)	16 (6.3)	133 (20.4)	<0.0001
Cardioembolism	113 (12.2)	29 (11.3)	79 (12.1)	0.741
Small vessel occlusion	183 (19.8)	51 (19.9)	129 (19.8)	0.963
Other determined etiology	50 (5.4)	25 (9.8)	25 (3.8)	<0.0001
Undetermined etiology	425 (45.9)	135 (52.7)	286 (43.9)	0.016

Data are presented as N(%), unless for age and body mass index, where mean (SD) are shown.
CVD indicates cardiovascular disease.

with levels of VWF:Ag after multivariate analysis (P < 0.0001; P < 0.0001, respectively). Patients with blood group O, with a mean VWF:Ag levels of 1.24 IU/ ml, had a similar carotid calcification volume compared with patients with blood group non-O, with a mean VWF:Ag level of 1.61 IU/ml (6.4 vs. 5.3 mm^3, P = 0.25).

Calcification volume of the aortic arch and of the carotid arteries were highly correlated (R = 0.69; P < 0.0001). Patients with calcifications in both arteries were older (67 vs. 49 years, P < 0.0001) and had more cardiovascular risk factors than those without calcifications (Table 1). This was similar in the separate arteries. Assessing the mean VWF:Ag level in patients using both calcification volume, showed the highest levels in patients with both the highest calcification volume (Fig. 2). The association between the calcification volume and VWF:Ag levels was seen both in patients with blood group O and non-O and this was not statistically significant different (P for interaction = 0.11).

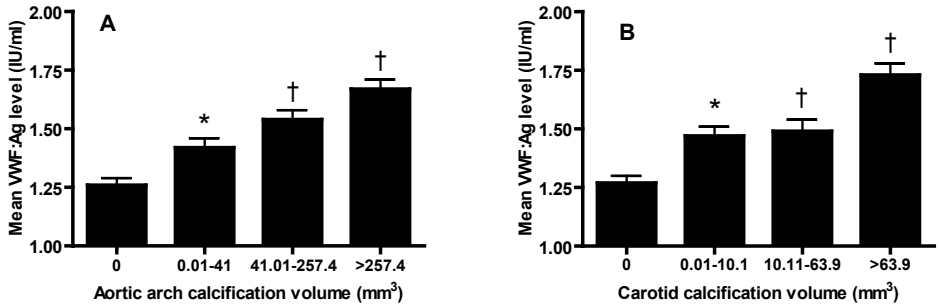


Figure 1. Levels of mean VWF:Ag per group of calcification volume. **A.** Mean and standard error (SE) VWF:Ag levels (in IU/ml) in the aortic arch calcification volume groups (*P = 0.006 compared with the first group; †P < 0.0001 compared with the first group). **B.** Mean and standard error (SE) VWF:Ag levels (in IU/ml) in the carotid calcification volume groups (*P = 0.001 compared with the first group; †P < 0.0001 compared with the first group).

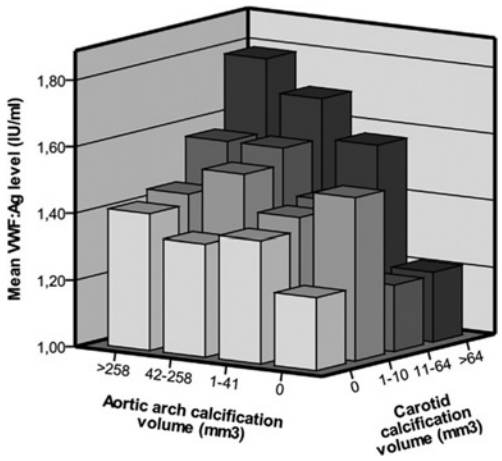


Figure 2. Relationship between aortic arch and carotid calcification volume and mean VWF:Ag levels The relation between aortic arch and carotid calcification volume groups and mean VWF:Ag levels (IU/ml).

Etiologic subtypes of TIA or stroke and levels of VWF:Ag

Levels of VWF:Ag differed significantly between etiologic subtypes of TIA and ischemic stroke (P = 0.006; Fig. 3). Levels of VWF:Ag were significantly increased in patients with a large artery atherosclerosis of TIA or ischemic stroke, compared with the other subtypes (1.59 vs. 1.40 IU/ml, P < 0.0001). Patients with a small vessel occlusion etiology had significantly lower levels of VWF:Ag compared with the other patients (1.34 vs. 1.45 IU/ml, P = 0.009).

Patients with a definite atherothrombotic stroke using the A-S- C-O score variant had significantly higher VWF:Ag levels (1.58 IU/ ml) compared with patients with a probable atherothrombotic stroke (1.40 IU/ml; P = 0.001), other cause of stroke (1.24 IU/ml; P = 0.009) and unknown cause of stroke (1.32 IU/ml; P < 0.0001). Patients with a definite lacunar stroke had significantly higher VWF:Ag levels (1.66 IU/ml) compared with probable atherothrombotic (P = 0.04), other cause of stroke (P = 0.013) and with unknown cause of stroke (P = 0.009).

VWF levels were significantly correlated with the NIHSS score at admission (R = 0.183, P < 0.0001) and with functional outcome of the patients, as determined by the modified Rankin Scale (mRS) at discharge (R = 0.222, P < 0.0001). Patients with an unfavorable outcome (mRS > 2) had significantly higher VWF:Ag levels compared with patients with a favorable outcome (mRS ≤ 2) (1.64 IU/ml vs. 1.41 IU/ml, P < 0.0001). However, these patients did not have a significant higher calcification volume in both the aortic arch (23.1 vs. 17.0 mm^3, P = 0.17) and carotid arteries (6.6 vs. 5.6 mm^3, P = 0.45), compared with patients with a favorable outcome. Patients with a high VWF:Ag level (>1.43 IU/ml) had a higher risk of an unfavorable outcome compared with patients with a low VWF:Ag level (≤ 1.43 IU/ml; OR 1.45, P < 0.0001) and this difference remained after additionally adjustment for aortic arch and carotid calcification volume. However, there was no significant difference in outcome between patients with blood group O and non-O (OR 0.95, P = 0.40) Table 2.

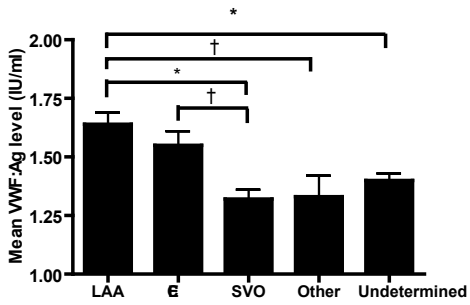


Figure 3. Levels of mean VWF:Ag per etiologic subtypes of TIA and ischemic stroke. Mean and standard error (SE) VWF:Ag levels (IU/ml) in the etiologic subtypes of TIA and ischemic stroke according to the TOAST criteria. * indicates P < 0.0001; †P < 0.05. LAA indicates large artery atherosclerosis; CE, cardio embolism; SVO, small vessel occlusion; Other, stroke of other determined etiology; Undetermined, cerebral ischemia of undetermined etiology.

Table 2. Association between calcification volume and VWF:Ag levels.

Calcification volume (mm^3)	Mean VWF:Ag (IU/ml) model 1	Mean VWF:Ag (IU/ml) model 2
Aortic arch		
0	1.26 ± 0.03	1.36 ± 0.03
0.01-41	1.42 ± 0.04	1.41 ± 0.04
41.01-257.4	1.54 ± 0.04	1.48 ± 0.04
>257.4	1.67 ± 0.04	1.54 ± 0.05
P for trend	<0.0001	0.03
Carotid artery		
0	1.27 ± 0.03	1.34 ± 0.03
0.01-10.1	1.47 ± 0.04	1.43 ± 0.04
10.11-63.9	1.49 ± 0.05	1.46 ± 0.04
>63.9	1.73 ± 0.05	1.61 ± 0.05
P for trend	<0.0001	<0.0001

Mean VWF:Ag levels (mean ± SE) per calcification volume subgroup.
Model 1: univariate. Model 2: adjusted for age, sex, blood group and cardiovascular risk factors.

Discussion

The main result of our study is a strong positive association between the extent of atherosclerosis, determined by the calcification volume in both the aortic arch and the carotid arteries, and VWF:Ag levels in patients with ischemic stroke. In addition, we observed that levels of VWF were significantly higher in patients with a large artery atherosclerosis type of ischemic stroke compared to other stroke subtypes.

To the best of our knowledge, our study is the first to investigate the extent of atherosclerosis, using newly developed quantitative measurements, and VWF levels in patients with TIA or ischemic stroke. We found similar positive associations in both aortic arch calcification volume and carotid calcification volume with VWF:Ag levels. Furthermore, patients with the highest score for both the aortic arch calcification volume and carotid calcification volume had the highest VWF:Ag levels. VWF:Ag levels were strongly associated with large artery atherosclerosis.

It is still debated whether VWF itself plays a pathogenetic role in atherogenesis. Despite the fact that several animal models indicated that VWF may lead to a reduction of atherosclerosis, multiple human studies failed to confirm this observation.[27,28] In these studies a similar extent of atherosclerosis in individuals with severe von Willebrand disease (VWD), characterized by reduced levels of VWF, was shown compared with controls.[29-33]

It is well known that genetic variations, both within the VWF gene and outside the VWF gene, strongly determine VWF levels.[11,12,34-36] One of the main genetic determinants of VWF levels is the ABO blood group, resulting in 25-30% lower levels in individuals with blood group O compared to non-O.[37] Also in our study, ischemic stroke patients with blood group O had 30% lower VWF:Ag levels compared to pa-

tients with blood group non-O, but did not have lower calcification volumes. This finding suggests that VWF levels do not have a major pathogenetic role in atherosclerosis. In addition, it is still debatable whether blood group is a risk factor for ischemic stroke.[38,39] An earlier study of our group already showed that, despite the fact that several genetic variations were strongly associated with VWF levels, the genetic variations did not influence the risk of ischemic stroke.[11] This suggests that VWF levels are merely a marker of atherosclerosis and thereby determine the previously found association between increased VWF:Ag levels and risk of stroke, as atherosclerosis is a well-known important risk factor for TIA and ischemic stroke.

Patients with a small vessel occlusion ischemic stroke had lower levels of VWF:Ag than the other stroke patients. This was comparable with another study, which showed that patients with a small vessel disease type of stroke, had lower VWF levels compared with the large vessel disease and cardio embolism group.[7] These lower VWF levels may be explained by the fact that in these patients atherosclerosis does not play a major role in the etiology of stroke. However, the small arterioles of the brain are presumed to be affected by various vascular risk factors.[40] This may also lead to endothelial activation, but only to certain subtypes of lacunar infarcts.[41] However, lacunar stroke described in the A-S-C-O score variant was associated with high VWF:Ag levels. This might be explained by the difference in the number of patients (N = 22 A-S- C-O score, N = 183 TOAST classification) and a difference in definition. In this study, VWF:Ag levels were associated with stroke severity, determined by the NIHSS at admission and with poor outcome, determined by the mRS at discharge. This is in agreement with one previous study in patients with acute ischemic and hemorrhagic stroke in which high VWF levels were associated with a poor modified Rankin score.[42] Recently we showed in the prospective population-based Rotterdam study that increased VWF levels are a predictor of stroke.[9] In this study we have shown that increased VWF levels and not blood group are a predictor of stroke outcome suggesting that VWF levels could serve as a risk marker of stroke outcome. Additionally, because levels of VWF are increased due to presence of atherosclerosis, VWF could also serve as a useful marker of atherosclerosis and thereby as a risk marker of stroke.

Some methodological issues have to be addressed. Strengths of our study are the large number of ischemic stroke patients of all ages, with availability of well-documented clinical information, extensive scoring (>96%) of MDCT angiography of the aortic arch and the carotid arteries, and the availability of plasma of nearly all consecutive patients.

Furthermore, we determined calcifications of the aortic arch and carotid arteries using dedicated commercially available software in which calcifications of the aortic arch and carotid arteries can be reproducible quantified in a specified range. So far only limited data was available regarding calcifications in the carotid arteries as a marker of atherosclerosis. In this study, we found a significantly higher prevalence of cardiovascular risk factors in patients with calcifications in the aortic arch and carotid arteries than in patients without calcifications. This is in agreement with another study, which showed an independent relationship between several cardiovascular risk factors and carotid calcifications and similar risk factor profiles were found in different vessel beds including the coronary arteries, aortic arch and carotid arteries.[17] Calcifications in the aortic arch and carotid arteries were associated with the presence of stroke and luminal stenosis in previous studies.[18,19,43,44] This all shows

that calcifications in the aortic arch and carotid arteries can be used as a marker of atherosclerosis.

A potential drawback of our study is that calcifications below the threshold of 600 HU or outside the scan range were not detected. Another possible limitation concerns the fact that calcifications are one of the components of an atherosclerotic plaque and a soft atherosclerotic plaque that contains no calcification is therefore not detected.[43] However, it has been shown that the majority (82%) of atherosclerotic plaques contains some calcifications and therefore this would only minimally affect our results.[25] In addition, time between stroke and blood sampling was variable. However, there was no significant correlation between VWF levels and time from event till blood sampling. This suggests that the time of blood drawn did not influence the levels of VWF. Furthermore, patients with significant comorbidity, very severe stroke or those who died within 24 h were not included in this study, resulting in a relatively less severely affected cohort.

Conclusion

In conclusion, our study in patients with TIA or ischemic stroke indicates that the extent of atherosclerosis, determined by calcifications in the aortic arch and carotid arteries, is strongly associated with VWF levels. In addition, highest VWF levels are found in large vessel disease, whereas the levels are lower in small vessel disease. Furthermore, our study suggests that VWF may have prognostic value in patients with ischemic stroke.

Supplementary information

Lacunar stroke is defined as a small deep infarct on scan <15 mm in the territory corresponding to symptoms in a patient with a clinical syndrome compatible with small deep infarct. Definite atherothrombotic stroke is defined by (1) an ipsilateral internal carotid stenosis ≥50% or (2) an ipsilateral stenosis ≥50% in another intra/extracranial artery (also in vertebrobasilar system if applicable), or (3) mobile thrombus in the aortic arch. Probable atherothrombotic stroke is defined as patients with ipsilateral internal carotid or other intra/extracranial artery <50% stenosis, contralateral stenosis; patients with ≥2 of the following risk factors for atherothrombotic disease: hypertension (as defined above), diabetes mellitus, smoking at time of event, high cholesterol. Definite cardiac cause is defined as patients with atrial fibrillation, atrial flutter, sick sinus syndrome, prosthetic valve, mitral stenosis, recent myocardial infarction (<6 weeks), left ventricular thrombus, atrial myxoma, infective endocarditis, non-ischemic dilating cardiomyopathy, non-bacterial thrombotic endocarditis. Possible cardiac cause is defined by patients with calcific aorta stenosis, mitral valve prolaps, mitral annulus calcification, patent foramen ovale, atrial septal aneurysm, ventricular aneurysm, ventricular septal defect, other structural cardiac abnormalities not mentioned above. Definite hematologic cause is defined as patients with disseminated intravascular coagulation, myeloproliferative disorders, essential thrombocythemia, polycythemia vera and antiphospholipid syndrome (the full syndrome). Possible hematologic cause is defined as patients with protein C deficiency, protein S deficiency, antithrombin deficiency, factor V Leiden, isolated lupus anticoagulans, single increased antiphospholipid antibodies (not confirmed with second increased measurement), and other hematologic causes. In other causes of stroke carotid or vertebral dissection, vasculitis, AVMs, Moyamoya, Fabry, other vascular causes, hemodynamic stroke, migrainous stroke, neoplasm and miscellaneous causes are included. Unknown cause stroke is defined as patients whom, based on all available data, cannot be categorized into either of the above categories.

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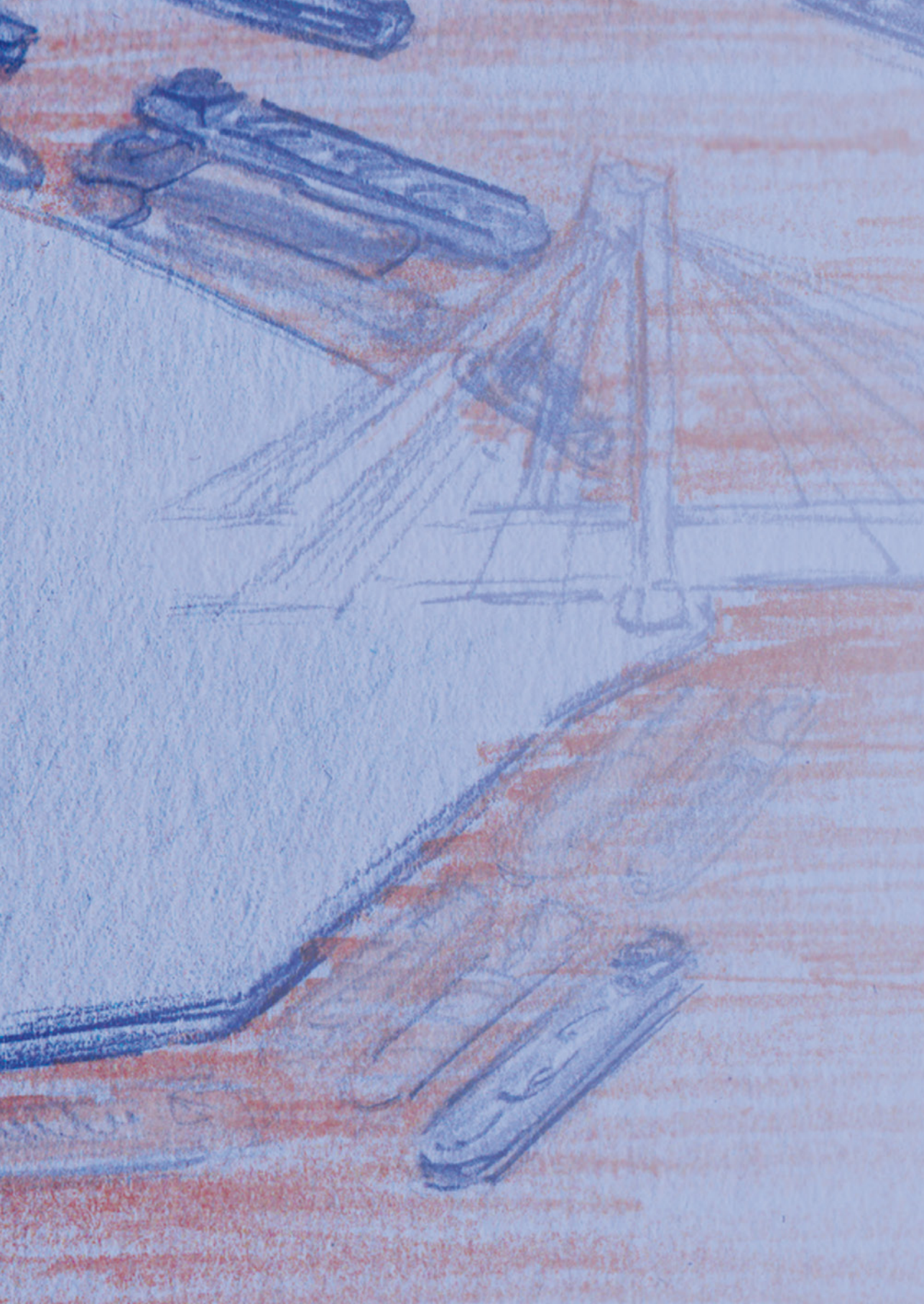

Chapter **2.2**

Imaging biomarkers of atherosclerosis are not associated with VWF:Ag levels or ADAMTS13 activity.

The Plaque At RISK study (PARISK)

A.C. van Dijk*, M.A.H. Sonneveld*, A.A.J. de Rotte, F.H.B.M. Schreuder, M.F. Chohan, M.T.B. Truijman, M.I. van Liem, T. Zadi, P.J. Koudstaal, F.W.G. Leebeek, R. Saxena, A.F.W. van der Steen, M.J.A.P. Daemen, P.J. Nederkoorn, J. Hendrikse, M.E. Kooi, M P.M. de Maat, A. van der Lugt

*Both authors contributed equally

Submitted

Abstract

Introduction: High Von Willebrand Factor (VWF) and low ADAMTS13 levels are associated with an increased risk of ischemic stroke and myocardial infarction. One of the candidate mechanisms underlying this association may be the increase of VWF levels by atherosclerosis. Therefore, we assessed the association between novel imaging biomarkers of the advanced atherosclerotic plaque and VWF levels and ADAMTS13 activity.

Methods: In 180 patients of the PARISK-study with a recent TIA or ischemic stroke and a symptomatic mild-to-moderate carotid artery stenosis (Plaque-At-RISK; clinicaltrials.gov NCT01208025), we measured VWF antigen (VWF:Ag) and ADAMTS13 activity. Imaging biomarkers of carotid atherosclerosis were determined by MDCTA (n=158) and MRI (n=169). In this cross-sectional analysis, we used linear regression analysis to assess the association between imaging biomarkers and VWF:Ag levels and ADAMTS13 activity.

Results: Age and blood group were associated with VWF:Ag (ß=0.01 IU/ml, p=0.001; ß=0.22 IU/ml, p<0.001). Age and time between event and blood withdrawal were inversely associated with ADAMTS13 (ß=-0.60%, p=0.002; ß=-0.13%, p=0.008). None of the imaging biomarkers were associated with VWF:Ag or ADAMTS13.

Conclusion: In our study, we found no association between imaging biomarkers of advanced atherosclerosis and VWF and ADAMTS13 in patients. It remains unclear whether the blood biomarkers mark widespread atherosclerosis or have a more complex role in atherosclerotic plaque development.

Introduction

Atherosclerosis of the carotid arteries is an important risk factor for ischemic strokes. With improved imaging techniques like multidetector-row computed tomography (MDCTA) and magnetic resonance imaging (MRI), atherosclerotic burden can be quantified by for example degree of stenosis, maximum vessel wall area or calcification volume; and characteristics of the vulnerable – rupture-prone – plaque can be visualized like plaque ulceration, intraplaque hemorrhage (IPH) and lipid core.[1, 2] Vulnerable plaque rupture is crucial in the pathophysiological cascade from atherosclerotic plaque development to thrombus formation and eventually ischemic stroke or TIA.

Von Willebrand Factor (VWF) has an important function in primary hemostasis via its role in platelet adhesion and aggregation, and increases as a result of endothelial damage. A Disintegrin And Metalloprotease with ThromboSpondin motif repeats 13 (ADAMTS13) cleaves large VWF multimers into smaller and less prothrombotic forms. Levels of VWF are increased in ischemic stroke patients and the highest levels of VWF are found in large vessel disease and cardio-embolic strokes.[3, 4] High VWF and low ADAMTS13 levels are associated with an increased risk of ischemic stroke and myocardial infarction.[5-7] One of the candidate mechanisms underlying the association between VWF and ischemic stroke may be atherosclerosis. Previous studies suggest that atherosclerosis (and not only thrombus formation) is a determinant of VWF levels.[8] On the other hand, in vitro and in vivo studies suggest that VWF might contribute to the pathogenesis of atherosclerosis.[9] In addition, studies on animals with a VWF deficiency suggest that VWF deficiency has a protective effect against atherosclerosis and VWF may be a determinant of atherosclerosis.[9-11] When taking all these studies together, the data on this topic are inconclusive.[9, 12, 13] We expect that, due to the role of ADAMTS13 in the metabolism of VWF, atherosclerosis might play a role in the association between ADAMTS13 and ischemic events as well.

The aim of our study was to evaluate the association between novel imaging biomarkers of the atherosclerotic plaque and VWF and ADAMTS13 in patients with a recent TIA or ischemic stroke and a symptomatic mild-to-moderate carotid artery stenosis to help clarify the association between atherosclerosis, VWF and ADAMTS13.

Methods

Study population

Patients were derived from the PARISK-study (Plaque-At-RISK; clinical trials.gov NCT01208025); details of the study design have been previously described.[14] The PARISK-study is a prospective multicenter cohort study using non-invasive plaque imaging to identify patients with an ipsilateral mild-to-moderate carotid artery stenosis (30-69%) with an increased risk of recurrent stroke. All included patients had a recent TIA, including amaurosis fugax, or minor stroke in the carotid artery territory prior to inclusion. Institutional Review Board approval was obtained and all patients gave written informed consent.

Between September 2010 and December 2014, 240 patients were included in the PARISK-study; 180 patients had either a MDCTA (n=158) or MRI (n=169) of the carotid arteries, and had an available blood sample.

MDCTA and 3T MRI data acquisition and analysis

Standardized, previously described, contrast-enhanced MDCTA and multi-sequence contrast-enhanced MRI protocols were used.[14] All imaging studies were evaluated by trained readers blinded for clinical data and other imaging tests.[15]

MDCTA images were reviewed using dedicated 3D analysis software (Leonardo and syngo.via; Siemens, Erlangen, Germany). First, image quality was rated on a 3-point scale; poor (not eligible for analysis), moderate and good (eligible for analysis).[15] The most severe stenosis in the symptomatic carotid bifurcation and internal carotid artery was measured according to the ECST criteria, perpendicular to the central lumen line.[16] Additionally, we defined plaque ulceration as an extension of contrast material of >1mm into the atherosclerotic plaque on at least 2 orthogonal planes.[17, 18] Finally, a custom-made plug-in for the freely available Image J software (National Institutes of Health, Bethesda, Maryland) was used to quantify calcifications in the symptomatic carotid artery within 3 cm proximal and distal to the bifurcation. We used a threshold of 600 HU to differentiate calcifications from contrast material in the lumen; calcification volume was expressed in cubic millimeters. A detailed description of the measurements is provided elsewhere.[19]

MR images were evaluated with dedicated vessel wall analysis software (Vesselmass, Department of Radiology, Leiden University Medical Center, Netherlands). Image quality was rated on a 5-point scale; low SNR (not eligible for analysis) to marginal and high SNR (eligible for analysis).[15, 20] MR images were automatically registered by delineating the lumen and outer vessel wall of the symptomatic carotid artery. Registration was manually corrected if needed. Plaque components of the symptomatic carotid artery (lipid, calcifications, IPH) were manually segmented. Fifteen transverse adjoining slices of 2 mm each covering the entire plaque were annotated. Maximum vessel wall area and plaque component volumes of the symptomatic carotid artery were derived from these annotations.

Blood sampling, VWF levels and ADAMTS13 activity measurements

Citrated blood was centrifuged at 2000 g for 10 minutes; then the plasma was centrifuged at 14000 g for 10 minutes and stored in aliquots at -80°C. VWF:Antigen (VWF:Ag) levels were measured with an in-house ELISA, using polyclonal rabbit anti-human VWF antibodies (Dakocytomation, Glostrup, Denmark) for catching and tagging. ADAMTS13 activity was measured using the Fluorescence Resonance Energy Transfer Substrate VWF 73 (FRETS-VWF73).[21] The inter- and intra-assay coefficient of variation for VWF:Ag levels and ADAMTS13 activity were 8.7% and 1.9% (VWF:Ag); 13.1% and 2.9% (ADAMTS13), respectively.

Statistical analysis

In this cross-sectional analysis, linear regression models were used to investigate the association between imaging biomarkers of the symptomatic carotid artery and VWF:Ag levels or ADAMTS13 activity, respectively. VWF:Ag levels were not normally distributed and therefore log-transformed. All quantitative imaging biomarkers were divided into tertiles. Adjustments were made for age and gender (model 1), and additionally for cardiovascular risk factors (model 2; age, gender, current smoking, hypertension, hypercholesterolemia, diabetes mellitus, cardiovascular history, BMI). In the analyses with VWF:Ag, we also adjusted for blood group. The analyses were repeated after adding interval event-blood withdrawal to the covariates. Statistical analyses were performed using STATA software (version 13.1, StataCorp, College Station, Texas). $P<0.05$ was considered statistically significant.

Results

Baseline clinical characteristics, VWF:Ag levels, ADAMTS13 activity and imaging characteristics are shown in Table 1. Mean age was 68 years, 74% were male and prevalence of cardiovascular risk factors was high. We found a median VWF:Ag level of 1.45 IU/ml [1.10 - 1.81] and a mean ADAMTS13 activity of 98.6% (± 22.8%). There was no correlation between VWF:Ag levels and ADAMTS13 activity ($R=0.06$, $P=0.42$). Mean symptomatic carotid artery stenosis (ECST) was 55 ± 15 %.

Table 1. Clinical characteristics, blood measurements and imaging biomarkers*

Clinical characteristic (n=180)	
Age (years)	68 ± 9
Male	133 (74%)
Current smoking	39 (22%)
BMI	26.7 ± 4.4
Hypertension	127 (71%)
Hypercholesterolemia	139 (77%)
Diabetes Mellitus	44 (24%)
History of CVD and PAD	85 (47%)
Classification event	
TIA	77 (43%)
Stroke	80 (44%)
Amaurosis fugax	23 (13%)

Continuation of Table 1.

Blood measurements (n=180)	
Interval event-blood withdrawal (days)	47 [32-67]
VWF:Ag (IU/ml)	1.45 [1.10-1.81]
ADAMTS13 activity (%)	98.6 ± 22.8
Blood group non-O	106 (59%)
Imaging biomarkers (symptomatic artery)	
Degree of stenosis (ECST) (%)†	55 ± 15
MDCTA (n=158)	
Interval event–MDCTA (days)	32 [12-54]
Presence plaque ulceration	43 (27%)
Presence calcifications	142 (90%)
Calcification volume (mm^3)	25.9 [5.1-80.7]
MRI (n=169)	
Interval event–MRI (days)	47 [30-67]
Maximum vessel wall area (mm^2)	72.7 [57.2-88.1]
Presence IPH	66 (39%)
IPH volume (mm^3)	0.0 [0.0-39.7]
Presence lipid	106 (63%)
Lipid volume (mm^3)	26.2 [0-146.0]

* Data are mean ± SD, absolute numbers of patients (%), or median [25th–75th percentile]; † If MDCTA was absent, degree of stenosis was assessed at MRI (n=22); CVD, cardiovascular disease; PAD, peripheral arterial disease; TIA, transient ischemic attack; ECST, European Carotid Surgery Trial; IPH, intraplaque hemorrhage

Increasing age was associated with higher VWF:Ag levels (ß= 0.01IU/ml/year, p=0.001) and individuals with blood group non-O had higher VWF:Ag levels compared with individuals with blood group O (ß= 0.22 IU/ml, p<0.001). None of the other clinical characteristics were significantly associated with VWF:Ag levels. Age was inversely associated with ADAMTS13 activity (ß= -0.60%/year, p=0.002). We also found an inverse association between the time from event to blood withdrawal and ADAMTS13 activity (ß= -0.13%/day, p=0.008). None of the other clinical characteristics were associated with ADAMTS13 activity.

None of the qualitative and quantitative measures of atherosclerosis were associated with VWF:Ag levels or ADAMTS13 activity in model 1 and 2 (Table 2). Additional adjustment for interval event–blood withdrawal did not change the results.

Table 2. Imaging characteristics and VWF:Ag levels and ADAMTS13 activity

Characteristic	VWF (model 1)*		ADAMTS13 (model 1)*	
	Beta [95% CI]	P value	Beta [95% CI]	P value
Degree of stenosis (ECST)†	-0.02 [-0.09;0.04]	0.53	-2.29 [-6.30;1.71]	0.26
Maximum vessel wall area†	-0.03 [-0.11;0.05]	0.41	2.50 [-2.11;7.10]	0.29
Calcification volume†	0.00 [-0.07;0.08]	0.91	-1.87 [-6.44;2.71]	0.42
Lipid volume†	-0.05 [-0.12;0.02]	0.14	1.43 [-2.76;5.62]	0.50
IPH volume†	-0.04 [-0.10;0.02]	0.18	0.70 [-3.11;4.51]	0.72
Plaque ulceration	-0.12 [-0.24;0.01]	0.08	0.56 [-7.40;8.52]	0.89

* Adjusted for age and gender; additionally for blood group non-O in case of VWF; † in tertiles; ECST, European Carotid Surgery Trial; IPH, intraplaque hemorrhage

Discussion

This study does not show associations between novel imaging biomarkers of atherosclerosis and VWF:Ag levels or ADAMTS13 activity in patients with a recent TIA or ischemic stroke and a symptomatic mild-to-moderate carotid artery stenosis.

The strength of our study is that we characterized the carotid atherosclerotic plaque using two image modalities (MDCTA and MRI) and assessed atherosclerotic burden as well as vulnerable plaque characteristics. Furthermore, this is the first study investigating the relationship between novel imaging biomarkers with VWF:Ag levels and ADAMTS13 activity. A limitation of our study was the cross-sectional design, which precludes the unraveling of cause and effect. We had a median delay of 47 days between clinical event and blood sampling/imaging, which might have influenced the association via a change in plaque composition and VWF levels.[22] VWF is known to be increased in the acute phase of an event.[23] However, imaging and blood sampling were performed at the same moment and we found no significant correlation between the interval event-blood withdrawal and VWF:Ag. We found a slight correlation between the interval event-blood withdrawal and ADAMTS13 activity, however this might be a chance finding. Nonetheless, adjustment for the interval did not influence the association between imaging and blood biomarkers.

In a previous study, we found a strong correlation between calcification volume in the aortic arch and carotid arteries and VWF levels in patients with an ischemic stroke or TIA. In accordance to literature we also found in this previous study significantly higher VWF levels in patients with large artery atherosclerosis compared to other etiological subtypes of TIA or stroke.[3, 4, 24] No previous studies are known investigating the association between ADAMTS13 and atherosclerosis. In the current study, all patients had a carotid artery stenosis and we assessed plaque volume as well as vulnerable plaque characteristics; no associations were found between any of the novel imaging biomarkers and VWF and ADAMTS13. It might be that VWF levels are

differently associated with plaque volume measurements than with vulnerable plaque characteristics. For instance, in acute coronary syndrome patients, the presence of atherosclerosis measured by IVUS was associated with VWF:Ag levels, but VWF:Ag levels were not associated with high risk, prone-to-rupture atherosclerotic lesions.[25] However, due to the clear role of VWF in thrombus formation and the less clear role of VWF and ADAMTS13 in atherosclerotic plaque development, we expected to find the opposite. It seems that neither the local disturbance of blood flow nor the disruptive plaque surface in the carotid bifurcation causes an increase in VWF or a decrease in ADAMTS13. It may be that the systemic alteration of the endothelial layer due to widespread atherosclerotic disease causes the change in VWF. If this is the case, blood coagulation markers are then a marker of a widespread atherosclerotic disease and not a modifiable risk marker for secondary prevention. However, a complex role of VWF and ADAMTS13 in atherosclerotic plaque development cannot be ruled out.

Conclusions

Imaging biomarkers of atherosclerosis were not associated with VWF:Ag levels or ADAMTS13 activity in patients with a recent TIA or ischemic stroke and a symptomatic mild-to-moderate carotid artery stenosis. Whether the blood biomarkers are simply a marker of widespread atherosclerosis or have a more complex role in atherosclerotic plaque development, remains unclear.

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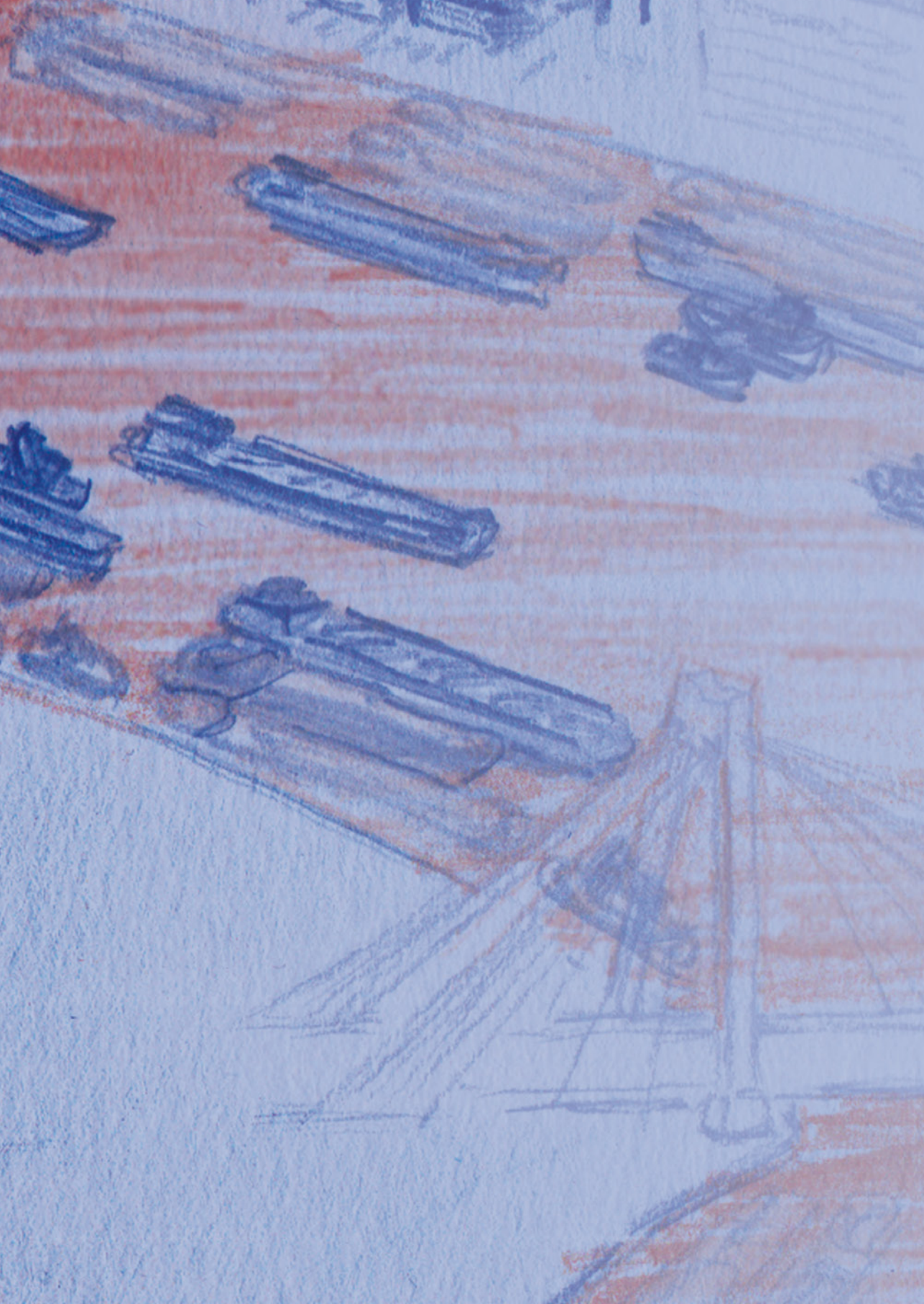

Chapter **2.3**

No association found between Von Willebrand factor and plaque ulceration in carotid artery atherosclerosis

T. Zadi, M.A.H. Sonneveld, A.C. van Dijk, B. Hussain, P.J. Koudstaal, F.W.G. Leebeek, M.P.M. de Maat, A. van der Lugt

Submitted

Abstract

Background and Purpose: Atherosclerotic plaque development may result in a specific morphologic buildup of plaque components, the so-called vulnerable plaque, which is mechanically unstable and can lead to plaque rupture, ending in an ischemic stroke. Plaque ulceration is considered a result from plaque rupture. An increased plasma level of von Willebrand Factor (VWF) is a result of endothelial dysfunction and is associated with an increased risk of ischemic stroke. We investigated the relationship between carotid artery plaque ulceration and VWF levels in patients with ischemic stroke.

Methods: We included 985 patients with TIA or ischemic stroke. Carotid imaging was performed with multidetector computed tomography angiography. Both carotid arteries were evaluated for volume of calcifications (mm^3) and the presence of plaque ulceration, defined as extension of contrast material of >1 mm into the surrounding atherosclerotic plaque on at least two orthogonal planes. Linear regression analysis was used to analyze the relationship between ulceration and VWF:Antigen (VWF:Ag) levels. Adjustments were made for age and gender (model I) and additionally for blood group, calcification volume and cardiovascular risk factors (model II).

Results: A significant correlation was found between plaque ulceration and VWF levels, after correction for age and gender (ß=0.066 [0.003-0.129]; p=0.040). After additional adjustment for blood group, calcification volume and cardiovascular risk factors, plaque ulceration was not significantly associated with VWF levels (ß=0.027 [-0.036-0.089]; p=0.403).

Conclusion: Atherosclerotic plaque ulceration in the carotid arteries is not, independently of calcification volume, related to higher levels of VWF in patients with TIA or ischemic stroke.

Introduction

Atherosclerotic plaque development starts with endothelial dysfunction, which leads to intimal thickening that finally ends in lipid accumulation beneath a fibrous cap, and formation of calcifications.[1-3] Atherosclerotic plaques with a specific morphologic buildup of plaque components, the so-called vulnerable plaque, are mechanically unstable, which can lead to plaque rupture. Carotid plaque rupture can lead to platelet aggregation, thrombus formation and embolization of thrombus and/or plaque material into the distal intracranial arteries, which causes an ischemic stroke.[4] Previous studies have shown a strong correlation between carotid artery plaque ulceration and histologic characteristics of plaque instability, such as intraplaque hemorrhage and plaque rupture.[5] New accurate imaging techniques like Computed Tomography Angiography (CTA) and Magnetic Resonance Imaging (MRI) can identify atherosclerotic plaque composition, such as lipid-rich necrotic core, intraplaque hemorrhage and calcifications with a high accuracy.[6-8] In addition, plaque ulceration, which is an independent predictor of ischemic stroke, can be detected with CTA and MRI as well.[9, 10,11]

Von Willebrand Factor (VWF) is a plasma glycoprotein, produced by endothelium, megakaryocytes and sub-endothelial connective tissue. VWF is involved in platelet adhesion and aggregation, leads to thrombus formation.[12] An increased level of VWF is seen as a result of adverse changes to endothelium[13] and is associated with an increased risk of ischemic stroke.[14] It is yet unknown whether atherosclerotic plaque leads to changes in hemostasis with a subsequent increase in ischemic stroke risk or whether atherosclerotic plaque and hemostatic changes independently increase ischemic stroke risk. In a previous study we evaluated the association between degree of calcification volume and VWF levels, and concluded that extent of calcification volumes in the carotid arteries and aortic arch are strongly associated with VWF levels. [15]As plaque ulceration is considered as an important step in the pathophysiology of ischemic stroke, we investigated whether there is a relationship between plaque ulceration in the carotid artery and VWF levels in patients with ischemic stroke.

Methods

Study population

This study is embedded in the Erasmus Stroke Study, an ongoing registry of patients with acute transient ischemic attack (TIA) or ischemic stroke treated at a single university medical center, Erasmus MC Rotterdam. In the current study we included 985 patients who were evaluated between July 2005 and November 2010 and had undergone blood sampling, genotyping and a CTA of the carotid bifurcation. All participants provided written informed consent. The study was approved by the Medical Ethics Committee of the Erasmus MC.

Baseline characteristics

Hypertension, hypercholesterolemia and diabetes mellitus were defined as the use of drugs (respectively antihypertensive, anti-cholesterol drugs and oral and/or parenteral antidiabetic drugs) before inclusion to the study. Patients documented with a history of ischemic heart disease (such as myocardial infarction, angina pectoris or cardiac revascularization therapy), peripheral arterial diseases, atrial fibrillation, TIA or ischemic stroke and/or the use of platelet aggregation inhibitors were considered as patients with a history of ischemic cardiovascular diseases. Body mass index (BMI) was used as a measurement of body fat, based on height and weight, applied to adult men and women. Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS) and classified by subtypes by using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria, at admission at the stroke unit or at the outpatient clinic. More information can be found elsewhere. [16, 17]

CTA acquisition and analysis

Imaging was performed with a 16, 64 or 128 slice multidetector CT scanner (Sensation 16, Sensation 64, Definition, Definition AS+ or Definition flash, Siemens Medical Solutions, Erlangen, Germany), using a standardized and optimized contrast-enhanced protocol (120 kVp, 180-200 mAs, collimation 16 x 0.75 mm; 32 x 2 x 0.6 mm; 64 x 2 x 0.6 mm, pitch <1), at a median of 5 days (interquartile range 2-14 days) after onset of symptoms. All patient received 80 ml contrast material (320 mg/mL iodixanol, Visipaque, Amersham Health, Little Chalfont, UK) , followed by 45 ml saline bolus chaser, both at an injection rate of 4 or 5 ml/s. Synchronization between data acquisition and the passage of contrast material was achieved by real-time bolus tracking at the level of the ascending aorta. The CTA scan reached from the ascending aorta to the intracranial vasculature. Parameters for image reconstructions were: field of view of 120 mm, matrix size 512 x 512, slice thickness 0.75 or 1.0 mm, increment 0.4-0.6 mm, with an intermediate reconstruction algorithm.

Experienced observers blinded for clinical data analyzed the CTA images. Calcification volume in the carotid arteries was quantified with dedicated commercially available software (Syngo CalciumScoring, Siemens) and expressed in cubic millimeters (mm^3). The carotid arteries were measured at both sides within three cm proximal and distal of the bifurcation. Calcifications were differentiated from contrast material in the lumen by using a threshold of 600 Hounsfield Units. Second, MDCTA images were transferred to a workstation equipped with dedicated 3D analysis software (Leonardo and syngo.via; Siemens, Erlangen, Germany). The multiplanar reformatting application allowed analysis of both carotid arteries in oblique, coronal, and sagittal planes. Both carotid arteries were evaluated for presence of plaque ulceration, defined as extension of contrast material of >1 mm in the surrounding atherosclerotic plaque on at least two orthogonal planes. [5, 18]

Blood samples and VWF measurement

Patients were subjected to blood sampling approximately 6 days after onset of symptoms (interquartile range 3-14 days). The citrated blood samples were centrifuged at room temperature for 15 min at 1700xg and stored within 2 hours at -80 °C. MagNA Pure (Roche Diagnostics) was used to isolate DNA from blood and likewise stored at -80 °C. An in-house ELISA was used to determine VWF:Ag levels, using rabbit anti-human VWF antibodies (DakoCytomation, Glostrop, Denmark) for catching and tagging. No significant correlation was found between VWF levels and time from event till blood sampling (ß – 0.0001 IU/ml per day; p = 0.33). Reference standard plasma was calibrated against the international standard (Cryocheck Reference, Kordia, Leiden, the Netherlands). In most of the patients, blood group was assessed with a standard test of blood group antibodies. In the remaining patients, we genotyped rs687289, which can be used to discriminate blood group O from non-O status, using custom TaqMan Genotyping Assays (Applied Biosystems, Foster City, CA, USA)[15].

Statistical Analysis

The distributions of VWF levels and calcification volumes in the carotid arteries were normalized by logarithmic transformation. For the analysis of calcification volume, we added 1.0 mm^3 to the nontransformed values to deal with participants with a calcification volume of zero. The data are presented as geometric means and 95% confidence interval (CI). Linear regression analysis was used to analyze the relationship between ulceration, calcification volume and cardiovascular risk factors and VWF levels. Ulceration was scored as present if one of the carotid arteries showed a plaque ulceration. The average calcification volume of both carotid arteries was used. Model I was adjusted for age and gender. Model II was additionally adjusted for blood group, calcification volume, and for cardiovascular risk factors with a p value < 0.05 in model I. The linear regression analysis was repeated for patients with large artery atherosclerosis according to the TOAST criteria. All analyses were performed using SPSS version 21.0 (IBM, Somers, NY, USA). A P value <0.05 was considered to indicate statistical significance.

Results

21 of the 985 patients were excluded due to lack of information about the carotid bifurcation, as a result of missing CTA (n=12), major artifacts (n=7) or poor contrast enhancement (n=2). The baseline characteristics of our remaining 964 study participants are shown in Table 1. Mean age at baseline was 62 ± 14 year and 46% of the patients were female. Mean VWF:Ag levels were 1.6 ± 0.7 IU/ml. Table 2 shows the imaging characteristics of the 1928 carotid arteries and in 964 patients. Plaque ulceration was seen in 7% (136/1928) of the carotid arteries and in 128 patients. Plaque calcifications were present in 48% of the vessels with a volume of 1.68 ± 1.89 mm^3.

Table 1. Clinical characteristics. Total cohort: n = 964

Clinical characteristic	
Age, years	62 ± 14
Female	462 (47%)
Body mass index (kg/m^2)	27.3 ± 13.1
Hypertension	531 (54%)
Hypercholesterolemia	354 (36%)
Diabetes mellitus	140 (14%)
History of CVD	644 (65%)
Current smokers	299 (30%)
Blood group	
O	445 (45%)
Non-O	534 (54%)
VWF (IU/ml)	1.6 ± 0.7
TOAST classification	
Large artery atherosclerosis	163 (17%)
Cardio-embolism	116 (12%)
Small vessel occlusion	191 (20%)
Other determined etiology	54 (6%)
Undetermined	440 (46%)

Data are presented as n (%), unless for age and body mass index, where mean ± SD are shown.
CVD indicates cardiovascular disease. VWF indicates Von Willebrand Factor. TOAST indicates stroke subtype according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria. Data were missing on Body Mass Index (n=377), hypertension (n=1), hypercholesterolemia (n=1), diabetes mellitus (n=1), history of CD (n=2), current smokers (n=45) and blood group (n=2)

Table 2. Imaging characteristics

	Carotid arteries (n=1928)	**Patients (n=964)**
Carotid plaque ulceration	136 (14%)	128 (13%)
Plaque calcification	920 (48%)	558 (58%)

Data are presented as n (%)

The determinants of VWF levels are presented in Table 3. A significant correlation was seen between plaque ulceration and VWF levels, after correction for age and gender ß=0.066 [0.003 - 0.129]; p=0.040). After additional adjustment for blood group, calcification volume and all cardiovascular risk factors with p value <0.05 plaque ulceration was not significant associated with VWF levels (ß=0.027 [-0.036 - 0.089]; p=0.403). Reanalysis in the patients with large artery atherosclerosis revealed no association between plaque ulceration and VWF levels.

Table 3. Determinants of Von Willebrand Factor.

Characteristics	Model I [a] Beta [95% CI]	p value	Model II [b] Beta [95% CI]	p value
Age, years	.008 [.007;.010]	<0.001	.005 [.003;.007]	.000
Female	.048 [-.002;.098]	.062	-	-
Body mass index (kg/m^2)	.001 [-.001;.004]	.329	-	-
Hypertension	.080 [.028;.133]	.003	.044 [-.007;.095]	.090
Hypercholesterolemia	.049 [-.004;.103]	.072	-	-
Diabetes mellitus	.074 [.002;.145]	.044	.047 [-.021;.114]	.175
History of CVD	.010 [-.044;.064]	.716	-	-
Current smoking	.010 [-.046;.066]	.735	-	-
Blood group O	-.266 [-.313;-.218]	.000	-.264 [-.311;-.217]	.000
TOAST classification				
Large artery atherosclerosis	.133 [.066;.200]	.000	.079 [-.039;.197]	.190
Cardio-embolism	.081 [.004;.159]	.040	.057 [-.065;.178]	.361
Small vessel occlusion	-.081 [-.143; -.018]	.012	-.071 [-.184; .042]	.217
Undetermined	-.054[-.105;.004]	.035	-.014[-.119;.092]	.798
Carotid plaque ulceration	.066 [.003;.129]	.040	.027 [-.036;.089]	.403
Calcification volume (mm^3)	.067 [.041;.092]	.000	.053 [.028;.079]	.000

[a] Adjusted for age and gender; [b] Adjusted for age, gender, blood group, calcification volume and all cardiovascular risk factors with p value <0.05 in model I.

Discussion

In the current study we evaluated whether plaque ulceration was related to an increased VWF level. Our findings showed no significant correlation between plaque ulceration and increased VWF levels after adjustment for age, gender, blood group and calcification volume.

Previous studies have demonstrated a strong correlation between ischemic stroke and a higher VWF levels [4]. Lip et al has shown that a high level of VWF has a prognostic value in patients with ischemic heart disease and inflammatory vascular disease. However, there is limited evidence that a higher level of VWF induces progression of vascular diseases[13]. Wieberdink et al. studied the correlation between the risk of stroke and VWF levels in the general population, and found a significant correlation also after adjustment for additional confounders.[19] Similar results were found in the study of Sonneveld et al., in which the association between the extent of atherosclerosis in the aortic arch and the carotid arteries and VWF levels was showed in patients with TIA or ischemic stroke. Higher levels of VWF were also associated with

a poor outcome.[15] This supports the hypothesis that VWF levels could be an index of endothelial damage in vascular disease. [20]

As high VWF levels increases the risk of ischemic stroke, [19] the question arises whether atherosclerotic plaque leads to changes in hemostasis and a subsequent increased risk of ischemic stroke, or whether both atherosclerotic plaque and hemostatic changes independently increase risk of ischemic stroke. The absence of an association between plaque ulceration and VWF levels after adjustment for calcifications does not support the hypothesis that VWF levels are affected by the presence of plaque ulceration and that a higher level of VWF an intermediate risk factor is in the pathway between atherosclerotic disease and ischemic stroke.

Strengths of our study are the large group of ischemic stroke patients, with availability of well-documented clinical information and plasma of almost all patients. The carotid arteries were analyzed with CTA, which gave us the opportunity to evaluate the vulnerable plaque characteristic plaque ulceration as well as a measure of atherosclerosis (calcification volume).

A limitation of our study is that patients with significant comorbidity, very severe stroke or those who died within 24h, were not included in this study. This resulted in a relatively less severely affected cohort and as a result less patients with ulcerations are included in this study resulting in dilution of a possible association.

In conclusion, atherosclerotic plaque ulceration in the carotid arteries is not, independently of calcification volume, related to higher levels of VWF in patients with TIA or ischemic stroke.

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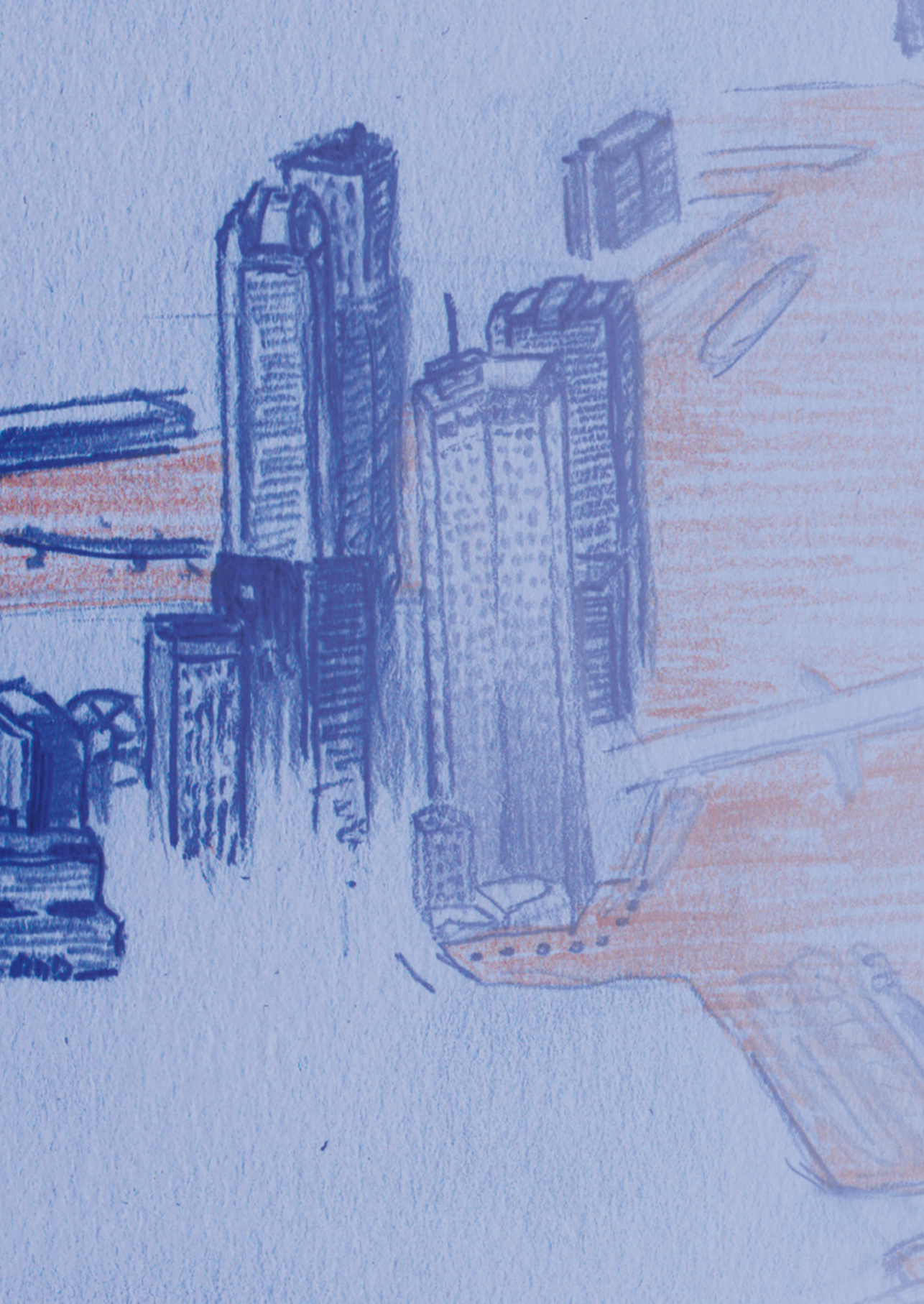
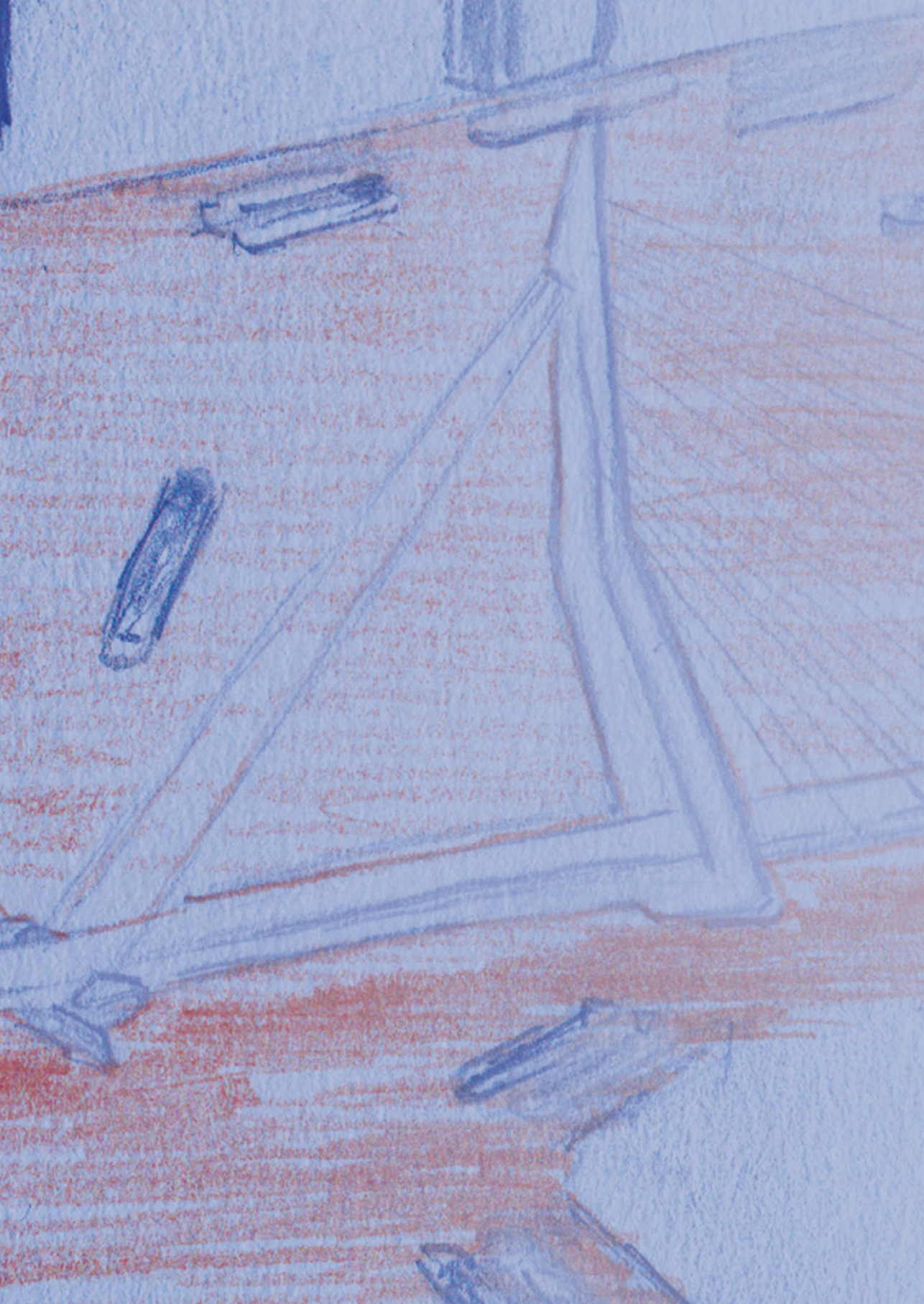


Chapter **3**

Comparison of non-invasive plaque imaging techniques



Chapter **3.1**

Intraplaque hemorrhage and the plaque surface in carotid atherosclerosis.

The Plaque At RISK study (PARISK)

A.C. van Dijk, M.T.B. Truijman, B. Hussain, T. Zadi, G. Saiedie, A.A.J. de Rotte, M.I. Liem, A.F.W. van der Steen, M.J.A.P. Daemen, P.J. Koudstaal, P.J. Nederkoorn, J. Hendrikse, M.E. Kooi, A. van der Lugt

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Abstract

Background and purpose: An important characteristic of vulnerable plaque, intraplaque hemorrhage, may predict plaque rupture. Plaque rupture can be visible on noninvasive imaging as a disruption of the plaque surface. We investigated the association between intraplaque hemorrhage and disruption of the plaque surface.

Materials and methods: We selected the first 100 patients of the Plaque At RISK study, an ongoing prospective noninvasive plaque imaging study in patients with mild-to-moderate atherosclerotic lesions in the carotid artery. In carotid artery plaques, disruption of the plaque surface (defined as ulcerated plaques and/or fissured fibrous cap) and intraplaque hemorrhage were assessed by using MDCTA and 3T MR imaging, respectively. We used a X2 test and multivariable logistic regression to assess the association between intraplaque hemorrhage and disrupted plaque surface.

Results: One hundred forty-nine carotid arteries in 78 patients could be used for the current analyses. Intraplaque hemorrhage and plaque ulcerations were more prevalent in symptomatic compared with contralateral vessels (hemorrhage, 38% versus 11%; $P < .001$; and ulcerations, 27% versus 7%; $P = .001$). Fissured fibrous cap was more prevalent in symptomatic compared with contralateral vessels (13% versus 4%; $P = .06$). After adjustment for age, sex, diabetes mellitus, and degree of stenosis, intraplaque hemorrhage was associated with disrupted plaque surface (OR, 3.13; 95% CI, 1.25–7.84) in all vessels.

Conclusions: Intraplaque hemorrhage is associated with disruption of the plaque surface in patients with a carotid artery stenosis of <70%. Serial studies are needed to investigate whether intraplaque hemorrhage indeed increases the risk of plaque rupture and subsequent ischemic stroke during follow-up.

Introduction

The need to identify patients with mild-to-moderate carotid artery stenosis and an increased stroke risk who might benefit from surgical treatment has shifted research interest from assessment of the degree of carotid stenosis to assessment of vulnerable plaque characteristics.[1] Vulnerable plaques are atherosclerotic plaques more prone to rupture and are associated with a higher risk for thromboembolism and ischemic stroke.[2,3] Intraplaque hemorrhage is an important characteristic of the vulnerable plaque.[4] Prevalence of intraplaque hemorrhage has been shown to be higher in symptomatic than in asymptomatic lesions.[5] Moreover, the presence of intraplaque hemorrhage in carotid artery disease is associated with an increased risk of cerebral ischemic events.[6-8]

The pathophysiologic mechanism leading to intraplaque hemorrhage is a topic of debate. However, a common viewpoint is that small leaky neovessels in the atherosclerotic plaques are a likely source of intraplaque hemorrhage.[5,9,10] The presence of intraplaque hemorrhage is thought to initiate several biologic processes like phagocytosis and local inflammation, leading to the release of proteolytic enzymes, deposition of free cholesterol and subsequently plaque growth, plaque destabilization, and possible plaque rupture.[5,9-12] Plaque rupture can be visible on imaging as a disruption of the atherosclerotic plaque surface (plaque ulceration and/or a fissured fibrous cap).[13,14]

A previous study reported that plaque ulceration on CTA was useful for the prediction of intraplaque hemorrhage on MR imaging in a broad group of symptomatic patients referred for carotid artery imaging.[15] Ulcerated plaques themselves are independently associated with an increased risk of ipsilateral ischemic events as well.[16,17]

The aim of the current study was to investigate the association between intraplaque hemorrhage, as assessed on MR imaging, and disruption of the plaque surface, assessed on MDCTA, in symptomatic patients with a carotid artery stenosis of <70%.

Materials and methods

Study population

Patients were derived from the Plaque At RISK (PARISK) study (clinical trials.gov, NCT01208025). Details of the PARISK study are previously described.[18] The PARISK study is an ongoing prospective multicenter cohort study focusing on the identification of patients with mild-to-moderate carotid artery stenosis with an increased risk of recurrent stroke by using noninvasive plaque imaging. Eligible for inclusion are patients with a TIA, including amaurosis fugax, or minor stroke in the carotid artery territory and a mild-to-moderate stenosis (30%– 69%) of the ipsilateral internal carotid artery. "TIA" was defined as an episode of temporary and focal cerebral dysfunction of vascular origin, lasting for a maximum 24 hours, leaving no persistent neurologic deficits. "Minor stroke" was defined as an episode of temporary and focal cerebral dysfunction of vascular origin, lasting for >24 hours or a nondisabling stroke with a

modified Rankin Scale score of ≤3. "Amaurosis fugax" was defined as a sudden loss of vision of presumed vascular origin and confined to 1 eye. The degree of stenosis was determined with clinically obtained Doppler sonography or MDCTA. The upper cutoff value of 70% was based on the NASCET criteria. The lower cutoff value was an atherosclerotic plaque with a thickness of at least 2–3 mm, which corresponds to a European Carotid Surgery Trial (ECST) stenosis of 30%.[19] Exclusion criteria were a probable cardiac source of embolism, a clotting disorder, severe comorbidity, standard contraindications for MR imaging, a documented allergy to MR imaging or CT contrast agents, or a renal clearance of <30 mL/min. Institutional review board approval was obtained in all university hospitals, and all patients gave written informed consent. For the current analyses, we selected the first 100 included patients.

Cardiovascular risk factors

"Hypercholesterolemia" was defined as fasting total cholesterol of >5 mmol/L or the use of cholesterol-lowering medication at the time of the TIA or ischemic stroke. We defined "hypertension" as systolic blood pressure of >140 mm Hg or a diastolic blood pressure of >90 mm Hg during 2 episodes of at least 15 minutes of continuous noninvasive blood pressure measurement or treatment with antihypertensive medication. "Diabetes mellitus" was defined as a fasting serum glucose level of >6.9 mmol/L, 2-hour postload glucose level of >11.0 mmol/L, or the use of antidiabetic medication. We assessed smoking status at the time of the TIA or ischemic stroke and dichotomized it into current smoker or no current smoker. In addition, we recorded body mass index, the use of cardiovascular medications, and medical history.

MDCTA data acquisition and analysis

We performed image acquisition by using a standardized protocol, as discussed in the study design article.[18] All MDCTA studies were evaluated by trained readers blinded to clinical data and other imaging tests. The MDCTA images were transferred to a workstation equipped with dedicated 3D analysis software (Leonardo and syngo. via; Siemens, Erlangen, Germany). The multiplanar reformatting application allowed analysis of both carotid arteries in oblique, coronal, and sagittal planes. Image quality was rated on a 3-point scale: 1) poor, defined as low contrast and major artifacts and not eligible for analysis; 2) moderate, defined as moderate artifacts and eligible for analysis; and 3) good, defined as few or no artifacts and eligible for analysis.

First, we evaluated the presence of an atherosclerotic plaque in both carotid arteries, defined as the presence of calcifications and/or thickening of the vessel wall (>≈1 mm). If present, disruption of the plaque surface was assessed in both arteries at the same time. "Disruption of the plaque surface," defined as the presence of plaque ulceration and/or a fissured fibrous cap, was assessed by 2 independent observers (B.H., 12 months, and A.C.v.D., 4 years of experience); discrepancies were solved by consensus and/or an experienced third observer (A.v.d.L, 10 years of experi-

ence). We defined "plaque ulceration" as an extension of contrast material of >1 mm into the atherosclerotic plaque on at least 2 orthogonal planes (Fig 1).[13,20] We defined "fissured fibrous cap" according to the criteria of Saba and Mallarini, extension of contrast material of <1 mm into the atherosclerotic plaque and an angle of >230° with the lumen (Fig 2).[21]

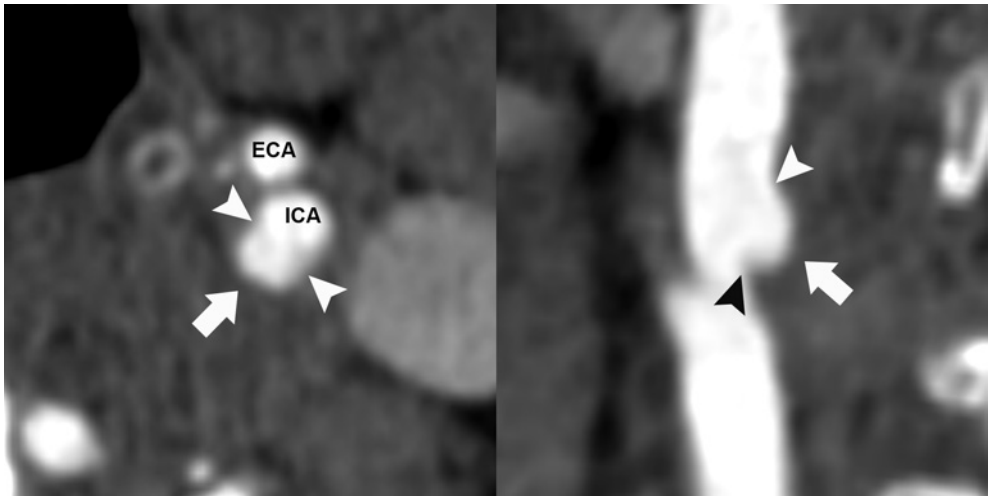

Figure 1. Plaque ulceration on MDCTA. Transversal (left) and longitudinal (right) MDCTA images of the carotid bifurcation. A "plaque ulceration," defined as the extension of contrast material in the atherosclerotic plaque, is visible on both planes (arrows; arrowheads indicate the edges of the plaque ulceration). ECA indicates external carotid artery.

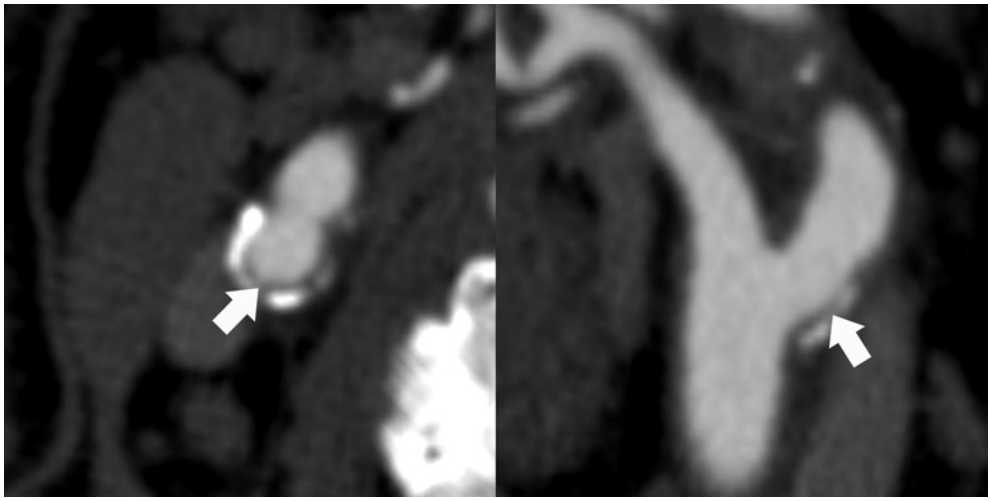

Figure 2. Fissured fibrous cap on MDCTA. Transversal (left) and longitudinal (right) MDCTA images of the carotid bifurcation. A "fissured fibrous cap," defined as an extension of contrast material of <1 mm into the atherosclerotic plaque and an angle of >230° with the lumen, is visible only on the transversal plane (arrows).

Interobserver variability for the initial review was moderate (K = 0.41) for the detection of disruption of the plaque surface (plaque ulceration and/or fissured fibrous cap), moderate (K = 0.46) for the detection of plaque ulceration, and fair (K = 0.24) for the detection of fissured fibrous cap. Moreover, the most severe stenosis in the carotid bifurcations and internal carotid arteries was measured according to the ECST and NASCET criteria, perpendicular to the central lumen line.[19,22] A custom-made plug-in for the freely available ImageJ software (National Institutes of Health, Bethesda, Maryland) was used to quantify calcifications in both carotid arteries within 3 cm proximal and distal to the bifurcation. We used a threshold of 600 HU to differentiate calcifications from contrast material in the lumen; calcification volume was expressed in cubic millimeters. A detailed description of the measurements is provided elsewhere.[23]

MR imaging data acquisition and analysis

Image acquisition was performed on a 3T MR imaging system (Achieva; Philips Healthcare, Best, the Netherlands; or Discovery MR 750; GE Healthcare, Milwaukee, Wisconsin). A multi- sequence contrast-enhanced protocol was used; a detailed description of this protocol is provided in the study design article.[18] For this study, we used the 3D-T1W fat suppressed spoiled gradient echo sequence (GE Healthcare) or the 2D-T1W inversion recovery turbo field echo sequence (Philips Healthcare). A 3D volume of the extracranial carotid artery (GE Healthcare) or 15 transverse adjoining sections of 2 mm each covering the entire plaque (Philips Healthcare) were imaged.

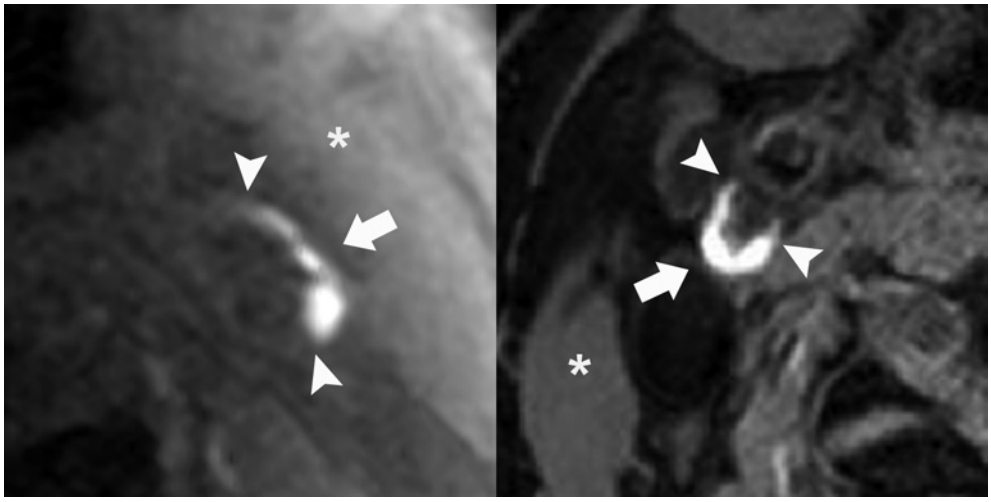

Figure 3. Intraplaque hemorrhage on MR imaging. Carotid bifurcations of 2 patients: left, the 3D-T1W fat suppressed spoiled gradient echo sequence (Discovery MR 750; GE Healthcare), and right, the 2D-T1W inversion recovery turbo field echo sequence (Achieva; Philips Healthcare). In both patients, intraplaque hemorrhage is present (arrows; arrowheads indicate the edges of the intraplaque hemorrhage), defined as a hyperintense signal in the atherosclerotic plaque compared with the sternocleidomastoid muscle (asterisk).

All MR imaging studies were evaluated by a trained reader (M.T.B.T., 4 years of experience) blinded to clinical data and other imaging tests. MR images were reviewed by using a standard DICOM viewer. Image quality was rated on a 5-point scale: 1) low SNR, limits use, arterial wall and vessel margins unidentifiable; 2) marginal SNR, arterial wall visible, with the substructure, lumen, and outer boundaries indistinct; 3) marginal SNR, wall structures identifiable with the lumen and outer boundaries partially obscured; 4) high SNR with minimal artifacts; vessel wall, lumen, and adventitial margins well-defined; and 5) high SNR without artifacts, wall architecture depicted in detail, lumen and adventitial boundary clearly defined.24 Intraplaque hemorrhage was scored in both arteries at the same time and was defined as a hyperintense signal in the plaque compared with the adjacent sternocleidomastoid muscle (Fig 3).[24] In 47 vessels, the presence of intraplaque hemorrhage was assessed by a second independent observer (A.C.v.D, 4 years of experience; the minimum interval between MDCTA and MR imaging scores was 8 months) to assess interobserver variability, and an excellent agreement was found (K = 0.95; 95% CI, 0.86 –1.00).

Statistical analysis

Baseline characteristics are shown for all patients; vessel characteristics are shown for all vessels and for symptomatic and contralateral vessels separately. Data are presented as mean ± SD, median (25th–75th percentile), or number of patients (percentage). Differences between symptomatic and contralateral vessels were evaluated by using a X2 test for categorical data and a Student t test or Mann-Whitney U test for continuous data. For the analysis of calcification volume, we used natural log-transformed values and added 1.0 mm3 to the nontransformed values to deal with participants with a calcification volume of zero. First, we used a X2 test to assess the association between intraplaque hemorrhage and disrupted plaque surface (ulcerated plaques and/or a fissured fibrous cap) in all vessels. We used all vessels because we assumed that the underlying pathophysiologic mechanism would be similar in symptomatic and contralateral vessels. Additionally, a logistic regression was used to further investigate the association between intraplaque hemorrhage, other plaque characteristics, and cardiovascular risk factors on the one hand and disrupted plaque surface on the other. We used a generalized estimation equation approach with an unstructured correlation matrix to adjust for the correlation between both carotid arteries in each patient. Adjustments were made for age and sex (model 1) supplemented with all variables with a P value < .10 in model 1 (model 2, included the degree of stenosis according to the ECST criteria to correct for differences in stenosis).

Analyses were repeated to assess the association between intraplaque hemorrhage and ulcerated plaques alone. In addition, analyses were repeated for the symptomatic artery. The location of intraplaque hemorrhage and disrupted plaque surface was visually correlated by 2 independent observers after manual alignment of the MR imaging and MDCTA scans based on vessel geometry and the location of the plaque. Statistical analyses were performed by using STATA software (Version 13.1; StataCorp, College Station, Texas). P < .05 was considered statistically significant.

Results

Patient characteristics

In 20 of the 100 patients, MDCTA (n = 18) or MR imaging (n = 2) of the carotid arteries had not been performed due to contra-indications. Two patients were excluded due to inferior image quality of the MDCTA. Of the remaining 78 patients, 149 vessels could be used for the current analyses. Seven contralateral vessels were excluded from analysis due to the absence of plaque (n = 5) or occlusion of the carotid artery (n = 2). Baseline characteristics are shown in Table 1. Forty-one of the 78 patients (53%) had an ischemic stroke; 37 (47%) patients had a TIA, including 7 with amaurosis fugax.

Table 1. Clinical characteristics of patients (n=78) [a]

Clinical characteristic	
Age (years)	67 ± 9
Male sex	57 (73%)
Classification event	
TIA	30 (38%)
Stroke	41 (53%)
Amaurosis fugax	7 (9%)
Hypercholesterolemia	43 (55%)
Hypertension	56 (72%)
Diabetes Mellitus	19 (24%)
Current smoking	
No	58 (74%)
Yes	20 (26%)
Body mass index	25.2 [24.3-28.4]
Current use	
Antiplatelet therapy	36 (46%)
Oral anticoagulants	0 (0%)
Statins	36 (46%)
Antihypertensive medication	50 (64%)
Antidiabetic medication	13 (17%)
History	
Ischemic stroke or TIA	13 (17%)
Ischemic heart disease	16 (21%)
Peripheral arterial disease	15 (19%)

[a] Data are mean ± SD, absolute numbers of patients (%) or median [25th-75th percentile]

Vessel characteristics

The median interval between the neurologic event and MDCTA was 32 days (25th to 75th percentile, 14 –56 days); the median interval between the event and MR imaging was 44 days (25th to 75th percentile, 27– 62 days). Characteristics of all vessels and symptomatic and contralateral vessels separately are shown in Table 2. The mean severity of stenosis was 51% ± 17% (ECST). The prevalence of intraplaque hemorrhage in the symptomatic and contralateral vessels was 38% and 11%, respectively. Twenty-six of the 149 vessels (17%) showed plaque ulceration; the prevalence in the symptomatic vessels and the contralateral vessels was 27% and 7%, respectively. In 13 of the 149 vessels (9%), a fissured fibrous cap was present; most were found in the symptomatic vessels (n = 10), but the difference was not significant (P = .06).

Table 2. Vessel characteristics[a]

Vessel characteristic	All vessels	Symptomatic vessels	Contralateral vessels	P value, symptomatic vs contralateral vessels
No.	149	78	71	
Plaque ulceration	26 (17%)	21 (27%)	5 (7%)	.001[b]
Fissured fibrous cap	13 (9%)	10 (13%)	3 (4%)	.06
Calcium volume (mm^3)	21.0 [3.6-56.2]	24.2 [6.2-71.8]	17.2 [1.9-53.1]	.09
Degree of stenosis (ECST) (%)	51 ± 17	55 ± 17	47 ± 16	.004[b]
Degree of stenosis (NASCET) (%)	8 [0-32]	14 [0-35]	2 [0-26]	.03[b]
Intraplaque hemorrhage	38 (26%)	30 (38%)	8 (11%)	<.001[b]

[a] Data are absolute numbers of vessels (%), median [25th-75th percentile] or mean ± SD; [b] p<.05

Intraplaque hemorrhage and disrupted plaque surface

In Table 3, the association between the presence of intraplaque hemorrhage and disrupted plaque surface (ulcerated plaque and/or fissured fibrous cap) is shown. In vessels with intraplaque hemorrhage, a disrupted plaque surface was significantly more prevalent compared with vessels without intraplaque hemorrhage (45% versus 15%; P < .001). Table 4 shows the results of the multivariable logistic regression. After correction for age and sex, the presence of intraplaque hemorrhage was associated with a disrupted plaque surface (OR, 3.98; 95% CI, 1.73–9.16; P = .001). Additionally, we found an association between the degree of stenosis and disrupted plaque surface (OR per 10% increase 1.42; 95% CI, 1.09 –1.84; P = .009). After correction for age, sex, and all variables with P < .10, intraplaque hemorrhage was still signifi-

cantly associated with disrupted plaque surface (OR, 3.13; 95% CI, 1.25–7.84; P = .02). Diabetes mellitus was inversely associated with disrupted plaque surface (OR, 0.31; 95% CI, 0.11– 0.94; P = .04). Similar results were found when the analyses were repeated to assess the association between intraplaque hemorrhage and ulcerated plaque alone. The association between intraplaque hemorrhage and disrupted plaque surface was attenuated when the analyses were repeated in only the symptomatic arteries (Supplementary Tables 1–3). The location of the plaque ulceration and/or fissured fibrous cap was the same as that of the intraplaque hemorrhage in 16 of the 21 lesions (76%); an example is shown in Fig 4.

Table 3. Association of intraplaque hemorrhage and disrupted plaque surface in all vessels[a]

	Disrupted plaque[b]	**Intact plaque surface**	**Total**
Intraplaque hemorrhage present (n=38)	17 (45%)	21 (55%)	38
Intraplaque hemorrhage absent (n=111)	17 (15%)	94 (85%)	111
Total	34	115	149

[a] p<0.001; [b] defined as ulcerated plaques and/or fissured fibrous cap

Table 4. Multivariable OR for association among clinical characteristics, vessel characteristics and disrupted plaque surface in all vessels

	Multivariable (Age, sex)		**Multivariable[a] (Age, sex, factors p<.10)**	
Characteristic	**OR [95% CI]**	**P value**	**OR [95% CI]**	**P value**
Age	1.05 [1.00-1.10]	.07	1.05 [1.00-1.10]	.07
Sex	0.42 [0.15-1.12]	.08	0.50 [0.17-1.42]	.19
Hypertension	1.98 [0.73-5.35]	.18		
Diabetes Mellitus	0.40 [0.14-1.10]	.08	0.31 [0.11-0.94]	.04[b]
Hypercholesterolemia	1.41 [0.63-3.15]	.40		
Current smoking	0.48 [0.16-1.46]	.20		
Intraplaque hemorrhage	3.98 [1.73-9.16]	.001[b]	3.13 [1.25-7.84]	.02[b]
Degree of stenosis (ECST, per 10%)	1.42 [1.09-1.84]	.009[b]	1.30 [1.00-1.69]	.07
Calcification volume	0.86 [0.69-1.08]	.21		

[a] We corrected for age, sex, diabetes mellitus and degree of stenosis (ECST); [b] P < .05

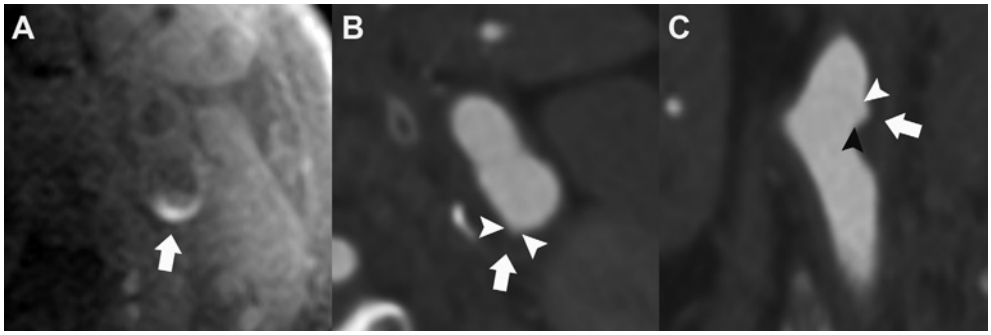

Figure 4. Correlated intraplaque hemorrhage on MR imaging and plaque ulceration on MDCTA. An example of MR imaging and MDCTA images of the left carotid bifurcation. On the 3D-T1W fat suppressed echo-spoiled gradient echo MR imaging sequence, intraplaque hemorrhage is visible in the atherosclerotic plaque (A, arrow). MDCTA images show a small ulceration on both the transversal and longitudinal plane at the site of the intraplaque hemorrhage (B and C, arrows; arrowheads indicate the edges of the plaque ulceration).

Discussion

This study shows that intraplaque hemorrhage is associated with a disrupted plaque surface (plaque ulceration and/or fissured fibrous cap) and plaque ulceration alone. In 76% of the vessels with both intraplaque hemorrhage and a disrupted plaque surface, intraplaque hemorrhage and plaque ulceration or fissured fibrous cap shared the same location. The association between these 2 vulnerable plaque characteristics may support the notion that intraplaque hemorrhage increases the risk of plaque rupture. However, because of the cross-sectional study design, serial studies are needed to further investigate the role of intraplaque hemorrhage in plaque rupture.

Some methodologic issues need to be discussed first. A strength of our prospective study is that most vessels showed a mild stenosis. Most previous studies focused on patients with a moderate or severe carotid artery stenosis. However, plaque imaging in this patient group is less relevant for clinical decision making because patients with a moderate (male patients) or severe (all patients) symptomatic carotid artery stenosis are already eligible for carotid endarterectomy.[16] Moreover, the amount of calcification will probably be higher in patients with a moderate or severe carotid artery stenosis, complicating the detection of plaque surface morphology. Second, we reviewed MDCTA and MR images of all patients with a disrupted plaque surface and intraplaque hemorrhage to investigate whether the intraplaque hemorrhage and plaque disruption were at the same location. The finding that the location was indeed similar in 76% strengthens the association between the 2 vulnerable plaque characteristics.

A limitation of our multicenter study is that we used different MDCTA and MR imaging scanners. MDCTA scanning protocols, however, were similar. We used the 3D-T1W fat suppresssed spoiled gradient echo or the 2D-T1W inversion recovery turbo field echo MR imaging sequence to detect intraplaque hemorrhage. Bitar et al. and Cappendijk et al. used sequences like ours and found a good sensitivity, specific-

ity, and interobserver agreement for the detection of intraplaque hemorrhage in both sequences.[25,26] They also found good agreement between MR imaging– depicted intraplaque hemorrhage and histologic sections. In addition, our overall prevalence of intraplaque hemorrhage of 26% is in accordance with that in the literature.[4,5,15] Correction for MR imaging protocol used in the multivariate model did not change our results. A second limitation is the lack of histology. We used MDCTA instead of MR imaging for the evaluation of the plaque surface, because MDCTA has been shown to have a high sensitivity and specificity for the detection of plaque ulceration and has fewer limitations such as partial volume effects and flow artifacts that mimic plaques.[27,28] In addition, when we reviewed the MR images and MDCTA images side by side, plaque ulcerations were hardly visible on the MR images. Saba et al. showed a good sensitivity and specificity for the detection of plaque ulceration by using MDCTA and multiplanar reconstruction (sensitivity, 75.8%; specificity, 90.8%).[27] We found an overall prevalence of plaque ulceration of 17%. Similar prevalence of plaque ulceration was found in other studies, ranging from 13% to 22%.[15,20,29,30] Detection of the presence of a fissured fibrous cap has been studied less intensively. We found an overall prevalence of the presence of a fissured fibrous cap of 9%, in agreement with the 11% found by Saba and Mallarini.[20] Rupture of the fissured fibrous cap was significantly associated with cerebrovascular symptoms ($P = .003$).[20] Based on the good sensitivity and specificity of MDCTA for plaque ulcerations and the good agreement between MR imaging–depicted intraplaque hemorrhage and histology, we therefore conclude that it is legitimate to use imaging techniques such as MDCTA and MR imaging to study the relationship between intraplaque hemorrhage and the disruption of the plaque surface.[25,26] Moreover, the use of MDCTA and MR imaging provides the opportunity to investigate the relationship in a specific category of patients—that is, those with a mild-to-moderate carotid artery stenosis, patients who are treated medically and thus have no available histology. The final limitation of our study is that it is cross-sectional. Our results should therefore be confirmed in a serial study.

Rupture of an atherosclerotic carotid artery plaque is an important cause of ischemic stroke. Therefore, understanding the pathophysiology of plaque rupture is very important. Infiltration of macrophages in pathologic intima thickening plays a key role in the development of atherosclerotic plaques. The combination of macrophage infiltration, apoptosis, and hypoxia-induced necrosis leads to the development of more advanced atherosclerotic plaques with a lipid-rich necrotic core. Previous studies already showed an association between lipid-rich necrotic core volumes and plaque ulceration.[29-31] In these larger plaques, hypoxia and the inflammatory response are assumed to promote neovascularization. As stated previously, the common viewpoint is that these small leaky neovessels are responsible for the occurrence of intraplaque hemorrhage and subsequently the development of an unstable rupture-prone plaque.[5,9-12,32] Our results—if confirmed in serial studies— can support the pathophysiologic relation between intraplaque hemorrhage and disrupted plaque surface. An alternative viewpoint to explain this relationship is that repeated fissuring of the plaque and associated formation of nonocclusive luminal thrombus incorporated in the plaque could be the cause of intraplaque hemorrhage.[5] Nevertheless, observations that intraplaque hemorrhage is related to high attenuation of plaque neovessels, in the absence of plaque fissuring, supports the more common view of small leaky neovessels as the cause of intraplaque hemorrhage.[5,10]

Conclusions

We showed that intraplaque hemorrhage is associated with a disrupted plaque surface (plaque ulceration and/or fissured fibrous cap) in patients with a <70% carotid artery stenosis. Our findings suggest a strong association between these 2 vulnerable plaque characteristics. Nevertheless, because of the cross-sectional study design, additional serial studies are needed to evaluate whether intraplaque hemorrhage indeed increases the risk for plaque rupture and subsequent TIA or ischemic stroke.

Supplementary information

Supplementary Table I. Association intraplaque hemorrhage and disrupted plaque surface/plaque ulceration in symptomatic vessels

	Disrupted plaque[1]	Ulceration present
Intraplaque hemorrhage present (n=30)	15 (50%)	11 (37%)
Intraplaque hemorrhage absent (n=48)	12 (25%)	10 (21%)
p value	p=0.02	p=0.13

[1]defined as ulcerated plaques and/or fissured fibrous cap

Supplementary Table II. Multivariable OR for association between clinical characteristics, vessel characteristics and disrupted plaque surface in symptomatic vessels

	Multivariable (Age, gender)	
Characteristic	**OR [95% CI]**	**P value**
Age	1.04 [0.98-1.10]	0.24
Gender	0.52 [0.16-1.63]	0.26
Hypertension	1.87 [0.58-5.98]	0.29
Diabetes Mellitus	0.35 [0.10-1.24]	0.11
Hypercholesterolemia	1.21 [0.45-3.27]	0.71
Current smoking	0.31 [0.08-1.23]	0.10
Intraplaque hemorrhage	2.63 [0.97-7.09]	0.06
Degree of stenosis (ECST, per 10%)	1.30 [0.91-1.69]	0.12
Calcification volume (per mm^3)	0.76 [0.56-1.04]	0.09

ECST, European Carotid Surgery Trial

Supplementary Table III. Multivariable OR for association between clinical characteristics, vessel characteristics and plaque ulceration in symptomatic vessels

	Multivariable (Age, gender)	
Characteristic	**OR [95% CI]**	**P value**
Age	1.02 [0.96-1.09]	0.52
Gender	0.57 [0.17-1.95]	0.37
Hypertension	1.19 [0.36-3.92]	0.77
Diabetes Mellitus	0.08 [0.01-0.70]	0.02*
Hypercholesterolemia	0.62 [0.21-1.78]	0.37
Current smoking	0.25 [0.05-1.28]	0.10
Intraplaque hemorrhage	1.99 [0.70-5.65]	0.20
Degree of stenosis (ECST, per 10%)	1.09 [0.83-1.42]	0.53
Calcification volume (per mm^3)	0.66 [0.47-0.93]	0.02*

ECST, European Carotid Surgery Trial; * $p<0.05$

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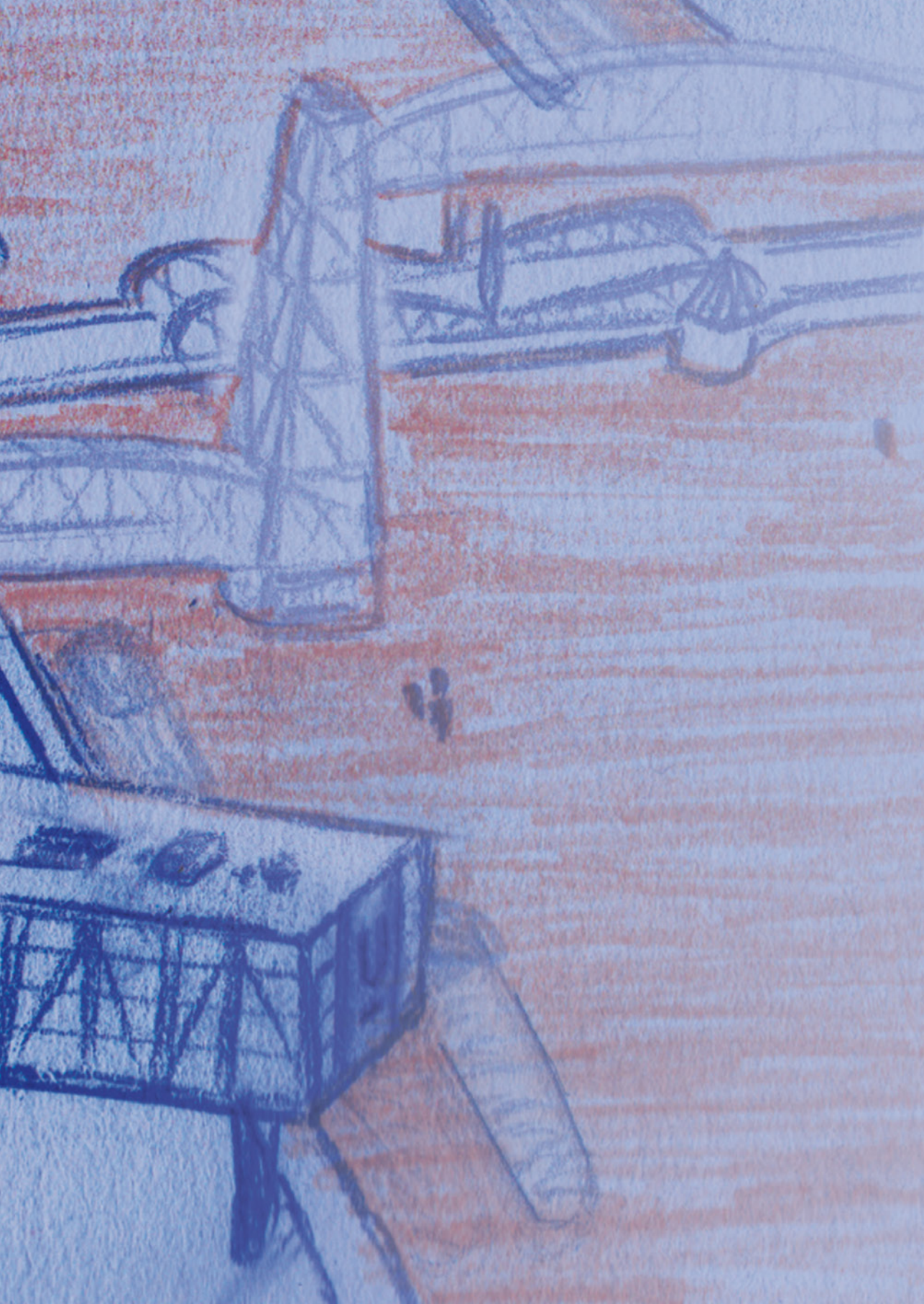

Chapter **3.2**

Imaging of atherosclerotic calcifications: MRI versus MDCTA.

The Plaque At RISK study (PARISK)

A.C. van Dijk, A.M.G. van Hattem, F.H.B.M. Schreuder, A.A.J. de Rotte, M.T.B. Truijman, M.I. Liem, T. Zadi, A.F.W. van der Steen, M.J.A.P. Daemen, P.J. Koudstaal, P.J. Nederkoorn, J. Hendrikse, M.E. Kooi, A. van der Lugt

Submitted

Abstract

Introduction: MRI is increasingly used for atherosclerotic plaque characterization. Previous studies suggest that MRI is less suitable than MDCTA for the detection of plaque calcifications. We investigated the agreement between MRI and MDCTA in the detection of calcifications in patients with carotid artery stenosis.

Methods: We assessed calcification volume (mm^3) in the symptomatic carotid artery in 185 patients of the PARISK-study with both MDCTA and multi-sequence contrast-enhanced MRI. Sensitivity and specificity of MRI to detect calcifications were estimated, using MDCTA as reference. We evaluated correlation and differences in calcification volume between MRI and MDCTA.

Results: Prevalence of calcifications on MRI and MDCTA was 90%. Sensitivity and specificity of MRI to detect calcifications were 93% (95%CI: 89-97%) and 39% (95%CI: 17-64%). Mean calcification volume on MRI was 58.7±71.0 mm^3 and similar to MDCTA (58.1±87.7mm^3; p=0.88). However, absolute individual differences could be large (mean difference -0.6 mm^3 (range -154.6 to +279.4mm^3)).

Conclusions: MRI has good sensitivity to detect plaque calcifications. Overall agreement in calcification volume assessed with MRI and MDCTA was good, but individual differences were substantial. MRI presently cannot be recommended to estimate plaque calcification volume in individual patients with carotid artery stenosis.

Introduction

Currently, degree of carotid artery stenosis is the main imaging characteristic to select patients with an ischemic stroke for an invasive carotid endarterectomy (CEA). In the last two decades, evidence has accumulated that focusing on the composition and morphology of the atherosclerotic plaque may help to expand knowledge of the pathophysiology of ischemic stroke caused by large artery atherosclerosis and may aid in risk stratification.[1, 2] Extensive imaging research has been performed to characterize the atherosclerotic carotid plaque beyond degree of stenosis. Atherosclerosis is a chronic inflammatory disease of the large arteries with different stages of plaque development. In the more advanced atherosclerotic plaques, calcifications are formed.[3, 4] Carotid calcifications are associated with degree of carotid artery stenosis and are increasingly used as a marker of atherosclerosis.[5-7] (Macro)calcifications are considered to be plaque stabilizers, as shown by a decrease in cardiovascular risk with an increase in coronary calcification density.[8, 9] In addition, the proportion of plaque calcification is inversely associated with the occurrence of symptoms in patients with a ≥50% carotid artery stenosis according to the NASCET criteria.[10, 11] Therefore, plaque calcifications are an important characteristic of the atherosclerotic plaque and may become one of the imaging biomarkers to estimate stroke risk in patients with carotid stenosis.

Two non-invasive image modalities used for plaque imaging are Multi-Detector Computed Tomography Angiography (MDCTA) and Magnetic Resonance Imaging (MRI). MDCTA is already extensively used for the assessment of carotid artery stenosis. In addition, it is an excellent tool for the detection of atherosclerotic plaque ulceration and the detection and quantification of plaque calcifications.[6, 12-15] The Agatston score (measure of calcification burden assessed at CT) is already used extensively in clinical and research practice.[13, 16] Studies have shown that calcification volume measurements assessed on CT are even more reproducible than Agatston score.[13, 16-18] Nevertheless, histological validation studies of plaque calcifications in the carotid artery assessed at CT are sparse and it is known that CT probably overestimates calcification volume due blooming effects.[15, 19-21] MRI of the carotid atherosclerotic plaque is not yet performed in clinical practice, but due to the excellent soft tissue contrast, it is a very promising image modality to assess plaque composition (lipid-rich necrotic core, intraplaque hemorrhage, calcifications, fibrous tissue).[22] Although atherosclerotic calcifications can be assessed both with MDCTA and MRI, to date MRI showed lower diagnostic accuracy in detecting and quantifying atherosclerotic calcifications.[22-25] MRI plaque imaging techniques are, however, rapidly improving. In the current study, we aimed to assess the current diagnostic accuracy of MRI plaque imaging compared to MDCTA in detecting calcifications, in patients with a recently symptomatic carotid artery stenosis. In addition, we investigated the correlation between calcification volumes assessed at MRI and MDCTA.

Methods

Study population

Patients were derived from the PARISK-study (Plaque-At-RISK; clinical trials.gov NCT01208025). Details of the study design have been previously described.[26] The PARISK-study is a prospective multicenter diagnostic cohort study using non-invasive plaque imaging to identify patients with an ipsilateral carotid plaque of at least 2-3 mm and a mild-to-moderate carotid artery stenosis (<70%) with an increased risk of recurrent stroke. All included patients had a recent TIA, including amaurosis fugax, or minor stroke in the carotid artery territory prior to inclusion. Institutional Review Board approval was obtained and all patients gave written informed consent.

MDCTA data acquisition and analysis

We performed image acquisition using a standardized protocol, as discussed in the study design paper.[26] All MDCTA studies were evaluated by trained readers blinded for clinical data and other imaging tests. Image quality was rated on a three point scale; poor (1) defined as low contrast and major artefacts and not eligible for analysis, moderate (2) defined as moderate artefacts and eligible for analysis and good (3) defined as little or no artefacts and eligible for analysis. The most severe stenosis in the carotid bifurcation and internal carotid artery was measured according to the ECST criteria as well as the NASCET criteria, perpendicular to the central lumen line.[11, 27] A custom-made plug-in for the freely available software ImageJ (Rasband, National Institute of Mental Health, Bethesda, MD) was used to semi-automatically quantify calcifications in the symptomatic carotid artery within 3 cm proximal and distal of the bifurcation to ensure the complete scan range of MRI (variable segments of common and internal carotid artery) was covered. We used a threshold of 600 Hounsfield units to differentiate calcifications from contrast material in the lumen. Calcification volume was expressed in millimeter cubed. A detailed description of the measurements is provided elsewhere.[28]

MRI data acquisition and analysis

Image acquisition was performed on a 3T MRI system (General Electric (GE) Healthcare, Milwaukee, MI, United States of America or Philips Healthcare, Best, Netherlands). A multi-sequence contrast-enhanced protocol was used. A detailed description of this protocol is provided in the study design paper.[26] All MRI carotid artery studies were evaluated by trained readers blinded for clinical data and other imaging tests using dedicated vessel wall analysis software (Vesselmass, Department of Radiology, Leiden University Medical Center, Netherlands).

Information of all five MRI carotid artery sequences was used (GE; 3D-FSPGR, 3D-T1w-FS-SPGR, 2D-T2w-DIR-FSE, 2D-T1w-DIR-FSE pre- and postcontrast; Philips;

3D-TOF-FFE, 3D-T1w-IR-TFE, 2D-T2w TSE, 2D-T1w-QIR-TSE pre- and postcontrast). MR images were automatically registered by delineating the lumen and outer vessel wall. Registration was manually corrected if needed. Plaque components (lipid-rich necrotic core, calcifications, intraplaque hemorrhage) were manually segmented. Calcifications were defined as areas with hypointense signal relative to the signal of the adjacent sternocleidomastoid muscle in at least two different weightings. Fifteen transverse adjoining slices of 2 mm each (total carotid artery coverage of 3 cm) covering the entire plaque (various segments of common and internal carotid artery) were annotated. Calcification volumes (expressed in millimeter cubed) could be derived from these annotations.

Statistical analysis

Baseline clinical characteristics are listed for all patients. Imaging characteristics are shown for the symptomatic carotid artery. Data are presented as mean ± SD, median [25th-75th percentile] or number of patients (%). A 2x2 table was used to calculate sensitivity and specificity including 95% confidence intervals (95% CIs) for MRI to detect calcifications with MDCTA as the reference standard. Correlation between calcification volume on MRI and calcification volume on MDCTA was analyzed using a Pearson correlation coefficient. Difference in calcification volumes on MRI and MDCTA were evaluated with paired t-test and a Bland-Altman plot. Analyses were repeated for the two different MRI protocols acquired on two different scanners (GE versus Philips), because one of the sequences used for the detection of plaque calcifications differed between both protocols (3D-FSPGR vs 3D-TOF-FFE). Thereafter, individual MRI and MDCTA scans of patients with absence of calcifications on one modality and presence of calcifications on the other modality (mismatch in detection of calcifications) and patients with a difference of more than 50 mm^3 between calcification volumes of both image modalities were visually compared by an experienced observer (AD; 3 years) to assess possible reasons for these discrepancies. Statistical analyses were performed using STATA software (version 14.1, STATA). $P<0.05$ was considered statistically significant.

Results

Patient characteristics

Between September 2010 and December 2014, 240 patients were included in the PARISK-study. In 193 patients both an MDCTA and MRI of the carotid arteries was performed. Two patients did not undergo MDCTA and MRI of the carotid arteries due to contra-indications for both modalities (n=1) or a sudden neurological deficit during the MRI of the brain (n=1). One patients refused to further participate in the study after signing the informed consent; in 8 patients MRI was not performed and 36 patients lacked MDCTA due to contra-indications for the image modality. Of the 193

available MDCTA and MRI scans, MDCTA or MRI calcification scores were missing in 8 patients due to bad image quality (n=2 and n=5, respectively) and a technical problem with measuring the amount of calcification on MDCTA (n=1). Finally, 185 patients could be used for the current analyses. Baseline clinical and imaging characteristics are shown in Table 1.

Table 1. Baseline characteristics (n=185)

Characteristic	
Age (year)	68 ± 8
Male	131 (71%)
Classification event	
TIA	78 (42%)
Stroke	89 (48%)
Amaurosis fugax	18 (10%)
MRI protocol	
GE (3D-FSPGR)	46 (25%)
Philips (3D-TOF-FFE)	139 (75%)
Degree of stenosis, ECST (%)	55 [47;66]
Degree of stenosis, NASCET (%)	12 [0;30]
Interval event – MDCTA (days)	33 [16;55]
Interval event – MRI (days)	47 [30;66]
Interval MDCTA – MRI (days)	1 [0;15]
Presence calcifications MDCTA	167 (90%)
Calcification volume MDCTA (mm^3)	58.1 ± 87.7
Presence calcifications MRI	167 (90%)
Calcification volume MRI (mm^3)	58.7 ± 71.0

Data are mean ± SD, absolute numbers of patients (%), or median [25^{th};75th percentile]
TIA Transient Ischemic Attack, GE General Electric, ECST European Carotid Surgery Trial, NASCET North American Symptomatic Carotid Endarterectomy Trial

Sensitivity and specificity of MRI for detection of calcifications

Prevalence of calcifications on MRI as well as MDCTA was 90% (Table 1). In 156 of the 185 (84%) patients, calcifications were present on both MRI as well as MDCTA; in 7 (4%) patients calcifications were absent in both modalities (Table 2). Sensitivity of MRI to detect calcifications compared to MDCTA was 93% (95% CI: 89-97%); specificity of MRI to detect calcifications compared to MDCTA was 39% (95% CI: 17-64%). Repeating the analyses for the two different MRI protocols showed similar results.

Table 2. Comparison presence calcifications MDCTA and MRI (n=185)

	MDCTA calcifications present	MDCTA calcifications absent	Total
MRI calcifications present	156	11	167
MRI calcifications absent	11	7	18
Total	167	18	185

Comparison of calcification volumes on MRI and MDCTA

A good correlation between calcification volume on MRI and MDCTA was found (ρ=0.81; p<0.0001). Mean calcification volume on MRI was 58.7 ± 71.0 mm^3 and similar to mean calcification volume on MDCTA 58.1 ± 87.7 mm^3 (p=0.88). However, individual absolute differences between calcification volume on MRI and MDCTA were substantial (mean difference -0.6 ± 51.3 mm^3, range -154.6 to +279.4 mm^3; Figure 1). Repeating these analyses for the two different MRI protocols showed similar results.

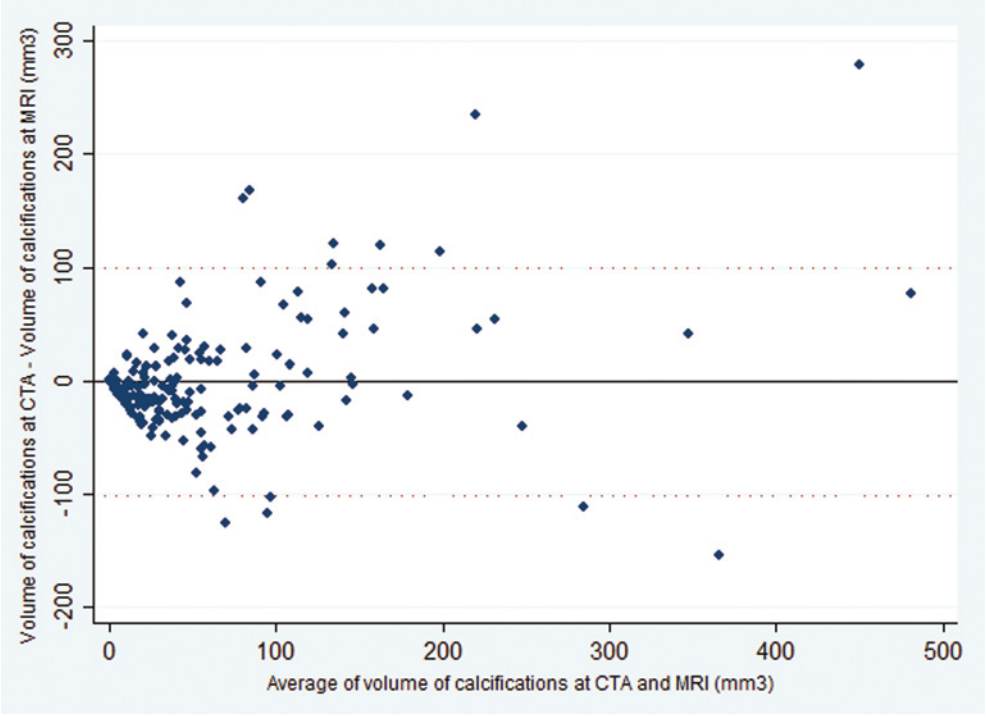

Figure 1. Bland-Altman plot of difference of MDCTA and MRI measurements of calcifications (n=185)

Possible reasons for a mismatch or large difference in MRI and MDCTA calcifications

In 51 patients (28%) a mismatch regarding the detection of calcifications (n=22) or a difference in calcification volume between MRI and MDCTA of more than 50 mm^3 (n=29) was found. In 29 of these patients, two possible reasons for the mismatch or difference were identified, and in one patient three reasons. A total of 82 possible reasons for the mismatch or large difference (in the 51 patients) are identified and presented in Table 3 and 4. MDCTA and MR images to illustrate these explanations for a mismatch in presence of calcifications or difference of more than 50 mm^3 are shown in Figure 2-5.

Table 3. Explanation of possible reasons of mismatch in presence or large difference in volume of calcifications

Reason	Explanation
1. Methodological	
600 HU threshold MDCTA	A threshold of 600 HU to differentiate calcifications from contrast material in the lumen leads to underestimation of MDCTA calcifications by missing or excluding low dense calcifications on MDCTA.
Extended scan range MDCTA	The assessment of calcifications on MDCTA within 3 cm proximal and distal of the bifurcation (6 cm in total) to make sure the complete scan range of MRI was covered (3 cm in total, including the entire atherosclerotic plaque but variable segments of common and internal carotid artery) leads to overestimation of calcifications on MDCTA.
2. Technical or interpretation	
Wrong interpretation MRI	Misinterpretation of MR images (for example perivascular tissue is incorrectly interpreted as calcifications or calcifications are erroneously interpreted as lumen) leads to overestimation or underestimation of calcifications on MRI.
Inferior image quality MRI	Inferior image quality and artefacts of MR images obscures calcifications on MRI and leads to underestimation of calcifications on MRI.
Wrong manual segmentation MRI	Misinterpretation leads to the drawing of (too) small or (too) broad calcification contours on MRI and as a result under- or overestimation of calcifications on MRI compared to MDCTA.
Blooming effect MDCTA	The blooming effect of calcifications on MDCTA leads to overestimation of calcifications on MDCTA.

Missed calcifications MRI	Due to very small volume of calcifications or slight differences in slice position of the different MRI sequences, calcifications are missed on MRI and therefore leads to underestimation of calcifications on MRI.
Shadowing by IPH on MRI	Bright signal of the intraplaque hemorrhage on MRI obscures small calcifications and therefore leads to underestimation of calcifications on MRI.
Including ECA calcifications on MDCTA	Inclusion of calcifications of the external carotid artery on MDCTA (partly due to limited soft tissue contrast) leads to overestimation of calcifications on MDCTA.

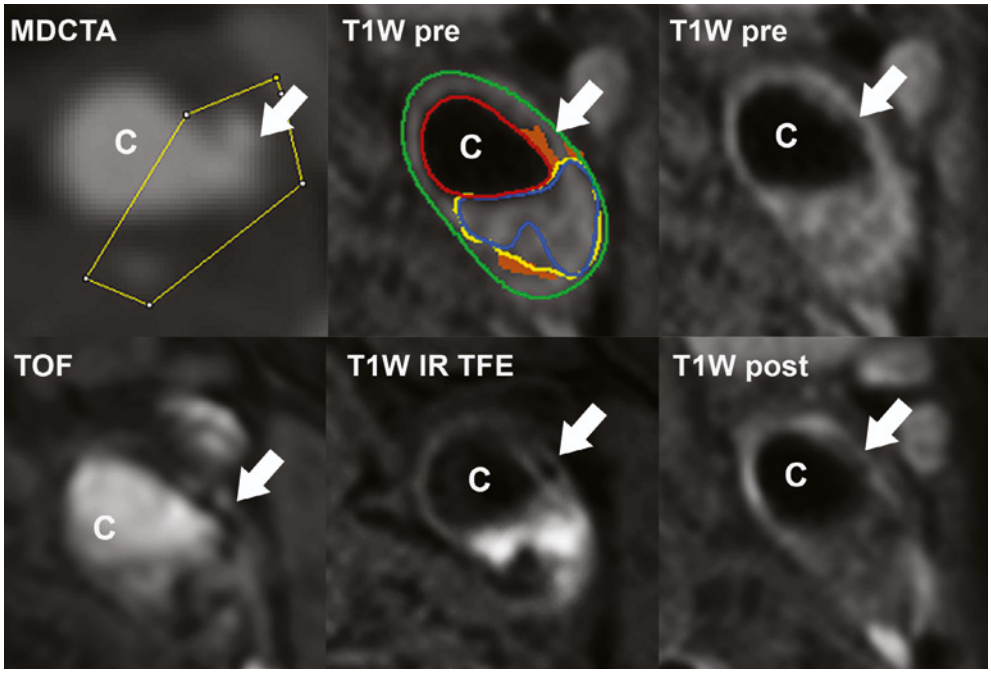

Figure 2. Example of how the use of a 600 HU threshold on MDCTA have led to underestimation of calcifications on MDCTA compared to MRI. Corresponding MDCTA and MR images of a patient in which the mismatch between MDCTA and MRI (MDCTA calcifications absent; MRI calcifications present) can be explained by the use of the 600 HU threshold on MDCTA: large calcification (arrow) is missed on MDCTA. C common carotid artery; arrow calcification.

Table 4. Overview of possible causes of mismatch in the detection of calcifications or large difference in calcification volume measurements. (n=82)

	Total	**Mismatch presence calcifications**	**Difference of >50 mm3**	**Consequence**
Methodological reasons				
600 HU threshold MDCTA (Fig. 2)	15	9	6	MDCTA underestimates
Extended scan range MDCTA	6	1	5	MDCTA overestimates
Technical or interpretation reasons				
Hypointensity on MRI incorrectly interpreted as calcification, probable component[1]:				MRI overestimates
Fibrous tissue	12	7	5	
Perivascular tissue	5	4	1	
Lipid-rich necrotic core	3	2	1	
Ulceration (Fig. 3)	2	2	0	
Lumen	1	0	1	
Total	16	9	7	
Inferior image quality MRI	9	8	1	MRI underestimates
Wrong manual segmentation MRI – contours too small (Fig. 4)	7	0	7	MRI underestimates
Blooming effect MDCTA	6	0	6	MDCTA overestimates
Calcifications MRI incorrectly interpreted as[2]:				MRI underestimates
Fibrous tissue	3	0	3	
Perivascular tissue	2	1	1	
Lumen	2	0	2	
Total	6	1	5	
Missed calcifications MRI	6	2	4	MRI underestimates
Wrong manual segmentation MRI – contours too broad	6	0	6	MRI overestimates
Shadowing by IPH on MRI (Fig. 5)	3	1	2	MRI underestimates
Including ECA calcifications on MDCTA	2	0	2	MDCTA overestimates

[1] In 5 patients hypointensity on MRI was misinterpreted as two different probable components; in 1 patient hypointensity on MRI was misinterpreted as three different probable components
[2] In 1 patient hypointensity on MRI was scored as two different components instead of calcification

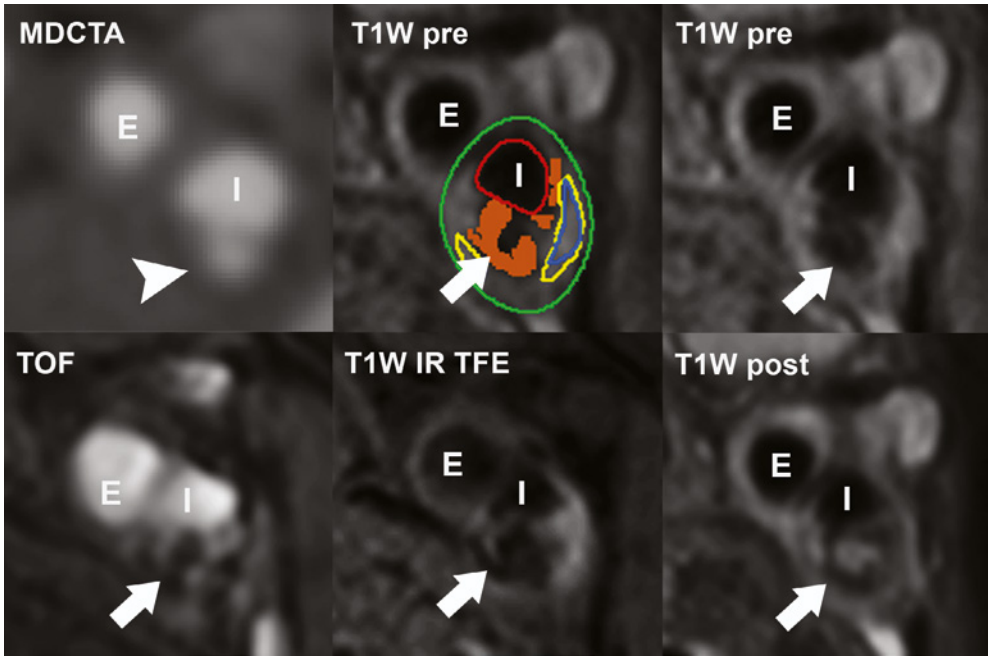

Figure 3. Example of how misinterpretation of the MRI have led to overestimation of calcifications on MRI compared to MDCTA. Corresponding MDCTA and MR images of a patient in which the mismatch between MDCTA and MRI (MDCTA calcification absent; MRI calcification present) can be explained by misinterpretation of the hypointensity on MRI: hypointensity is scored as calcification on MRI, whereas a plaque ulceration was identified on MDCTA (arrow head). E external carotid artery; I internal carotid artery; arrow calcification.

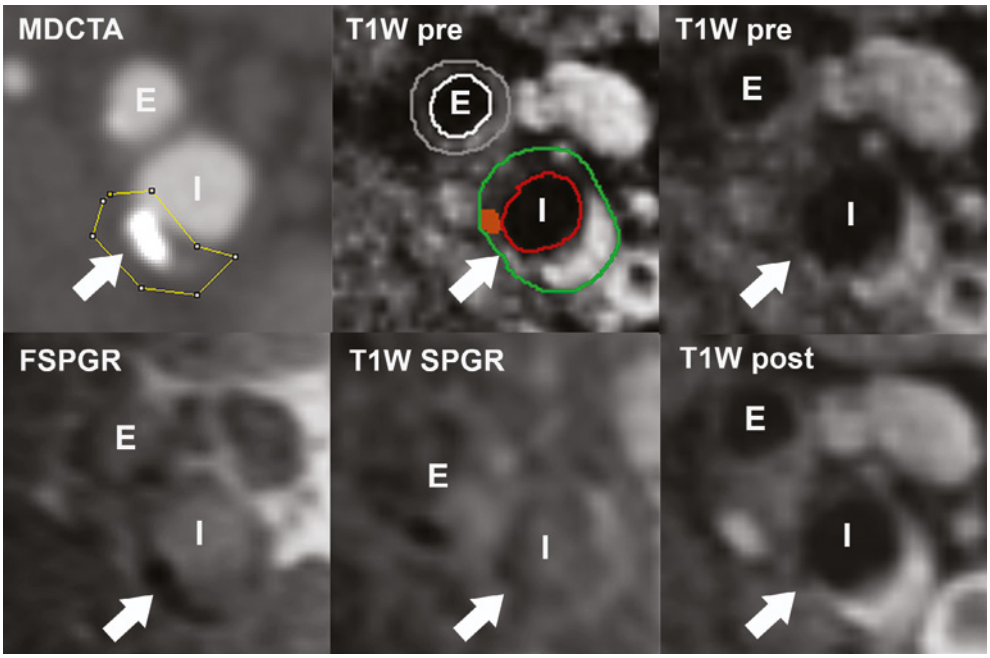

Figure 4. Example of how misinterpretation of the MRI have led to underestimation of calcifications on MRI compared to MDCTA. Corresponding MDCTA and MR images of a patient in which the difference between MDCTA and MRI (MDCTA calcification volume >50 mm^3 larger than MRI volume) can be explained by the drawing of calcification contours on MRI which seems to be too small. E external carotid artery; I internal carotid artery; arrow calcification.

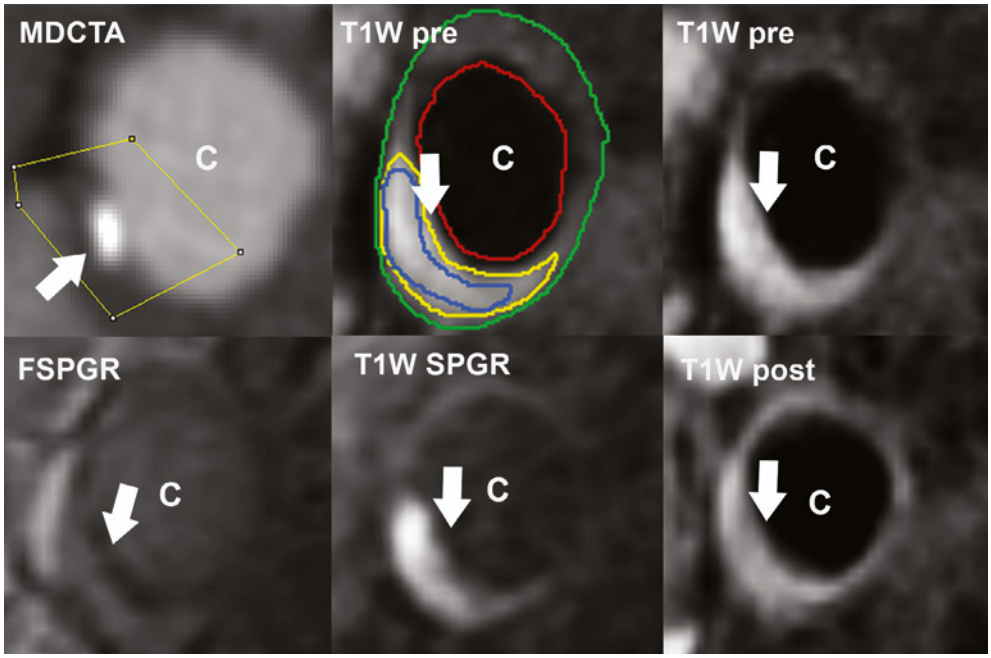

Figure 5. Example of how overshadowing of intraplaque hemorrhage on MRI have led to underestimation of calcifications on MRI compared to MDCTA. Corresponding MDCTA and MR images of a patient in which the mismatch between MDCTA and MRI (MDCTA calcifications present; MRI calcifications absent) can be explained by missing the calcification on MRI because overshadowing of the intraplaque hemorrhage (blue contour). C common carotid artery; arrow calcification.

Discussion

This study shows that MRI has a good sensitivity, but a low specificity for the detection of plaque calcifications compared with MDCTA as a reference standard. A good correlation was found between MRI and MDCTA calcification volumes and no significant difference was found between MRI and MDCTA estimates of calcification volumes. However, individual absolute differences could be substantial. Therefore, current MRI plaque imaging techniques cannot be recommended to estimate volume of plaque calcifications in individual patients with symptomatic carotid artery stenosis.

Some methodological issues need to be discussed first. A strength of our study is that we compared calcifications assessed on MRI and MDCTA in a large group of patients (n=185). In addition, we used patients of a multicenter study which made it possible to compare different MRI protocols, mirrors clinical practice and increases external validity. Finally, we explored reasons for the mismatch or large difference between MRI and MDCTA by reviewing individual cases retrospectively in order to identify possible future targets for improvement of the correlation between MRI and MDCTA derived calcifications. A limitation of our study is that MRI and MDCTA examination were performed mostly on different days due to the use of a contrast agent in both examinations. This led to a slight time interval between MRI and MDCTA in

some patients. However, calcium growth in the atherosclerotic plaque is a slow process.[3,29] Therefore, the effect of this delay between the two scans will be minimal. Another limitation is that we had to apply a threshold of 600 HU to automatically distinguish calcifications from the dense contrast material in the lumen on MDCTA. In non-contrast enhanced CT scans or studies which are able to exclude the high density lumen from the measurements, a threshold of 130 HU is applied.[13, 23] The use of a 600 HU threshold will have led to lower calcification size and missing small calcifications and therefore a subsequent underestimation of the calcification volume on MDCTA in comparison to a threshold of 130 HU and to MRI. At visual inspection, we found that in 15 patients (29%) the use of a 600 HU threshold was probably one of the reasons for the mismatch in detection of calcifications or a difference of more than 50 mm^3 between MRI and MDCTA. Finally, the evaluated longitudinal range differed between MRI and MDCTA (3 vs. 6 cm, respectively). The scan range on MRI was placed in such a way that the entire atherosclerotic plaque was covered. In practice this meant that variable ranges of the common and internal carotid artery were scanned. We evaluated MDCTA images within 3 cm proximal and distal of the bifurcation in order to ensure we included the complete scan range of MRI. However, in only 6 of the 51 patients with a mismatch in detecting calcifications or a large difference in calcification volume between MRI and MDCTA, MDCTA revealed calcifications outside the MRI scan range. Ababneh et al. showed that although calcified plaque is heterogeneously distributed in carotid endarterectomy tissue, most calcifications are found in the carotid bulb and adjacent internal and external carotid segments.[30] Automated registration of MDCTA and MR images would be optimal for head to head comparison, but is not trivial.[31]

Histological studies have shown that calcifications can be assessed with MRI with an overall sensitivity ranging from 76% to 92% and specificity ranging from 86% to 95%.[22] Kwee et al. compared calcification volumes assessed on CT with volumes on MRI and also found a moderate correlation between CT and MRI assessed calcification volume and also a considerable variation in absolute differences between CT and MRI measurements.[32] In contrast to our study, they found that calcification volume was significantly higher on CT than on MRI (246.3 mm^3 vs. 65.8 mm^3; $p<0.001$, respectively).[32] They were able to apply a threshold of 130 HU on MDCTA, leading to a consistent increase in the volume of calcifications on MDCTA compared to our MDCTA derived calcification volume (246.3 mm^3 vs. 58.1 mm^3, respectively). Their calcification volume on MRI was in the same range as our MRI derived calcification volume (65.8 mm^3 vs. 58.7 mm^3, respectively).

Besides the previous mentioned methodology related causes, we found other causes for a decreased MRI sensitivity or MRI misinterpretation. Low signal intensity regions on MRI were interpreted as calcification, but after comparison with MDCTA were found likely to be other tissue components like fibrous tissue, perivascular tissue, lipid-rich necrotic core, ulceration or lumen. In contrast, some hypointense regions on MRI were considered to be non-calcified but were retrospectively more likely to be a calcifications. Both situations have affected the detection and quantification of individual calcified spots. In addition, the image quality of MRI and wrong manual segmentation of calcifications (contours were likely drawn too small or too broad), or simply missing some calcifications on MRI, were all prevalent reasons for a mismatch in detection or a large difference in calcification volume between MRI and MDCTA.

We also found that the bright signal of intraplaque hemorrhage could obscure small calcifications. In most cases, we could retrospectively identify a small calcification on MRI when we compared it to MDCTA. However, some studies have shown that calcifications can have high signal intensity in some T1-weighted sequences.[25, 33, 34]

Although MRI shows an overall agreement with histology and MDCTA in the detection and quantification of plaque calcifications, the results for MRI assessed calcifications on individual patient level are disappointing. The findings in the present study may be used to identify complicated cases that could be used during training of MRI observers. We expect that agreement between MRI and MDCTA calcifications can be improved by an increase in image quality of MRI. In addition, the use of dedicated (automated) annotation tools can further improve agreement.[35] Otherwise, combining information from different image modalities for segmentation of the atherosclerotic plaque can be a future scenario.[31]

Summary

MRI has a good sensitivity for the detection of plaque calcifications with MDCTA as reference standard. An overall agreement was found in calcification volume assessed with MRI and MDCTA; however individual absolute differences could be substantial. Therefore, current MRI plaque imaging techniques cannot be recommended to estimate volume of plaque calcifications in individual patients with symptomatic carotid artery stenosis.

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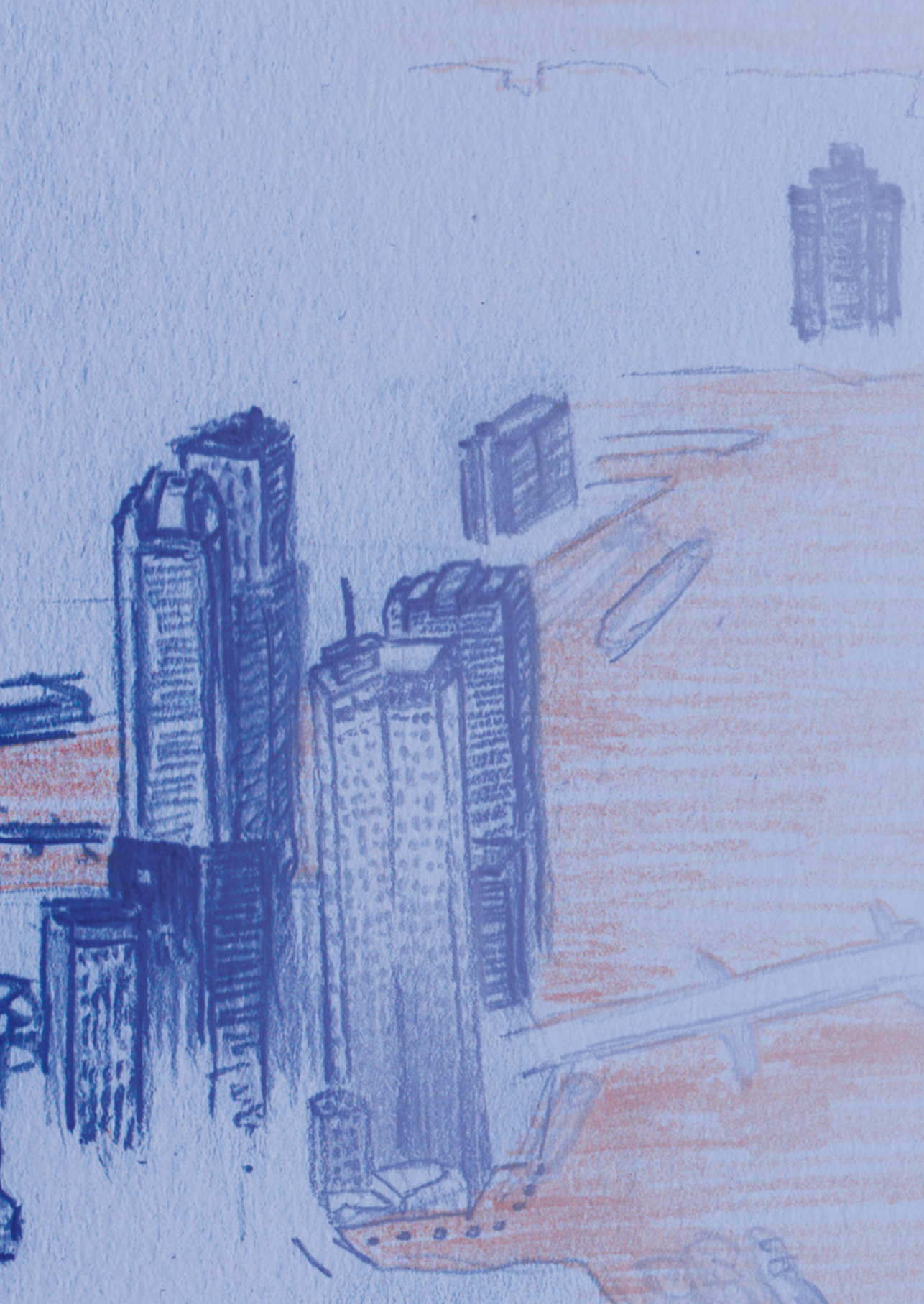

Chapter **3.3**

Usefulness of contrast-enhanced ultrasound for detection of carotid plaque ulceration in patients with symptomatic carotid atherosclerosis

G.L. ten Kate, A.C. van Dijk, S.C.H. van den Oord, B. Hussain, H.J.M. Verhagen, E.J.G. Sijbrands, A.F.W. van der Steen, A. van der Lugt, A.F.L. Schinkel

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Abstract

Previous data have indicated that carotid plaque ulceration is a strong predictor of cerebrovascular events. Standard ultrasound and color Doppler ultrasound (CDUS) scans have poor diagnostic accuracy for the detection of carotid plaque ulceration. The aim of the present prospective study was to assess the value of contrast-enhanced ultrasound (CEUS) scans for the detection of carotid plaque ulceration. The Institutional Ethics Committee approved the study protocol, and all patients provided informed consent. The patients had symptomatic stenosis of the internal carotid artery and underwent carotid computed tomographic angiography as part of their clinical evaluation. All patients underwent a CDUS examination in conjunction with CEUS. Carotid plaque ulceration was defined as the presence of ≥1 disruptions in the plaque-lumen border ≥1 x 1 mm. Carotid computed tomographic angiography was used as reference technique. The study population consisted of 20 patients (mean age 64 ± 9 years, 80% men), and 39 carotid arteries were included in the present analysis. Computed tomographic angiography demonstrated that the plaque surface was smooth in 15 (38%), irregular in 7 (18%) and ulcerated in 17 (44%) carotid arteries. The sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of CDUS for the detection of ulceration was 29%, 73%, 54%, 46%, and 57%, respectively. The sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of CEUS for the detection of ulceration was 88%, 59%, 72%, 63%, and 87%, respectively. CEUS had superior sensitivity and diagnostic accuracy for the assessment of carotid plaque ulceration compared with CDUS. CEUS improved the intrareader and inter-reader variability for the assessment of carotid plaque ulceration compared with CDUS. In conclusion, CEUS could be an additional method for the detection of carotid plaque ulceration. The role of CDUS for the assessment of carotid plaque ulceration seems limited.

Introduction

Previous studies have demonstrated that carotid plaque ulceration is a strong predictor of cerebrovascular events.[1,2] Ultrasound is the most frequently used imaging technique for the assessment of carotid atherosclerosis. It is readily available, of low cost, safe, and accurately identifies the presence of flow-limiting stenosis.[3-5] Nevertheless, ultrasound, including color Doppler ultrasound (CDUS), fails to accurately identify carotid plaque ulceration.[4-9] Contrast- enhanced ultrasound (CEUS) is an advanced form of ultrasound imaging using a microbubble contrast agent. CEUS provides a better delineation of the carotid lumen than does CDUS.[10-14] The use of CEUS for the detection of carotid plaque ulceration has not yet been studied. The aim of the present prospective study was to assess the value of CEUS for the detection of carotid plaque ulceration in patients with symptomatic carotid atherosclerosis. Carotid computed tomographic angiography (CTA) was used as the reference technique.

Methods

The Institutional Ethics Committee approved the study protocol. All patients provided informed consent. A total of 20 consecutive patients were included in the present study. All patients had symptomatic stenosis of the internal carotid artery and underwent carotid CTA as part of their clinical evaluation. All patients also underwent a carotid CDUS examination in conjunction with CEUS. The exclusion criteria included contraindications for the use of an ultra- sound contrast agent, such as unstable angina, acute cardiac failure, acute endocarditis, known right-to-left shunts, and a known allergy to microbubble contrast agents.

CDUS and CEUS were performed using a Philips iU-22 ultrasound system (Philips Medical Systems, Bothell, Washington), equipped with an L9-3 transducer. CDUS consisted of standard B-mode and color Doppler imaging and was performed using a standard scanning protocol.[15] In brief, both left and right carotid arteries were examined with the patient in the supine position with the head supported at a 45° angle turned to the contralateral side. The common carotid artery, carotid bulb, internal carotid artery, and external carotid artery were evaluated using CDUS and pulsed wave Doppler imaging. All anatomic sites were examined from different angles of view, and each site was scanned in the cross-sectional view and longitudinal view.

After the CDUS examination, CEUS was performed using intravenous administration of SonoVue contrast agent (Bracco SpA, Milan, Italy). The contrast mode of the ultrasound system, using amplitude modulation and a mechanical index of 0.06, was used to optimize the CEUS examination. The ultrasound contrast agent was injected in 0.5-ml boluses. Each contrast agent bolus was followed by a saline flush using 2.0 ml NaCl 0.9% solution. After administration of the contrast agent, high-quality contrast images could be obtained for approximately 1 minute. The bolus injection of the contrast agent was repeated when necessary. Both carotid arteries were examined using a standard acquisition protocol, and still frames and cine clips were digitally stored for offline analysis.

All studies were analyzed by 2 readers who were unaware of the clinical and CTA data. Discrepancies in their readings were resolved by consensus; if a consensus could not be reached, a third experienced reader was consulted. The CDUS and CEUS studies were analyzed separately. The image quality of the region of interest was scored using a 3-point scale as good, moderate, or poor image quality. The plaque border was visually scored as smooth, irregular (Figure 1), or ulcerated (Figures 2 and 3).[16] Carotid plaque ulceration was defined as the presence of ≥1 disruptions of the plaque-lumen border ≥1 x 1 mm. The presence of calcification was scored using a semiquantitative grading system: 0, no calcification; 1, limited calcification; 2, moderate calcification; and 3, severe calcification. For each study, the readers indicated whether the presence of calcification hindered the evaluation of the plaque surface morphology.

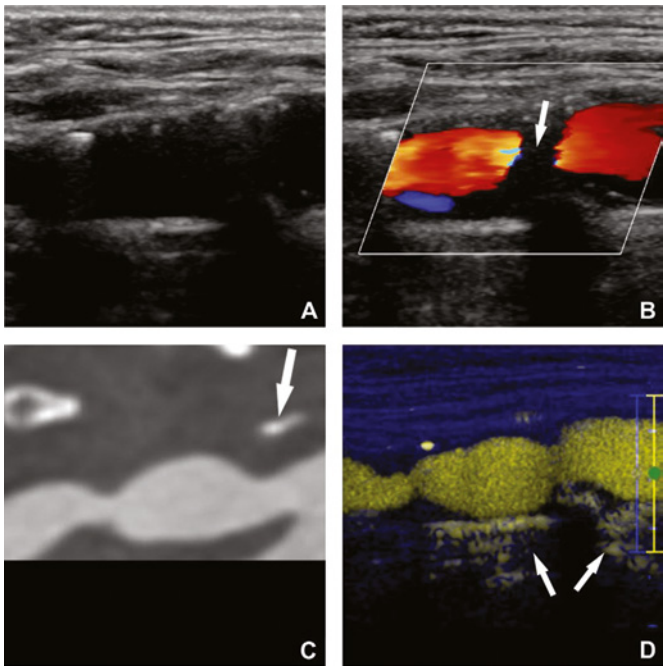

Figure 1. Longitudinal B-mode ultrasound (A), CDUS (B), CTA (C), and CEUS (D) images of carotid plaque classified as irregular using both CEUS and CTA. Calcification of carotid artery (C, arrow) caused acoustic shadow that hindered accurate color Doppler evaluation (B, arrow). A clear evaluation is possible with CEUS (D), providing information similar to that obtained with CTA (C). The enhancement underneath the vessel (D, arrows) is a pseudoenhancement artifact.

CTA was performed using a 128-slice, multidetector CT system (Definition AS+ or Definition flash [1 patient], Siemens Medical Solutions, Forchheim, Germany) using a contrast-enhanced CTA protocol (120 kVp, 180 mA, collimation 64x2x0.6 mm, pitch <1). The scan range extended from the ascending aorta to the intracranial circulation (3 cm above the sella turcica). Of the 20 patients, 19 received 80 ml of contrast agent (320 mg/ml iodixanol, Visipaque, Amersham Health, Little Chalfont, United Kingdom), followed by 45-ml saline bolus chaser, both at an injection rate of 5 ml/s. One patient received 60 ml of contrast agent, followed by a 45-ml saline bolus chaser, both at an injection rate of 4 ml/s. Real-time bolus tracking at the level of the ascending aorta was used to synchronize the passage of the contrast agent and data acquisition. Image reconstructions were made with a field of view of 120 to 170 mm, matrix size

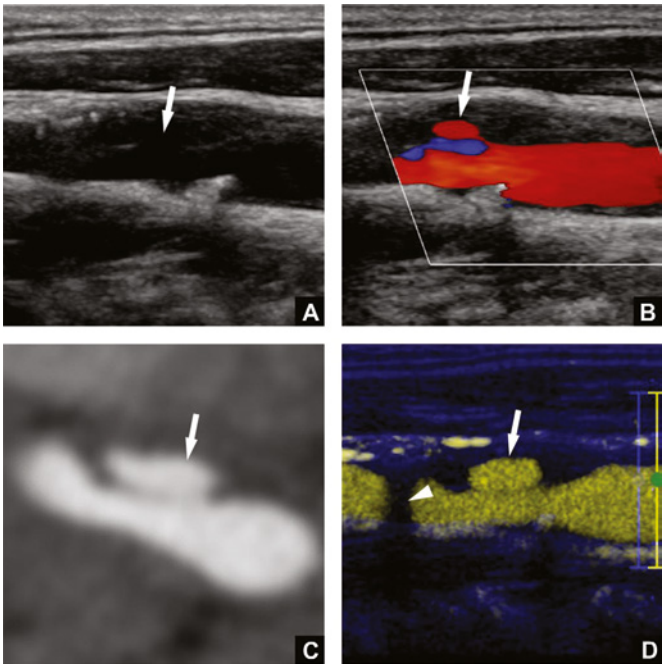

Figure 2. Longitudinal images of carotid plaque classified as ulcerated using CDUS, CEUS, and CTA. Longitudinal B-mode ultrasound (A), CDUS (B), CTA (C), and CEUS (D). A hypoechoic plaque with ulceration is present in carotid bulb (arrows). A small calcification causes shadowing that hindered visualization of a small part of the plaque surface (D, arrowhead).

512 x 512, slice thickness 1.0 mm, increment 0.6 mm, and an intermediate reconstruction algorithm.

All CTA studies were evaluated with the reviewers unaware of the clinical and ultrasound data. A 2-dimensional axial image viewer was used to assess the image quality and degree of stenosis of both carotid arteries. The image quality of the region of interest was scored using a 3-point scale as good, moderate, or poor image quality. The degree of stenosis was calculated using the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria perpendicular to the central lumen line.[17] Dedicated 3-dimensional analysis software with multiplanar reformatting was used to evaluate the plaque surface in the oblique, coronal, and sagittal plane. The plaque surface of all carotid arteries was independently evaluated by 2 readers. Discrepancies in their evaluation were solved by consensus; if consensus could not be reached, a third experienced reader was consulted. The CTA and ultrasound studies were analyzed by different readers. The surface of the plaque was scored as smooth, irregular, or ulcerated. Carotid plaque ulceration was defined as the presence of contrast extending beyond the vascular lumen ≥1 mm in ≥2 planes. The calcium volume was measured from 3 cm proximal to 3 cm distal of the flow divider using semiautomated quantification software. A threshold of 600 Hounsfield units was used to differentiate calcium from contrast.

Statistical analyses were performed using the Statistical Package for Social Sciences for Windows, version 17.0 (SPSS, Chicago, Illinois). Continuous variables are reported as the mean ± SD or median and interquartile range. Categorical variables are expressed as numbers and percentages. The CDUS and CEUS studies of the right

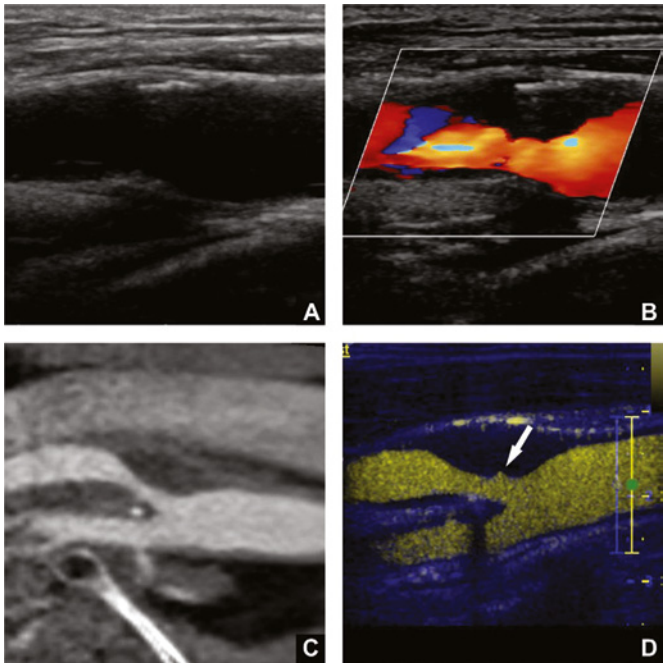

Figure 3. Longitudinal images of carotid plaque classified as ulcerated with CEUS and smooth with CTA. Longitudinal B-mode ultrasound (A), CDUS (B), CTA (C), and CEUS (D). A hypoechoic plaque was present in the near wall of the carotid artery, the full extent of which was not visible on B-mode ultrasound (A). CDUS (B) provided improved visualization of the plaque size but did not identify an ulcer. (D) CEUS demonstrated a small (≈1 x 2 mm) ulcer (arrow) not visible using CDUS or CTA (C).

and left carotid artery were analyzed independently, with the corresponding CTA study as the reference technique. The measurements of accuracy (sensitivity, specificity, accuracy, positive predictive value, and negative predictive value) were calculated. Differences in diagnostic accuracy were compared using McNemar's test. The CTA determined calcium volume and ultrasound calcium scores were compared using Spearman's rank correlation. The inter- reader and intrareader reproducibility was assessed using weighted k statistics. p Values <0.05 were considered statistically significant.

Results

The clinical characteristics of the study population (mean age 64 ± 9 years, 80% men) are listed in Table 1. All CDUS, CEUS, and CTA studies were performed without adverse reactions. One patient had previously undergone right-sided endarterectomy; this carotid artery was excluded from the analysis, resulting in 39 carotid arteries for the present analysis.

The image quality of the CDUS studies was good in 31 (79%), moderate in 6 (15%), and poor in 2 (5%) carotid arteries. CEUS resulted in improved image quality, with 37 (95%) studies of good, 2 (5%) of moderate, and 0 of poor quality. None of the ultrasound studies were judged to be uninterpretable. The CTA image quality was good in 37 (95%) and moderate in 2 (5%) arteries. No CTA scan was judged to be uninterpretable.

Table 1. Patient characteristics (n = 20)

Variable	Value
Men	16 (80)
Age (yrs)	64 ± 9
Body mass index (kg/m2)	29 ± 6
Hypertension	10 (50)
Diabetes mellitus	5 (25)
Smoker	9 (45)
Quit smoking	6 (30)
Dyslipidemia	7 (35)
Coronary artery bypass grafting	2 (10)
Myocardial infarction	1 (5)
Amaurosis fugax	5 (25)
Transient ischemic attack	8 (40)
Ischemic stroke	5 (25)
Retinal infarction	1 (5)
Pulsatile tinnitus	1 (5)
Platelet aggregation inhibitor use	19 (95)
ß-Blocker therapy	12 (60)
Statin therapy	19 (95)

Data are presented as mean ± SD or n (%).
Dyslipidemia was defined as total cholesterol ≥6.4 mmol/L (≥247 mg/dl).

CTA demonstrated that the plaque surface was smooth in 15 (38%), irregular in 7 (18%), and ulcerated in 17 (44%) carotid arteries (Table 2). The performance characteristics of CDUS for the evaluation of the carotid plaque surface are listed in Table 3. CDUS revealed 5 of 17 ulcerations (29%) identified at CTA, with 6 additional ulcerations detected that could not be confirmed. This resulted in a poor agreement between CDUS and CTA (k = 0.19, 95% confidence interval [CI] 0.00 to 0.42).

Table 2. Computed tomographic angiographic results (n = 39 carotid arteries)

Variable	Value
Mean stenosis (NASCET [%])	43 ± 24
<50% NASCET stenosis	18 (46)
50-69% NASCET stenosis	17 (44)
≥70% NASCET stenosis	4 (10)
Plaque surface morphology	
Ulcerated	17 (44)
Irregular	7 (18)
Smooth	15 (38)
Presence of calcium	35 (90)
Calcium volume (mm^3)	
Median	35.2
Interquartile range	7.1-127.4

Data are presented as mean ± SD or n (%), unless otherwise noted.
NASCET = North American Symptomatic Carotid Endarterectomy Trial.

Table 3. Standard ultrasound, including color Doppler evaluation of carotid plaque surface compared with computed tomographic angiography (CTA)

CDUS	CTA			Total
	Smooth	Irregular	Ulcerated	
Smooth	7 (19)	1 (3)	2 (5)	10
Irregular	6 (15)	2 (5)	10 (26)	18
Ulcerated	2 (5)	4 (10)	5 (13)	11
Total	15	7	17	39

Data are presented as n (%).

Table 4. Contrast-enhanced ultrasound (CEUS) of carotid plaque surface compared with computed tomographic angiography (CTA)

CEUS	CTA			Total
	Smooth	Irregular	Ulcerated	
Smooth	5 (13)	0	0	5
Irregular	7 (19)	1 (3)	2 (5)	10
Ulcerated	3 (8)	6 (15)	15 (38)	24
Total	15	7	17	39

Data are presented as n (%).

Table 5. Comparison of color Doppler ultrasound (CDUS) and contrast-enhanced ultrasound (CEUS) for detection of carotid plaque ulceration

Variable	Method	
	CDUS	CEUS
Sensitivity (%)	29	88
Specificity (%)	73	59
Accuracy (%)	54	72
PPV (%)	46	63
NPV (%)	57	87

NPV = negative predictive value; PPV = positive predictive value

Carotid CEUS detected 15 of 17 ulcerations (88%) identified at CTA and identified 9 additional ulcerations that were not confirmed at CTA (Table 4). The addition of CEUS to the CDUS study provided a substantial improvement in agreement between ultrasound and CTA (k = 0.42, 95% CI 0.26 to 0.62). CEUS significantly improved the sensitivity for identifying ulcerations (sensitivity 29% for CDUS vs 88% for CEUS, p <0.01), without significantly decreasing the specificity (specificity 73% for CDUS vs 59% for CEUS, p = 0.45). The diagnostic accuracy of CDUS and CEUS for the detection of carotid plaque ulceration is summarized in Table 5.

CTA demonstrated the presence of calcification in 35 carotid arteries (90%), with a median calcium volume of 35.2 mm3 (interquartile range 7.1 to 127.4). CDUS demonstrated no calcification (grade 0) in 3 (8%), limited calcification (grade 1) in 15 (38%), moderate calcification (grade 2) in 11 (28%), and severe calcification (grade 3) in 10 (26%) carotid arteries. CEUS demonstrated no calcification (grade 0) in 5 (13%), limited calcification (grade 1) in 11 (28%), moderate calcifications (grade

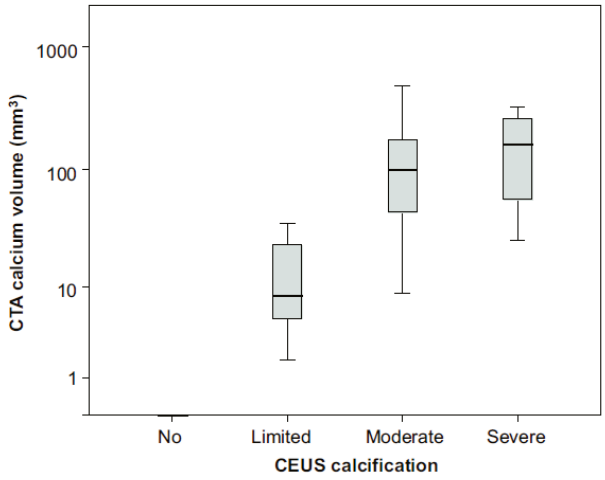

Figure 4. Box plot of semi-quantitative calcium grading obtained with CEUS compared with CTA determined calcium volume (logarithmic).

2) in 19 (49%), and severe calcification (grade 3) in 4 (10%) carotid arteries. Both the CDUS and CEUS semiquantitative calcium grading showed a strong correlation with the CTA deter- mined calcium volume (Spearman's r = 0.69, p <0.001, and r = 0.72, p <0.001, respectively; Figure 4). In none of the patients, did the observers judge the calcification to obscure the plaque such that an evaluation with ultrasound was not possible. The exclusion of studies with heavy calcification did not change the accuracy of ulceration detection.

The inter-reader and intrareader reproducibility of CDUS for the detection of plaque ulceration was moderate (intrareader variability, k = 0.38, 95% CI 0.15 to 0.61; intrareader variability, k = 0.61, 95% CI 0.40 to 0.81). CEUS improved the inter-reader and intrareader variability for the assessment of carotid plaque ulceration compared with CDUS (inter-reader variability, k = 0.65, 95% CI 0.42 to 0.88; intrareader variability, k = 0.69, 95% CI 0.52 to 0.85).

Discussion

The main finding of the present study was that CEUS could be an additional method for the detection of carotid plaque ulceration in patients with symptomatic carotid atherosclerosis. CEUS had a significantly greater sensitivity and diagnostic accuracy for the assessment of carotid plaque ulceration compared with CDUS. CEUS improved the intrareader and inter-reader variability for the assessment of carotid plaque ulceration compared with CDUS. These findings suggest that the role of CDUS for the assessment of carotid plaque ulceration is limited. The results of the present study could have implications for the use of CEUS in the evaluation of patients with carotid plaques.

These results have confirmed the findings from previous studies using CDUS for the detection of carotid plaque ulceration. Previous studies have shown that CDUS has a limited accuracy for the detection of carotid plaque ulceration.[6-11] Saba et al[7] studied 103 carotid arteries with CDUS using the histologic examination as the reference method and demonstrated a sensitivity similar to our study (sensitivity 37.5%, specificity 91.5%). The poor sensitivity led the investigators to conclude that CDUS has no place in the evaluation of carotid plaque morphology.[7] They consequently recommended the use of CTA for this purpose.[7] The present results are in line with those from previous experimental studies. Sirlin et al[10] demonstrated that CEUS provides a more accurate delineation of the vascular lumen than CDUS. In that initial study, CEUS was performed without a contrast-specific image acquisition setting, but CEUS improved the depiction of the luminal and wall abnormalities in an in vivo rabbit model of atherosclerosis and in replicas of diseased human carotid arteries. Kono et al[11] showed that phase-inversion harmonic imaging further increased the sensitivity of ultrasound to a contrast agent, leading to improved visualization of the vascular lumen and tissues.

The present study is the first to evaluate the value of CEUS for the detection of carotid plaque ulceration. CEUS was relatively easily incorporated into the standard carotid ultra- sound acquisition protocol and significantly improved the accuracy

for the assessment of plaque ulceration. However, the agreement between CEUS and CTA in our study was not perfect. This could have been caused by several factors. First, the ultrasound acquisition might have been hindered because of calcified plaques owing to acoustic shadowing. CDUS and CEUS demonstrated severe calcium in 26% and 10% of the carotid arteries, respectively. The semiquantitative calcium scales obtained by ultrasound were in line with the semiautomated quantification of calcium using CTA. None of the ultrasound studies was judged to be uninterpretable, and the exclusion of severely calcified plaques did not change the diagnostic accuracy. However, we could not exclude that ulcerations could have been missed as a result of acoustic shadowing. Second, both CDUS and CEUS were performed using 2-dimensional acquisition, but CTA is a 3-dimensional imaging technique. The 2-dimensional acquisition might have decreased the accuracy of CEUS for the assessment of plaque ulceration. Third, in the present study, carotid CEUS identified 9 additional ulcerations that were not detected using CTA. This fact raises the question of whether CTA is a reliable reference standard for detecting plaque ulcerations. Because of the lower local and temporal resolution of CTA compared with ultrasound imaging, CEUS might even exceed the diagnostic performance for detecting plaque ulcerations compared with CTA. Finally, the present study was limited by the relatively small number of patients (n = 20) and carotid lesions (n = 39).

The results of our study should be viewed in the context of risk assessment of patients with atherosclerosis of the carotid artery. The presence of ulceration is 1 of the specific risk features of the unstable atherosclerotic plaque.[18] Carotid plaque ulceration can lead to embolism and occlusion, and previous studies have indicated that ulceration is an important risk marker for neurologic symptoms and acute ischemic stroke.[1,2] These studies have focused on plaque ulceration in patients with symptomatic carotid atherosclerosis. Additional studies are needed to assess the predictive value of carotid plaque ulceration in asymptomatic patients. The detection of plaque ulceration using CEUS could be implemented in clinical practice for the identification of plaques at high risk of causing thromboembolic events. The assessment of these unstable or vulnerable plaques could help to further improve the identification of patients who might benefit from aggressive medical therapy or carotid surgery. The challenge for the future is to develop accurate risk stratification models.

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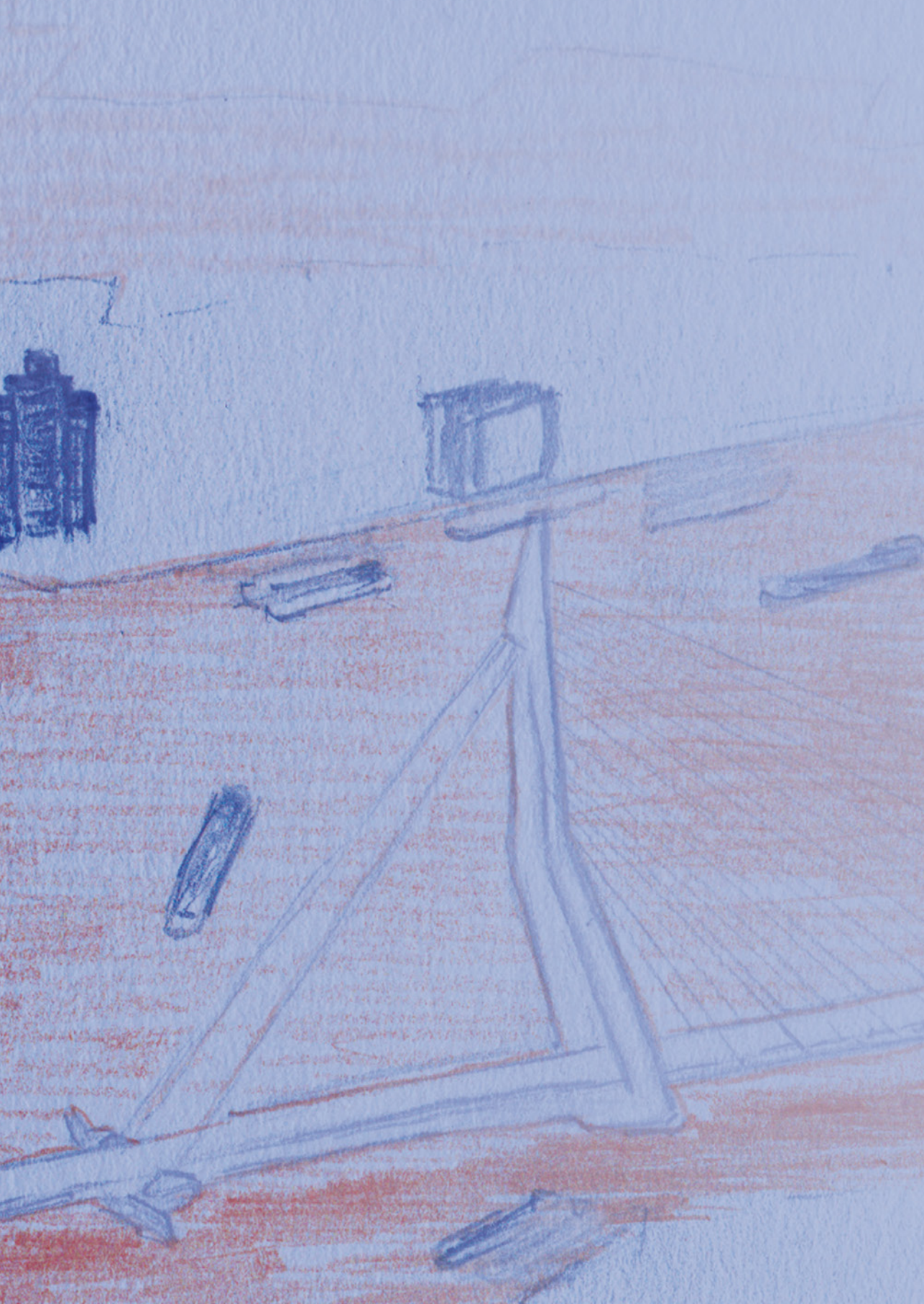
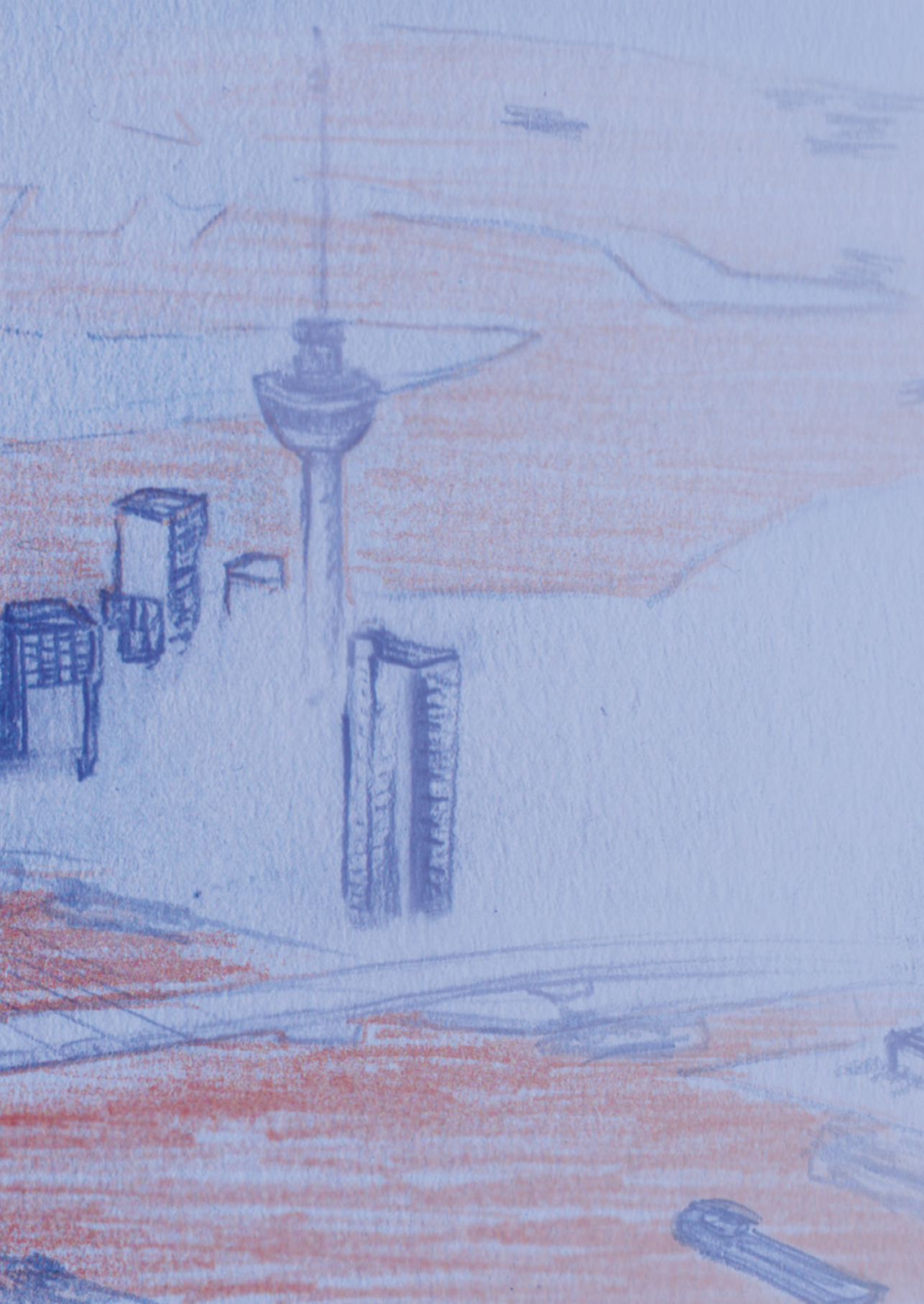


Chapter **4**

Clinical studies



Chapter **4.1**

Association between arterial calcifications and nonlacunar and lacunar ischemic strokes

A.C. van Dijk, S. Fonville, T. Zadi, A.M.G. van Hattem, G. Saiedie, P.J. Koudstaal, A. van der Lugt

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Abstract

Background and Purpose: Nonlacunar cerebral infarcts are presumed to be caused by thromboembolism from the heart or extracranial arteries, whereas lacunar infarcts are thought to be caused by small vessel disease. We investigated to what extent arterial calcifications differ between nonlacunar and lacunar ischemic strokes.

Methods: We studied 820 consecutive patients with transient ischemic attack or ischemic stroke in the anterior circulation who underwent multidetector computed tomography angiography and had no rare cause of stroke. The presence of likely cardioembolic pathogenesis was determined according to the Trial of Org 10172 in Acute Stroke Treatment criteria. The remaining 708 patients were categorized as nonlacunar or lacunar strokes, either transient ischemic attacks or strokes, based on clinical symptoms corrected by brain imaging results. We measured volume of calcifications in the aortic arch, symptomatic extracranial and intracranial carotid artery using multidetector computed tomography angiography. The difference in calcifications between nonlacunar and lacunar strokes was assessed with a multivariable logistic regression analysis. We adjusted for degree of symptomatic carotid artery stenosis and cardiovascular risk factors.

Results: We found an independent association between volume of aortic arch calcifications and nonlacunar ischemic strokes (adjusted odds ratio [95% confidence interval], 1.11 [1.02–1.21]). No independent associations between extracranial and intracranial carotid artery calcifications and nonlacunar strokes were present.

Conclusions: The only difference we found between nonlacunar and lacunar strokes was a higher calcification volume in the aortic arch in nonlacunar strokes. Our findings only partially confirm the notion of distinct etiologies and suggest that the potential role of other plaque components, plaque morphology, and aortic arch calcifications in ischemic stroke subtypes awaits further evaluation.

Introduction

Nonlacunar infarcts are presumed to be caused by thromboembolism from the heart or large extracranial arteries, whereas lacunar infarcts are considered to be caused by the occlusion of small perforating arteries. Jackson et al[1,2] found that cardiovascular risk factors, such as hypertension and diabetes mellitus, are equally common in nonlacunar and lacunar infarcts, but atrial fibrillation or cardiac valve disease and significant carotid artery stenosis were more frequent in non- lacunar infarcts compared with lacunar infarcts, supporting the notion of separate etiologies. Others have found a lower risk of early stroke recurrence and myocardial infarction in patients with lacunar versus nonlacunar infarcts.[3,4] Imaging of the extracranial and intracranial arteries allows assessment of not only the presence of atherosclerosis, but also the atherosclerotic plaque surface and plaque composition. Homburg et al[5] showed that plaque ulceration is associated with nonlacunar ischemic stroke, suggesting indeed that nonlacunar and lacunar infarcts have different types of atherosclerotic disease and supporting the notion of different underlying pathophysiology.

Calcifications are present in the more severe atherosclerotic plaques and are easily detectable on multidetector computed tomography angiography (MDCTA) and can therefore be used as a marker of atherosclerosis.[6] We investigated whether and to what extent calcifications in the aortic arch, symptomatic extracranial and intracranial carotid artery differ between nonlacunar and lacunar ischemic strokes.

Methods

Study population

Patients were derived from an ongoing prospective registry of all patients with neurovascular diseases admitted to our hospital (Erasmus Stroke Study [ESS]). MDCT of the brain and MDCTA are part of the clinical work-up. Detailed clinical information, imaging, blood samples, and DNA are collected from all participants. Written informed consent was obtained from all patients, as approved by the Institutional Ethics Committee. All patients with an ischemic event, either transient ischemic attack (TIA) or stroke, in the anterior circulation were included in this study. Patients with a rare cause of stroke, carotid artery dissection, absent MDCTA, or amaurosis fugax were excluded.

Stroke classification

First, the presence of a likely cardioembolic pathogenesis according to the Trial of Org 10172 in Acute Stroke Treatment criteria was determined.[7] We categorized all remaining ischemic events, either TIAs or strokes, as nonlacunar or lacunar strokes based on the clinical Oxfordshire Community Stroke Project criteria and corrected for relevant infarcts on MDCT scan of the brain.[8] Lacunar ischemic strokes were defined

as pure motor strokes, pure sensory strokes, sensory-motor strokes, or ataxic hemiparesis.[8,9] Patients who were not categorized as lacunar strokes were categorized as nonlacunar strokes. In both the nonlacunar and the lacunar stroke groups, TIAs and ischemic strokes were represented.

Cardiovascular risk factors

Hypercholesterolemia was defined as fasting total cholesterol >5 mmol/L or the use of cholesterol-lowering medication at the time of the TIA or ischemic stroke. Hypertension was defined as systolic blood pressure >140 mm Hg or a diastolic blood pressure >90 mm Hg during 2 episodes of ≥15 minutes of continuous noninvasive blood pressure measurement or treatment with antihypertensive medication. Diabetes mellitus was defined as fasting plasma glucose level >6.9 mmol/L or a 2-hour postload glucose level >11.0 mmol/L or the use of antidiabetic medication. Smoking status was assessed at the time of the ischemic event and dichotomized into current smoker or no current smoker.

MDCT and MDCTA data acquisition

Image acquisition was performed using a 16-slice, 64-slice, or 128-slice MDCT system (Sensation 16, Sensation 64, Definition, Definition AS+ or Definition Flash, Siemens Medical Solutions, Erlangen, Germany) using a standardized optimized contrast-enhanced proto- col (120 kVp; 180–200 reference mAs; collimation 16×0.75 mm, 32×2×0.6 mm, or 64×2×0.6 mm; pitch <1).

The MDCTA scan ranged from the ascending aorta to the intracranial circulation (3 cm above the sella turcica). All patients received 80 mL of contrast agent (320 mg/mL iodixanol, Visipaque, Amersham Health, Little Chalfont, United Kingdom), followed by 45-mL saline bolus chaser, both at an injection rate of 4 to 5 mL/s. Real-time bolus tracking at the level of the ascending aorta was used to synchronize passage of contrast agent and data acquisition. Image reconstructions were made with field of view of 120 mm, matrix size 512×512, slice thickness 1.0 or 0.75 mm, increment 0.6 to 0.4 mm, and an intermediate reconstruction algorithm.

The MDCT brain scan ranged from the foramen magnum to the vertex. Image reconstructions were made with a 200 to 250 mm field of view, matrix size 512×512, slice thickness 3 to 5 mm, and an inter- mediate reconstruction algorithm.

MDCT and MDCTA data analysis

All MDCT and MDCTA studies were evaluated by trained readers blinded for clinical data. Relevant cerebral infarctions on MDCT of the brain were classified as small deep, large deep, end zone, border- line, or multiple infarcts and dichotomized into

nonlacunar strokes (large deep, end zone, borderline, and multiple infarcts) or lacunar strokes (small deep infarcts).[10]

The most severe stenosis in the symptomatic carotid bifurcation and internal carotid artery was measured on MDCTA according to the North American Symptomatic Carotid Endarterectomy Trial criteria.[11] The aortic arch was defined as the origin of the aortic arch to the first 1 cm of its branches. The extracranial carotid artery was defined as the carotid artery within 3 cm proximal and distal of the bifurcation. The intracranial internal carotid artery was defined as the horizontal segment of the petrous internal carotid artery to the top of the internal carotid artery. Calcifications in the aortic arch and symptomatic extracranial carotid artery were quantified semiautomatically using dedicated commercially available software (Syngo CalciumScoring, Siemens). A custom-made plug-in for the freely available software ImageJ (Rasband, National Institute of Mental Health, Bethesda, MD) was used to quantify calcifications in the symptomatic intracranial carotid artery, because of the close relationship of the intracranial carotid artery and the skull, automatic quantification was not possible. A threshold of 600 Hounsfield units was used to differentiate calcifications from contrast material in the lumen; calcification volume was expressed in millimeter cubed. A detailed description of the measurements is provided elsewhere.[12-15]

An intraclass correlation coefficient and coefficient of variation for the aortic arch, extracranial and intracranial carotid artery scoring method was assessed in 29 MDCTA examinations based on the ratings of 2 observers. The intraclass correlation coefficient was 1.00 and the coefficient of variation 10% to 11% for all 3 vessel beds.

Statistics

We compared cardiovascular risk factors, degree of symptomatic carotid artery stenosis, and extent of calcifications between patients with nonlacunar strokes, lacunar strokes, and cardioembolic strokes. Data are presented as mean ± SD, medians with interquartile range, or number of patients with percentages (%). Differences between categorical data were analyzed with a X^2 test; differences between continuous data were analyzed using a Student t test or Mann–Whitney U test. In the analyses with calcification as continuous measure, we used natural log-transformed values and added 1.0 mm^3 to the non-transformed values to deal with participants with a calcification volume of zero. We estimated the extent of misclassification of ischemic stroke subtype according to the criteria of Jackson et al.[2]

For the purpose of this study we focused on the comparison between nonlacunar and lacunar ischemic strokes. Cardiovascular risk factors, degree of symptomatic carotid artery stenosis (per 10%), presence and volume of calcifications (per mm^3) among nonlacunar versus lacunar ischemic strokes were analyzed with a univariable logistic regression model. Thereafter, a multivariable logistic regression analysis was performed to assess the difference in calcifications between nonlacunar and lacunar ischemic strokes. Adjustments were made for age, sex, and degree of symptomatic carotid artery stenosis (model 1) supplemented with cardiovascular risk factors (model 2).

Statistical analyses were performed with STATA software (version 12.0, STATA). $P<0.05$ was considered statistically significant.

Results

Patient characteristics

Between December 2005 and September 2010, 1597 patients with a TIA or ischemic stroke were registered in the ESS. Reasons for exclusion in the present study are shown in the Figure. Subsequently, the aortic arch, the symptomatic extracranial and intracranial carotid artery could be analyzed in 813, 794, and 788 patients, respectively.

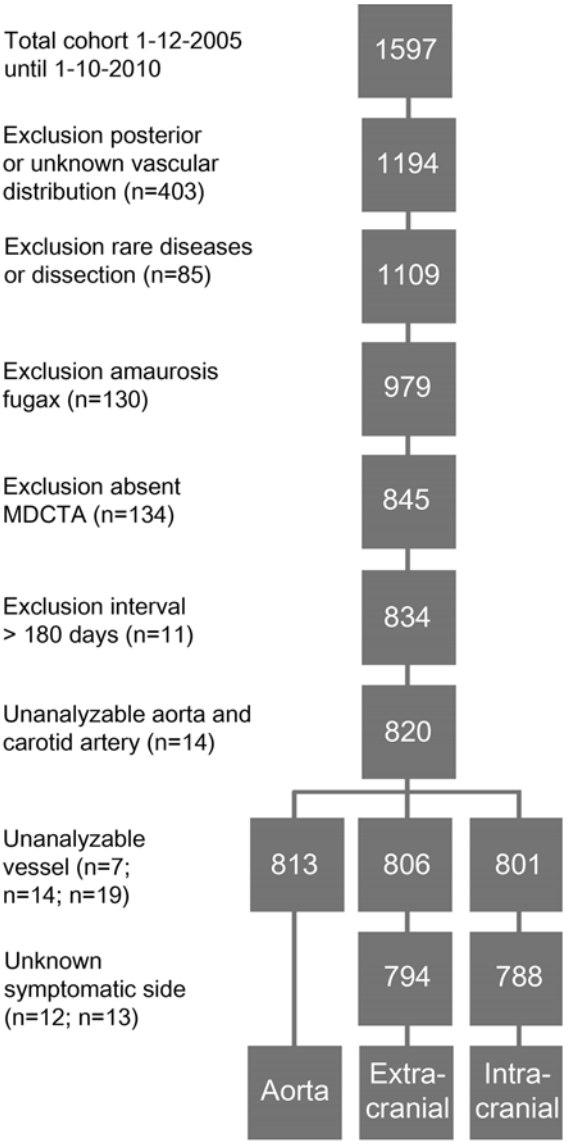

Figure. Overview study cohort and exclusion criteria.

Excluded patients without a MDCTA (n=134) were significantly older than patients with a MDCTA (mean age, 73 versus 63 years; P<0.001), had a stroke more frequently instead of a TIA (prevalence stroke 77% versus 68%; P=0.04), and had a higher total National Institute of Health Stroke Scale score on admission (median score, 5 versus 2; P<0.001).

Cardioembolic stroke was present in 112 patients. Of the remaining 708 patients, 365 (52%) had a nonlacunar and 343 (48%) a lacunar ischemic stroke. Characteristics of the study population are shown in Table 1. In 199 of the 708 patients (28%), a relevant infarct was visible on the MDCT of the brain; in 132 (36%) of the nonlacunar stroke patients and in 67 (20%) of the lacunar stroke patients, respectively. A total of 35 patients were reclassified using their brain imaging. Extrapolating this percentage to the patients without an infarct on brain imaging, 90 of the 708 patients were estimated to be misclassified (13%), with a slightly higher proportion in the lacunar strokes compared with the nonlacunar strokes (21% versus 8%). Patients with a nonlacunar stroke were significantly older than patients with a lacunar stroke, were less frequent men, and were less often a current smoker. About the other cardiovascular risk factors no significant differences were found between the 2 groups.

Table 1. Baseline characteristics in patients with nonlacunar and lacunar ischemic strokes (n=708)

Characteristics	Nonlacunar ischemic strokes	Lacunar ischemic strokes	P value
n	365 (52%)	343 (48%)	
Age, y	64 ± 14	61 ± 13	<0.001
Men	164 (45%)	187 (55%)	0.01
Hypercholesterolemia	261 (72%)	257 (75%)	0.33
Hypertension	254 (70%)	248 (72%)	0.46
Diabetes mellitus	121 (33%)	126 (37%)	0.32
Current smoking	114 (31%)	133 (39%)	0.04
Use of statins	122 (33%)	116 (34%)	0.62
History of stroke	95 (26%)	76 (22%)	0.30
Type of event			0.07
TIA	103 (28%)	119 (35%)	
Stroke	260 (71%)	223 (65%)	
Unknown	2 (0.5%)	1 (0.3%)	

TIA indicates transient ischemic attack.

Arterial calcifications

Presence and volume of aortic arch calcifications were significantly higher in patients with a nonlacunar stroke compared with patients with a lacunar stroke (prevalence 73% versus 62%; P<0.01 and median volume 62.2 versus 10.5 mm3; P<0.001; Table 2). In patients with a nonlacunar stroke, prevalence of calcifications was 56% and 59% in the symptomatic extracranial and intracranial carotid artery, respectively. Similar prevalences of calcifications were found in patients with a lacunar stroke. There was no significant difference in calcification volume in the symptomatic extracranial and intracranial carotid artery between patients with a nonlacunar and a lacunar stroke.

A significant difference in severity of symptomatic carotid artery stenosis was found between patients with a nonlacunar and a lacunar stroke. In patients with a nonlacunar ischemic stroke, more occlusions were found compared with patients with a lacunar ischemic stroke (10% versus 1%; Table 2).

Calcifications in cardioembolic strokes seemed in between (aortic arch) or lower (extracranial and intracranial carotid artery) than that of nonlacunar and lacunar strokes (Supplementary Table I).

Table 2. Vessel characteristics in patients with nonlacunar and lacunar ischemic strokes (n=708)

Characteristics	Nonlacunar ischemic strokes	Lacunar ischemic strokes	P value
n	365 (52%)	343 (48%)	
Presence of calcifications* †			
Aortic arch	261 (73%)	211 (62%)	<0.01
Symptomatic extracranial carotid	198 (56%)	169 (50%)	0.11
Symptomatic intracranial carotid	210 (59%)	178 (54%)	0.13
Calcification volume (median)†			
Aortic arch	62.2 [0-408.8]	10.5 [0-148.6]	<0.001
Symptomatic extracranial carotid	1.1 [0-27]	0.2 [0-16.7]	0.06
Symptomatic intracranial carotid	2.2 [0-20.7]	0.7 [0-16.5]	0.06
Maximum stenosis symptomatic carotid			<0.001
0%	206 (56%)	244 (71%)	
1-49%	69 (19%)	54 (16%)	
50-69%	18 (5%)	19 (6%)	
70-99%	26 (7%)	21 (6%)	
100%	36 (10%)	3 (1%)	
unknown	10 (3%)	2 (1%)	

* Presence of calcifications is defined as a calcification volume >0 mm^3.
† Of the nonlacunar ischemic strokes, calcifications were assessed in the aortic arch of 360 patients, the symptomatic extracranial and intracranial carotid artery of 351 and 353 patients, respectively. Of the lacunar ischemic strokes, calcifications were assessed in the aortic arch of 341 patients, the symptomatic extracranial and intracranial carotid artery of 336 and 331 patients, respectively.

Table 3. Determinants of nonlacunar versus lacunar ischemic strokes (n=708)

Characteristics	Univariable		Multivariable Model 1 (age, sex, symptomatic carotid stenosis)		Multivariable model 2 (age, sex, symptomatic carotid stenosis, cardiovascular risk factors)	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Age, y	1.02 (1.01-1.03)	<0.01	1.01 (1.00-1.02)	0.02	1.02 (1.00-1.03)	0.01
Men	0.68 (0.51-0.92)	0.01	0.57 (0.42-0.78)	<0.001	0.55 (0.40-0.75)	<0.001
Hypercholesterolemia	0.85 (0.61-1.18)	0.33	0.66 (0.46-0.94)	0.02	0.71 (0.50-1.03)	0.07
Hypertension	0.88 (0.64-1.22)	0.46	0.66 (0.47-0.94)	0.02	0.67 (0.46-0.96)	0.03
Diabetes mellitus	0.85 (0.63-1.16)	0.32	0.75 (0.54-1.04)	0.08	0.76 (0.55-1.06)	0.11
Current smoking	0.72 (0.53-0.98)	0.04	0.80 (0.57-1.11)	0.18	0.74 (0.53-1.04)	0.09
History of stroke	1.28 (0.93-1.75)	0.13	1.24 (0.91-1.69)	0.17	1.26 (0.87-1.84)	0.22
Presence of calcifications*						
Aortic arch	1.62 (1.18-2.23)	<0.01	1.19 (0.78-1.82)	0.43	1.30 (0.84-2.02)	0.24
Symptomatic extracranial carotid	1.28 (0.95-1.73)	0.11	0.88 (0.61-1.27)	0.50	0.93 (0.64-1.34)	0.69
Symptomatic intracranial carotid	1.26 (0.93-1.71)	0.13	0.97 (0.68-1.39)	0.87	1.04 (0.72-1.50)	0.82
Calcification volume (per mm^3)						
Aortic arch	1.14 (1.08-1.21)	<0.001	1.09 (1.01-1.19)	0.03	1.11 (1.02-1.21)	0.02
Symptomatic extracranial carotid	1.08 (1.00-1.17)	0.06	0.98 (0.88-1.08)	0.62	0.90 (0.89-1.10)	0.85
Symptomatic intracranial carotid	1.09 (1.00-1.19)	0.06	1.00 (0.90-1.11)	0.95	1.02 (0.92-1.14)	0.69
Maximum stenosis (per 10%)						
Symptomatic carotid	1.12 (1.07-1.17)	<0.001	1.11 (1.06-1.17)	<0.001	1.13 (1.08-1.19)	<0.001

Values represent odds ratio (95% confidence interval). * Presence of calcifications is defined as a calcification volume >0 mm^3

Risk factors for nonlacunar ischemic strokes

A comparison of risk factors between nonlacunar and lacunar ischemic strokes is shown in Table 3. After adjusting for age, sex, symptomatic carotid artery stenosis, and cardiovascular risk factors, age, symp-tomatic carotid artery stenosis, and volume of the aortic arch calcifications (adjusted odds ratio, 1.11; 95% CI, 1.02–1.21) were independently associated with nonlacunar strokes. Male sex and hypertension were independently associated with lacunar strokes. Volumes of extracranial and intracranial carotid artery calcifications were not independently associated with nonlacunar ischemic strokes.

Discussion

This study shows a high overall prevalence of vascular calcifications in both nonlacunar and lacunar ischemic strokes. We found no significant difference in symptomatic extracranial and intracranial carotid artery calcification volume between nonlacunar and lacunar ischemic strokes, but only a significantly higher calcification volume in the aortic arch in nonlacunar strokes compared with lacunar strokes.

Previous studies have investigated the association between arterial calcifications and cerebral ischemic strokes on brain imaging.[12,16] Bos et al[12] found in a population study an independent association between calcification volume in the aortic arch, extracranial and intracranial carotid artery, and the presence of cerebral infarcts on MRI of the brain. In contrast, Babiarz et al[16] found no difference in cavernous calcifications between patients with a MRI-confirmed acute stroke and age-matched healthy controls without a stroke. This could partially be explained by the qualitative calcification scoring system used in that study with the inherent risk of misclassification. Few studies have investigated the association between arterial calcifications and ischemic stroke subtypes or the difference in arterial calcifications between ischemic stroke subtypes. Bos et al[12] found an association between extracranial and intracranial carotid artery calcifications and the presence of lacunar infarcts and an association between aortic arch calcifications and the presence of cortical infarcts. Our finding of an independent association between aortic arch calcifications and nonlacunar strokes combined with the common notion that severe atheroma in the aortic arch is an important risk factor for ischemic stroke, indicates that the potential role of aortic arch calcifications in ischemic stroke should be further explored.[17]

The role of carotid artery atherosclerosis in ischemic stroke is established but the exact pathophysiologic pathway is unclear. In the past decade, research focus has shifted from luminal stenosis to the composition and morphology of the atherosclerotic plaque. The so-called vulnerable plaque with a large lipid-rich necrotic core, a thin fibrous cap, inflammation, intraplaque hemorrhage, and plaque ulceration is more prone to rupture and can cause an ischemic cerebral event after embolization of atherosclerotic debris and thrombus.[18] Symptomatic carotid artery plaques are suggested to have a lower degree of calcification than asymptomatic plaques, implying that calcifications may play a stabilizing role.[19] Others have shown that size, number, and location of calcifications may influence plaque vulnerability.[20] As embolization

after rupture of a vulnerable plaque will predominantly lead to cortical infarcts, one hypothesis might be that patients with nonlacunar ischemic strokes are more likely to have vulnerable plaques than patients with lacunar strokes. Our study shows that carotid artery stenosis is more prevalent in nonlacunar ischemic strokes, but the similar extent of extracranial carotid artery calcification in nonlacunar and lacunar ischemic strokes as well as in cardioembolic strokes implies that other plaque components or plaque morphology may play a more important role. For instance, Homburg et al[5] showed that plaque ulceration, a characteristic of the vulnerable plaque, is associated with nonlacunar stroke.

Strengths of our study are that we studied calcifications as a marker of the atherosclerosis in a relatively large group of consecutive patients and that we assessed calcifications in multiple vessel beds. Coronary artery calcifications are generally accepted as a sensitive marker for atherosclerosis of the coronary arteries. The guidelines of the American heart Association and American College of Cardiology uses the coronary artery calcium score as a diagnostic tool for clinical decision making.[21] In contrast, calcifications of the aortic arch, extracranial and intracranial carotid arteries have been studied less extensively. Extracranial and intracranial carotid artery calcifications are associated with cardiovascular risk factors and history of stroke.[13,22,23] Furthermore, extracranial carotid artery calcifications are associated with carotid artery stenosis in asymptomatic patients, but in symptomatic patients this association is less clear.[24-26] Moreover, calcifications can be assessed easily and quantified at MDCTA, an imaging modality increasingly used in daily clinical work-up of patients with stroke, making them a suitable and robust marker of atherosclerosis.

Our study has also some limitations. First, stroke subtype was assessed similarly to Jackson et al[2,4] using clinical criteria and correcting for brain imaging results. Although this is probably the least biased method, we found a potential misclassification of 13% compared with 7% found by Jackson et al.[2] We found a slightly higher misclassification proportion in the lacunar strokes compared with the nonlacunar strokes, which may led to an overestimation of the prevalence of lacunar strokes and a possible underestimation of the true association between calcifications and nonlacunar stroke. A second limitation of our study is the absence of a MDCTA in 134 patients. Reasons for not performing a MDCTA in our hospital were severe ischemic stroke, which were probably nonlacunar strokes, and kidney failure. Moreover, patients without a MDCTA were significantly older than patients with a MDCTA where it is known that the prevalence of calcifications increases with age.[14] This may have led to an underestimation of the true association between calcifications and nonlacunar strokes.

In summary, we found no difference in extracranial and intracranial carotid artery calcifications between nonlacunar and lacunar ischemic strokes. However, we did find that a higher calcification volume in the aortic arch is independently associated with nonlacunar ischemic strokes. These findings only partially confirm the notion of distinct etiologies for nonlacunar and lacunar ischemic strokes and suggest that the potential role of other plaque components, plaque morphology, and aortic arch calcifications in ischemic stroke subtypes needs further evaluation.

Supplementary information

Supplementary Table 1. Baseline characteristics and calcifications (presence and volume) in patients with nonlacunar, lacunar and cardioembolic strokes (n=820)

Characteristics	Nonlacunar strokes	Lacunar strokes	Cardioembolic strokes	P value nonlacunar vs lacunar	P value nonlacunar vs cardioembolic	P value lacunar vs cardioembolic
Number	365 (45%)	343 (42%)	112 (14%)			
Age, yr	64±14	61±13	66±16	<0.001	0.33	<0.001
Male	164 (45%)	187 (55%)	66 (59%)	0.01	0.01	0.42
Hypercholesterolemia	261 (72%)	257 (75%)	74 (66%)	0.33	0.25	0.07
Hypertension	254 (70%)	248 (72%)	87 (78%)	0.46	0.11	0.26
Diabetes mellitus	121 (33%)	126 (37%)	45 (40%)	0.32	0.17	0.51
Current smoking	114 (31%)	133 (39%)	22 (20%)	0.04	0.02	<0.001
Use of statins	122 (33%)	116 (34%)	34 (30%)	0.62	0.58	0.18
History of stroke	95 (26%)	76 (22%)	27 (24%)	0.30	0.63	0.19
Classification event				0.07	0.56	0.49
TIA	103 (28%)	119 (35%)	35 (31%)			
Stroke	260 (71%)	223 (65%)	77 (69%)			
Unknown	2 (0.5%)	1 (0.3%)	0 (0%)			
Presence of calcium* †						
Aortic arch	261 (73%)	211 (62%)	72 (66%)	<0.01	0.19	0.43
Symptomatic extracranial carotid	198 (56%)	169 (50%)	50 (48%)	0.11	0.13	0.69
Symptomatic intracranial carotid	210 (59%)	178 (54%)	47 (45%)	0.13	0.01	0.13

Continuation of Supplementary Table I

Characteristics	Nonlacunar strokes	Lacunar strokes	Cardioembolic strokes	P value nonlacunar vs lacunar	P value nonlacunar vs cardioembolic	P value lacunar vs cardioembolic
Calcium volume (median) †						
Aortic arch	62.2 [0-408.8]	10.5 [0-148.6]	66.5 [0-264.3]	<0.001	0.32	0.02
Symptomatic extracranial carotid	1.1 [0-27]	0.2 [0-16.7]	0 [0-21.9]	0.06	0.52	0.54
Symptomatic intracranial carotid	2.2 [0-20.7]	0.7 [0-16.5]	0 [0-14.7]	0.06	0.06	0.52
Maximum stenosis symptomatic carotid				<0.001	<0.001	<0.01
0%	206 (56%)	244 (71%)	77 (69%)			
1-49%	69 (19%)	54 (16%)	27 (24%)			
50-69%	18 (5%)	19 (6%)	0 (0%)			
70-99%	26 (7%)	21 (6%)	0 (0%)			
100%	36 (10%)	3 (1%)	2 (2%)			
Unknown	10 (3%)	2 (1%)	6 (5%)			

* Presence of calcifications is defined as a calcification volume >0 mm3. † Of the nonlacunar ischemic strokes, calcifications were assessed in the aortic arch of 360 patients, the symptomatic extracranial and intracranial carotid artery of respectively 351 and 353 patients. Of the lacunar ischemic strokes, calcifications were assessed in the aortic arch of 341 patients, the symptomatic extracranial and intracranial carotid artery of respectively 336 and 331 patients. Of the cardioembolic strokes, calcifications were assessed in the aortic arch of 109 patients, the symptomatic extracranial and intracranial carotid artery of respectively 104 and 104 patient.

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Chapter **4.2**

Plaque At RISK (PARISK): prospective multicenter study to improve diagnosis of high-risk carotid plaques

M.T.B. Truijman, M.E. Kooi, A. C. van Dijk, A.A.J. de Rotte, A.G. van der Kolk, M.I. Liem, F.H.B.M. Schreuder, E. Boersma, W.H. Mess, R.J. van Oostenbrugge, P.J. Koudstaal, L.J. Kappelle, P.J. Nederkoorn, A.J. Nederveen, J. Hendrikse, A.F.W. van der Steen, M.J.A.P. Daemen, A. van der Lugt

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Abstract

Background: Patients with symptomatic carotid artery stenosis are at high risk for recurrent stroke. To date, the decision to perform carotid endarterectomy in patients with a recent cerebrovascular event is mainly based on degree of stenosis of the ipsilateral carotid artery. However, additional atherosclerotic plaque characteristics might be better predictors of stroke, allowing for more precise selection of patients for carotid endarterectomy.

Aims and hypothesis: We investigate the hypothesis that the assessment of carotid plaque characteristics with magnetic resonance imaging, multidetector-row computed tomography angiography, ultrasonography, and transcranial Doppler, either alone or in combination, may improve identification of a subgroup of patients with < 70% carotid artery stenosis with an increased risk of recurrent stroke.

Methods: The Plaque At RISK (PARISK) study is a prospective multicenter cohort study of patients with recent (<3 months) neurological symptoms due to ischemia in the territory of the carotid artery and < 70% ipsilateral carotid artery stenosis who are not scheduled for carotid endarterectomy or stenting. At baseline, 300 patients will undergo magnetic resonance imaging, multidetector-row computed tomography angiography, and ultrasonography examination of the carotid arteries. In addition, magnetic resonance imaging of the brain, ambulatory transcranial Doppler recording of the middle cerebral artery and blood withdrawal will be performed. After two years, imaging will be repeated in 150 patients. All patients undergo a follow-up brain magnetic resonance imaging, and there will be regular clinical follow-up until the end of the study.

Study outcomes: The combined primary end-point contains ipsilateral recurrent ischemic stroke or transient ischemic attack or new ipsilateral ischemic brain lesions on follow-up brain magnetic resonance imaging.

Introduction and rationale

Ischemic stroke is the second most common cause of death in Europe, accounting for almost 1.1 million deaths each year.[1] Atherosclerosis is an important cause of clinical cerebrovascular events. The pathophysiology can be ascribed to cerebral embolism from an atherosclerotic carotid plaque or hypoperfusion due to carotid luminal stenosis.

Currently, the decision to perform carotid endarterectomy (CEA) in patients with a recent cerebrovascular event is based on the degree of luminal stenosis. CEA is highly beneficial for symptomatic patients with an ipsilateral carotid artery stenosis of 70–99%.[2–4] However, the beneficial effect of surgery for symptomatic patients with an ipsilateral stenosis between 30% and 69% is only marginal. Several studies show that vulnerable plaque features are related to cerebral embolization.[5–7] Features of a vulnerable atherosclerotic plaque are a large lipid-rich necrotic core (LRNC), a thin or ruptured fibrous cap (FC), the presence of inflammatory cells, ulcerations, and intraplaque haemorrhage (IPH). Recent studies in symptomatic patients did show that IPH, LRNC, and a thin or ruptured FC as assessed with magnetic resonance imaging (MRI) are associated with cerebrovascular events.[8-10] For multidetector-row computed tomography (MDCT), it is known that ulcerations and large calcifications and LRNC are significantly more present in symptomatic patients with an atherosclerotic cause of ischemic stroke.[11] Prospective studies for MDCT have not been conducted yet. Several prospective studies have investigated the predictive value of ultrasonography (US) in the occurrence of recurrent ischemic stroke, with conflicting results.[12–14] Microemboli detected with transcranial Doppler (TCD) predict short-term ipsilateral ischemic stroke.[15] However, all these studies were single modality studies or had a relative small population size.[8-11,15] Therefore, we hypothesize that the assessment of markers of plaque vulnerability with MRI, MDCT angiography (MDCTA), US, TCD, either alone or in combination, improves the identification of a subgroup of patients in the < 70% carotid artery stenosis group with an increased risk of recurrent ischemic stroke.

Methods

Study design

The Plaque At RISK (PARISK) study (clinical trials.gov NCT01208025) is a prospective multicenter cohort study (Table 1), which investigates whether (a combination of) non- or minimally invasive imaging techniques enable us to identify patients with symptomatic carotid artery stenosis < 70%, who have an increased risk of recurrent stroke. All patients undergo multi-sequence 3·0 Tesla (T) MRI, MDCTA and US imaging of the carotid arteries, MRI of the brain and TCD recordings of the middle cerebral artery (MCA). Also blood will be drawn for the determination of different biomarkers. Imaging will be repeated two-years after inclusion.

Table 1. Participating centers in the Netherlands. In bold, the academic hospitals.

No. of center	Name	Abbreviation
1.	**Academic Medical Center Amsterdam** **(PJ Nederkoorn, MD, PhD)** Flevoziekenhuis, Almere (M Limburg, MD, PhD) Kennemer Gasthuis, Haarlem (M Weisfelt, MD, PhD) Slotervaartziekenhuis, Amsterdam (ND Kruyt, MD, PhD)	AMC
2.	**Erasmus Medical Center Rotterdam** **(A van der Lugt, MD, PhD; PJ Koudstaal, MD, PhD)** Maasstad Hospital, Rotterdam (R Saxena, MD, PhD) Sint Franciscus Gasthuis, Rotterdam (SLM Bakker, MD, PhD) Vlietland Hospital, Schiedam (JCB Verhey, MD) IJsselland Hospital, Capelle a/day IJsel (AD Wijnhoud, MD, PhD)	EMC
3.	**Maastricht University Medical Center** **(ME Kooi, PhD; WH Mess, MD, PhD; RJ van Oostenbrugge, MD, PhD)** Atrium Medical Center, Heerlen (T Schreuder, MD) Laurentius, Roermond (AG Korten, MD, PhD) Orbis Medical Center, Sittard (NP van Orshoven, MD, PhD) Viecuri Medical Center, Venlo (BJ Meems, MD, PhD)	MUMC
4.	**University Medical Center Utrecht** **(J Hendrikse, MD, PhD; LJ Kappelle, MD, PhD)** Diakonessenhuis, Utrecht (R Donders, MD, PhD) Sint Antonius ziekenhuis, Nieuwegein (S Tromp, MD, PhD) Ter Gooi ziekenhuizen, Hilversum Blaricum (J de Kruijk, MD, PhD)	UMCU

Study objectives

The primary objective of the PARISK study is to identify whether high-resolution MRI, MDCTA, US, and/or TCD enable us to predict future ischemic events in recent symptomatic patients with < 70% carotid stenosis. The secondary objectives are to study (1) determinants for plaque progression; (2) the relationship between plaque characteristics, microemboli, and vascular damage on brain MRI; and (3) the association between blood biomarkers and plaque parameters.

Patient population

Eligible for the study are patients with a transient ischemic attack (TIA), amaurosis fugax or minor stroke (modified Rankin scale ≤3) of the carotid artery territory and an atherosclerotic plaque with a < 70% stenosis of the ipsilateral internal carotid artery (ICA) who are not scheduled for a revascularization procedure. Patients need to be eligible for imaging and blood withdrawal within three-months after initial ischemic event. Exclusion criteria are a probable cardiac source of embolism, a clotting disorder, severe comorbidity, standard contraindications for MRI, a documented allergy for MRI or CT contrast agent or a renal clearance of <30 ml/min. Written informed consent will be obtained from all patients before enrolment.

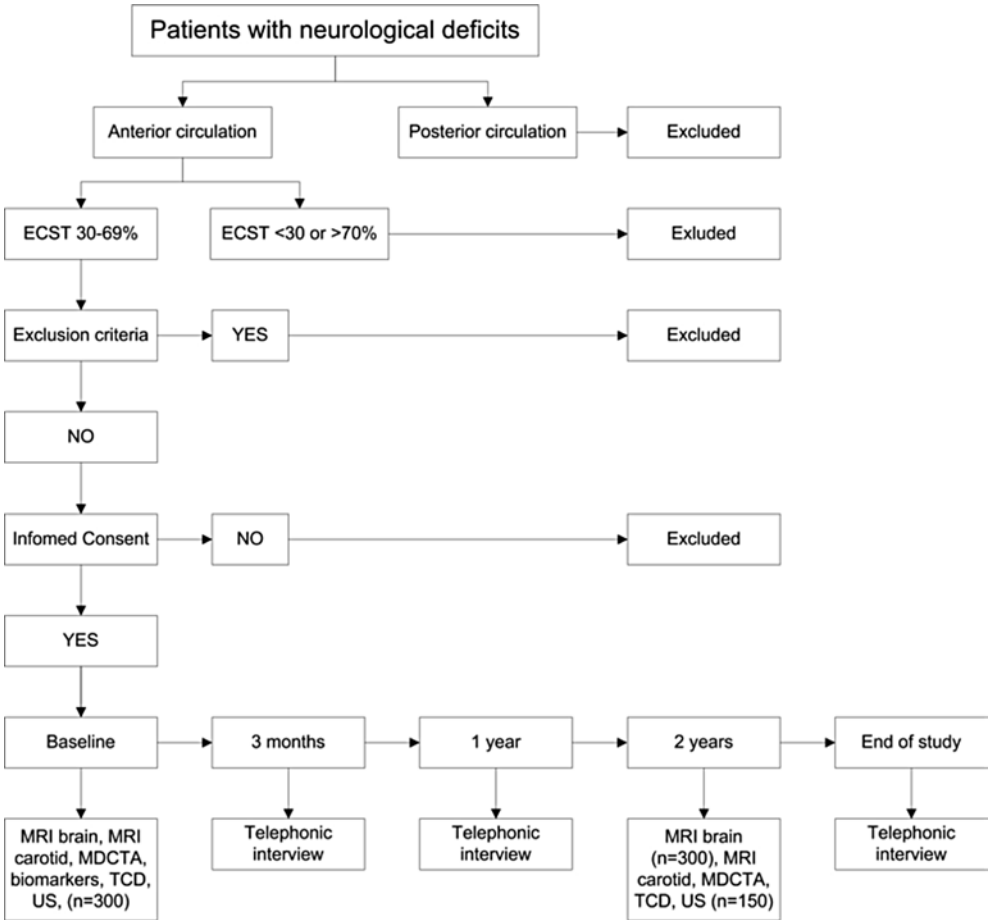

Figure 1. Flowchart of study protocol.

Study protocol

Degree of stenosis will be determined with clinically obtained Doppler US or CT angiography (CTA). The upper cutoff value of 70% is based on the North American Symptomatic Carotid Endarterectomy Trial criteria.[16] The lower cutoff value is an atherosclerotic plaque with a thickness of at least 2–3 mm, which corresponds to an European Carotid Surgery Trial stenosis of 30%.[3] A total of 300 patients will be included.

At baseline, clinical data such as age, sex, occurrence of last symptoms, medication use and cardiovascular risk factors are collected. Blood samples will be drawn. All noninvasive imaging examinations will be performed within a five-day time window.

Follow-up by telephone will be done after three-months, one year, and yearly until the end of the study in December 2014 (Fig. 1). During follow-up, clinical data such as daily functioning (modified Rankin scale), changes in medication use, cardiovascular risk factors, cardiovascular and cerebrovascular events and hospital admissions are collected. Two-years after inclusion, noninvasive imaging of the carotid artery will be repeated in the first 150 patients. Follow-up brain MRI will be performed in all patients after two-years.

MRI

Magnetic resonance imaging will be performed on 3·0-T whole body scanners. A dedicated eight-channel phased-array coil (Shanghai Chenguang Medical Technologies Co., Shanghai, China) is used for imaging of the carotid artery in three centers; a dedicated four-channel carotid phased-array coil with an angulated setup (Machnet B.V., Roden, the Netherlands) is used in one center. For brain imaging, dedicated head coils are used. First, brain MR images will be acquired using the sequences as listed in Table 2. Second, the carotid bifurcation will be identified by means of MR angiography without contrast enhancement. The atherosclerotic plaque will be imaged with a multi-sequence MR protocol as listed in Table 3. Fifteen transverse adjoining

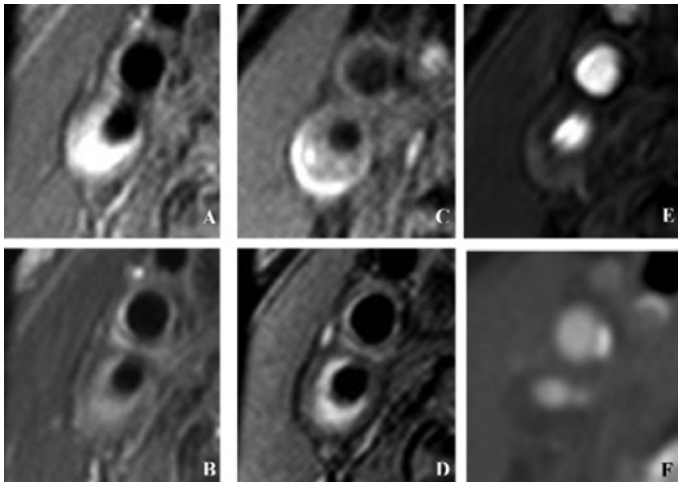

Figure 2 Co-registered pre- (A) and postcontrast (B) T1-w TSE, T1-w TFE (C), T2-w TSE (D) TOF (E) and MDCTA (F) of the internal carotid artery from a 66 year old male patient with transient dysarthria.

Table 2. Scan parameters: MR brain

Pulse sequence	T2w FSE	T2w TSE	FLAIR		T2*w SPGR	T2*w FFE	DWI	
Center	2	1,3,4	2	1,3,4	2	1,3,4	2[2]	1,3,4[2]
Acquisition format	2D		2D		3D	2D	2D	
TR (ms)	6900	3198	8800	11000	25	1653	5100	3835
TE (ms)	80	19/140	140	125	16	20	72	90
TI (ms)	-	-	2250	2800	-	-	-	-
Flip angle (°)	90		90		12	90	90	
No. of slices	48		48		94[1]	48	48	30
Slice thickness (mm)	3		3		1.6	3	3	5
Slice gap (mm)	0	0	0	0	0	0	0	0.1
FOV (mm)	230x190	230x190	230x230	230x190	230x190	230x190	230x230	208x153
Acquisition matrix	256x192	232x179	256x160	232x148	224x192	232x190	116x130	128x102
Acquired voxel size	0.89x 0.99	0.99x 1.06	0.90x 1.44	0.99x 1.28	1.03x 0.99	0.99x 1.00	1.98x1.77	1.63x1.5
Reconstruction matrix	256x256	240x240	256x256	240x240	256x256	240x240	256x256	128x128
Reconstructed voxel size	0.90x 0.74	0.96x 0.79	0.90x 0.90	0.96x 0.79	0.90x 0.74	0.96x 0.79	0.90x0.90	1.63x1.20
Echo train length	15	26	42	31	1	1	Single-shot	Single-shot
Parallel imaging	Yes		No	Yes	Yes		Yes	
No. of signal averages	1	2	1		1		2	1

[1]180 reconstructed slices of 0.8 mm [2]No of B-factors 3; FSE: fast spin echo, TSE: turbo spin echo, FLAIR: fluid attenuated inversion recovery, SPGR: spoiled gradient echo, FFE: fast field echo, DWI: diffusion weighted imaging, TR: repetition time, TE: echo time, TI: inversion time, FOV: field of view.

Table 3. Scan parameters: MR carotid arteries

Pulse sequence	**FSPGR**	**TOF FFE**	**SPGR**	**IR-TFE**[1]	**T2w DIR FSE**	**T2w TSE**	**T1w DIR FSE**	**T1wQIR TSE**
Center	2	1,3,4	2	1,3,4	2	1,3,4	2	1,3,4
Acquisition format	3D		3D		2D		2D	
Acquisition plane	coronal	transversal	coronal	transversal	transversal	transversal	transversal	transversal
TR (ms)	3.3	20	9	9.1	2 RR	4800	1 RR	800
TE (ms)	2.1	5	1.3	5.5	50	49	5.2	10
TI (ms)	n/a		n/a	304	Auto[2]	n/a	Auto[2]	282, 61
Flip angle (°)	5	20	30	15	-		-	
No. of slices	120	15	248	15	15		15	
Slice thickness (mm)	0.8	2	0.8	2	2		2	
FOV (cm)	160x160	160x160	160x160	160x128	140x160	160x160	140x160	160x160
Acquisition matrix	160x128	260x258	160x128	260x204	256x224	260x252	256x224	260x240
Acquired voxel size	1.00x1.25	0.62x 0.62	1.00x 1.25	0.62x 0.63	0.55x0.71	0.62x0.63	0.55x 0.71	0.62x 0.67
Reconstruction matrix	256x256	528x528	256x256	528x528	256x256	528x528	256x256	528x528
Reconstructed voxel size	0.63 x0.63	0.30x 0.30	0.63x 0.63	0.30x 0.24	0.55x0.63	0.30x0.30	0.55x 0.63	0.30x 0.30
Echo train length	1		1	51	24	12	12	10
Parallel imaging	No		No		No		No	
No. of signal averages	7	1	1	6	1		1	
ECG triggered	No	No	No	No	Yes	No	Yes	No
Fat suppression	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes

[1]shot interval time: 550 ms; [2]approximately TI adjusted to T_1 relaxation time of blood by scanner
FSPGR, fast spoiled gradient echo, TOF: time of flight, FFE: fast field echo, TFE: turbo field echo, DIR: double inversion recovery, FSE: fast spin echo, QIR: quadruple inversion recovery, TSE: turbo spin echo, TR: repetition time, TE: echo time, TI: inversion time, n/a: not applicable, FOV: field of view, NSA: number of signal averages

slices of 2 mm each, covering the entire plaque or a 3D volume of the extracranial carotid artery is used. Six-minutes after injection of 0·1 mmol/kg body weight of a gadolinium-based contrast agent the T1w DIR FSE (center 2) or T1w QIR TSE (center 1, 3, 4) are repeated to obtain postcontrast images (Fig. 2).

MDCTA

Image acquisition will be performed using a 16, 64, or 128 slice multi-detector row CT system using a standardized optimized contrast-enhanced MDCTA protocol (120 kVp, 150–180 mAs, collimation 16 × 0·75 mm or 64 × 2 × 0·6 mm, pitch <1). One center reconstructs 120 kVp images from the 100 kVp and 140 kVp images obtained with dual-energy MDCTA. The scan range extends from the ascending aorta to the intracranial circulation (3 cm above the sella turcica). All patients receive 80–85 ml of an iodinated contrast agent (300–320 mg/ml) followed by a 45 ml saline bolus chaser, both at an injection rate of 4 or 5 ml/s. Real-time bolus tracking at the level of the ascending aorta is used. Image reconstructions are made with a FOV of 120–160 mm, matrix size 512 × 512, slice thickness 1·0 mm, increment 0·6–0·7 mm and with intermediate reconstruction algorithms.

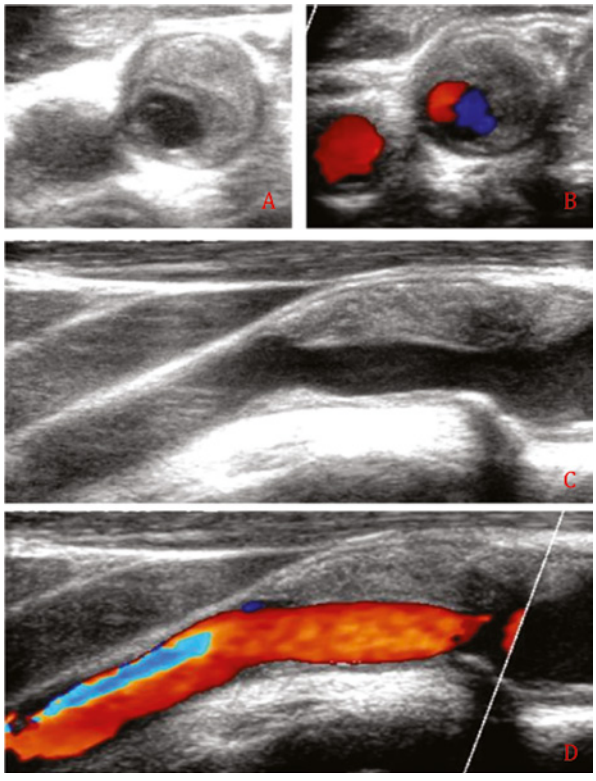

Figure 3 Transverse (A, B) en longitudinal (C, D) ultrasound images of the internal carotid artery in B-mode (A, C) and colour-coded (B, D). Images are from the same patient as figure 2.

Ultrasound

After 10 min of rest in supine position, duplex ultrasound examination (Fig. 3) will be performed by a well-trained US technician. The extracranial arteries are visualized with a 17-5 MHz linear array transducer. If the artery is located too deep, a 12-5 or a 9-3 MHz transducer will be used. The protocol consists of (1) longitudinal images of the common carotid artery (CCA), the carotid bulb, and the ICA in B-mode and color Doppler mode, (2) pulsed Doppler recordings of the CCA and the ICA with a sample volume of 1 mm, and (3) transversal recording from the CCA to the ICA in B-mode and color Doppler covering a total length of 8 cm. All images will be recorded from anterolateral and posterolateral views at a frame rate >40 Hz and with a cine loop of five seconds.

TCD

Transcranial Doppler measurements will be performed on the symptomatic side only. With conventional Doppler, the transtemporal window is investigated, as well as the depth of the main stem of the MCA. In case of a sufficient window, an ambulatory TCD system (TCD-X, Hemodynamics AG, Bern, Switzerland) is positioned at the location of the transtemporal bone window. When the signal of the MCA is detected, settings such as sample volume, gain, power, and depth are optimized by means of dedicated software on a laptop connected to the TCD-X. Afterward, the device is disconnected from the laptop and the four-hour recording will start.

Biomarkers

Nine milliliters (ml) of citrated plasma for platelet-rich plasma, 9 ml citrated plasma for platelet-poor plasma, 8·5 ml of serum, 10 ml of ethylenediaminetetraacetic acid plasma, and 4·5 ml acidified citrated plasma (Stabilyte) will be taken for the determination of different biomarkers. All samples will be processed within one-hour and stored in 0·5 ml tubes at −80 °C until analysis. Markers for thrombus generation and formation, fibrinolysis, endothelial function, and vascular inflammation will be assessed. Blood samples will be stored for 15 years. The informed consent form includes an additional question on the use of blood samples for extra testing (e.g. genetic testing) for research related to the main hypothesis.

Image data analysis

All data will be evaluated by trained readers blinded to the results of other image modalities, clinical data, and baseline/follow-up data. Each reader performs training on test sets and interobserver reproducibility will be assessed beforehand.

MRI evaluation of the carotid artery will be done using dedicated vessel wall analysis software (VesselMass, department of Radiology, Leiden University Medical Center, the Netherlands) as described previously.[17] MR images will be assessed for vessel wall and luminal area, LRNC area, calcification area, presence of IPH and FC status using previously published criteria.[18–20] MRI of the brain will be scored for cortical and lacunar infarcts, microbleeds, and the severity of white matter lesions according to Fazekas et al.[21]

All MDCTA data will be evaluated for the most severe stenosis in the carotid bifurcations and internal carotid arteries. Calcifications at extracranial carotid arteries within 3 cm proximal and distal of the bifurcation will be measures and expressed as calcification volume in mm^3. A threshold of 600 Hounsfield units will be used to differentiate calcifications from contrast material in the lumen. Plaque ulceration is defined as extension of contrast material of >1 mm into the atherosclerotic plaque on at least two orthogonal planes. Plaque volumes will be assessed with custom-made software.[22] Plaques will be subdivided into fatty plaques, mixed or calcified plaques based on attenuation values.[23]

Ultrasound data will be analyzed on software developed in-house, based on previously published algorithms.[24–26] The anatomical course of the carotid artery, the presence of plaques, and the degree of stenosis will be scored. Second, images will be quantified on appearance by using dedicated gray scale analysis software. The morphology of the plaque will be determined by manually contouring plaques to quantify its area, thickness, length, and echogenicity. In addition, the morphology of the artery will be characterized by end diastolic diameter and vessel wall thickness, and their inhomogeneities along the artery section. Finally, dynamic vessel wall characteristics will be quantified by distension, i.e. the change in diameter over a cardiac cycle, and its spatial inhomogeneity.

The four-hour transcranial Doppler recordings will be analyzed using semiautomatic software (Hemodynamics AG, Bern, Switzerland). All high-intensity transient signals will be verified by a trained reader. Based upon predefined criteria they will be identified as either microembolic signals (Fig. 4) or artifacts.[27]

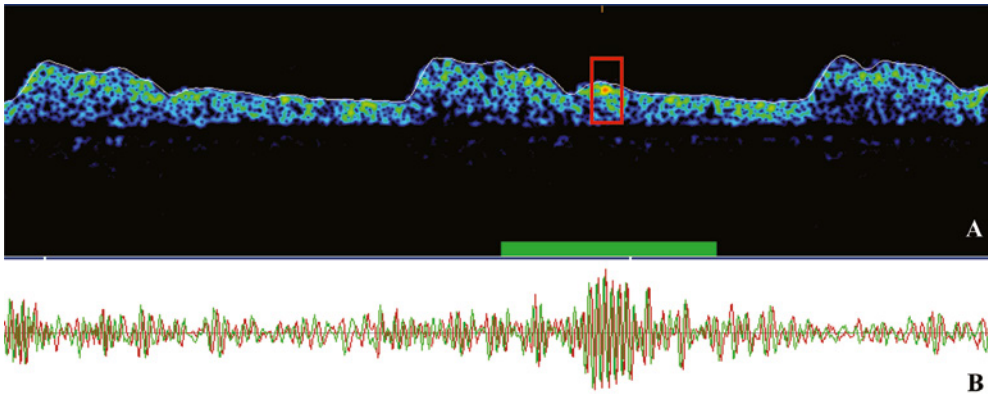

Figure 4 The red box indicates a typical example of a microembolic signal, both in the frequency (a) and time (b) domain.

Outcome measures

The primary end-point is an ipsilateral recurrent ischemic stroke, or TIA and/or new ipsilateral ischemic brain lesions on follow-up brain MRI. Clinical events are verified by a neurologist. Secondary end-point is new ipsilateral ischemic brain lesions on MRI.

Statistical analysis

Cox proportional hazard models will be used to compute hazard ratios with 95% confidence intervals for the association of plaque parameters with the primary and secondary end-points. In the primary analysis we will focus on four plaque parameters: (1) presence of IPH as assessed on ipsilateral MRI of carotid plaque, (2) ipsilateral carotid plaque ulceration as assessed on MDCTA, (3) ipsilateral carotid plaque volume as assessed on MRI, and (4) the proportion of calcifications with respect to the ipsilateral carotid plaque volume as assessed with MDCTA. First, we will analyze the data univariately, then we will adjust for age, sex, and major cardiovascular risk factors. Subsequently, we will adjust for the other plaque parameters. Additionally, we will investigate the association between number of microemboli, ultrasound gray scale values and vessel wall motion, volume of plaque components on MRI (calcifications, LRNC, fibrous tissue), FC status on MRI, plaque volume on MDCTA, and the end-points.

Sample size

The expected incidence of ischemic stroke is 2·5% per year based on the standard medical treatment of antiplatelet therapy, a statin and antihypertensive agents to obtain strict blood pressure control. This means in a group of 300 patients, with a follow-up of two-years, we expect an incidence rate of 15 ischemic strokes. In a recent study in one of our participating centers 14/72 (19%) symptomatic patients with 30–69% carotid stenosis had a new silent infarct on brain MRI after one-year. Assuming that this number will not increase with a follow-up of two-years, we expect that 57 of the 300 patients will demonstrate a new infarct on brain MRI. Based on this number we will be able to evaluate 6–11 imaging parameters for the primary analysis.

Data storage

All clinical information from the different centers will be stored in a sophisticated web-based database system (OpenClinica version 3·1.2, Community Edition, Boston, MA, USA). All image data from different centers are anonymized and centrally stored on a DICOM server (based on open source DCM4CHEE software) hosted by one of the participating centers. Image data are accessible for all partners.

Ethical and regulatory considerations

The PARISK study will be performed in the Netherlands and has been approved by the Medical Ethical Committees of the participating academic centers. All participants will provide written informed consent.

Discussion

We present the protocol of a prospective multicenter cohort study, PARISK, to identify imaging parameters that can improve risk prediction for stroke recurrence in recently symptomatic patients with < 70% carotid artery stenosis and help in the selection of patients for CEA. These imaging parameters will be determined using detailed imaging of the carotid arteries using 3T MRI, MDCTA, and US as well as TCD of the MCA.

Atherosclerotic plaques in carotid arteries have been studied intensively over the last decades. However, the PARISK study has a different approach, as compared with previously published studies. To our knowledge, it is the first prospective multicenter cohort study with multimodality imaging of the carotid artery. Patients referred to academic hospitals as well as to regional hospitals will be included and centrally imaged in the academic centers. This setting enables us to include a large number of patients in a relative short period of time. In the various academic hospitals different MRI and MDCT scanners are used, which reflects variation in clinical practice.

The multimodality approach allows us not only to study morphological parameters, such as the size of LRNC or FC status, but also biological and biomechanical parameters such as blood biomarkers and vessel wall motion. The ability to define which (combination of) parameters predicts a recurrent stroke makes this a highly clinical relevant prognostic cohort study. The event rate of stroke has significantly decreased in the last decade. Based on studies from Rothwell et al.[2] we originally expected 30 clinical end-points in 36 months of follow-up in our study population. However, this number will probably be much lower due to widespread use of statins, antiplatelet therapy, and blood pressure optimization.[28] For this reason, the PARISK trial uses a primary composite end-point that combines clinical events and silent brain infarcts on MRI.

Imaging studies have revealed that 30–50% of the TIAs are accompanied by ischemic lesions on diffusion weighted imaging MRI in the acute phase and that most of these lesions lead to permanent brain damage.[29,30] Vernooij et al.[31] showed in healthy participants that silent brain infarcts are much more prevalent than previously expected. These silent infarcts have the same risk factors as infarcts with neurological deficits as result and are considered to have the same pathophysiological mechanism.

Conclusion

The PARISK study represents a prospective multicenter study of symptomatic patients with recent (<3 months) neurological symptoms due to ischemia in the territory of the carotid artery and a < 70% ipsilateral carotid artery stenosis who are not scheduled for CEA or stenting. The primary objective of this study is to identify whether MRI, MDCTA, US, or TCD or a combination of these techniques enable us to identify patients with an increased stroke risk. This would highly influence clinical decision making.

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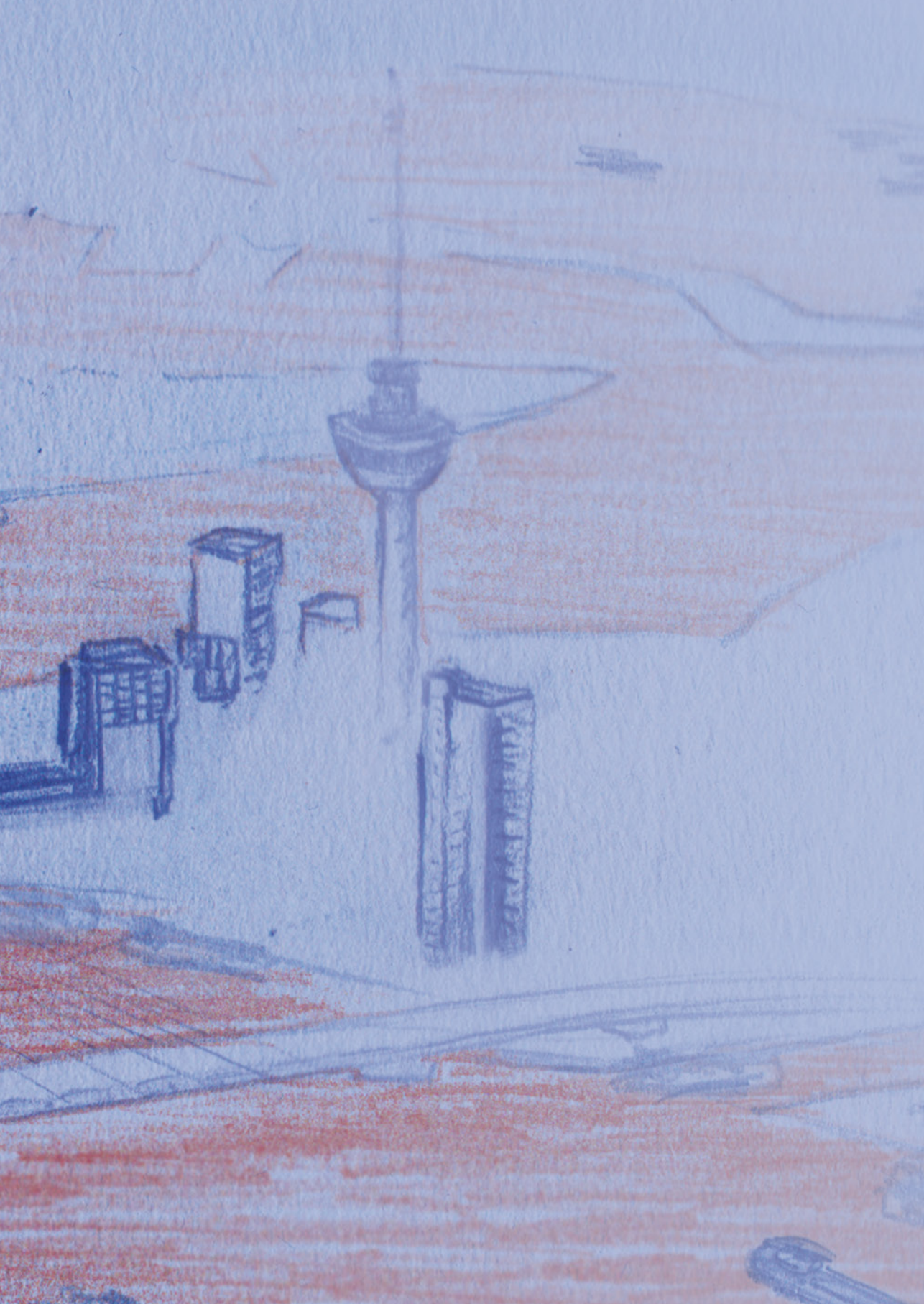

Chapter **5**

General discussion

General discussion

Rupture is one of the major hazards of an atherosclerotic plaque and can lead to an ischemic stroke or TIA. Ischemic stroke is an important cause of disability and cardiovascular death. Imaging techniques like contrast-enhanced ultrasound (CEUS), multidetector-row computed tomography angiography (MDCTA) and magnetic resonance imaging (MRI) have made it possible to quantify atherosclerotic burden by degree of stenosis, maximum vessel wall area or calcification volume. In addition, characteristics of the vulnerable, i.e.rupture-prone, plaque can be visualized as plaque ulceration, intraplaque hemorrhage (IPH) and lipid core. In this thesis, I focus on extracted imaging biomarkers of the atherosclerotic plaque in the carotid artery acquired using CEUS, MDCTA and MRI for a better understanding of plaque pathophysiology and the role of blood coagulation. In addition, I evaluate which image modalities and which imaging biomarkers can be valuable in the clinical setting.

Atherosclerosis and coagulation

Von Willebrand factor (VWF) plasma levels increase as a result of endothelial damage and VWF plays an important role in the initial steps of thrombus formation.[1] Levels of VWF are increased in ischemic stroke patients and the highest levels of VWF are found in patients with large vessel disease and cardio-embolic strokes.[2, 3] In addition, high levels of VWF are associated with an increased risk of ischemic stroke.[4] It has been suggested that because endothelial activation is related to atherosclerosis and an association has been found between the intima-media thickness and increased VWF levels in healthy individuals, atherosclerosis itself (and not only thrombus formation) may be a determinant of VWF levels.[5] In contrast, several in vitro and in vivo studies that for example evaluated interactions between VWF/Weibel-Palade bodies (which secret VWF) and low-density lipoproteins (LDL), smooth muscle cells (SMCs) and inflammation, suggest VWF might contribute to the pathogenesis of atherosclerosis.[6] In addition, animal studies have shown that absence of VWF may have a protective effect against the development of atherosclerosis which indicate that VWF could be a determinant of atherosclerosis.[6-8] However, results of patients studies are inconclusive. On the one hand, Bilora et al. found that patients with hemophilia A and von Willebrand disease had fewer carotid plaques and a smaller degree of carotid stenosis than normal subjects of the same sex and age.[6, 9] On the other hand, Sramek et al found no substantial differences in intima-media thickness between Von Willebrand disease patients and healthy controls.[6, 10] To conclude, the exact mechanism of the interaction between VWF and atherosclerosis seems complex and remains unclear.

A Disintegrin And Metalloprotease with Thrombo Spondin motif repeats 13 (ADAMTS13) has been studies less intensively than VWF. ADAMTS13 cleaves large VWF multimers into smaller and lower prothrombotic forms, and low ADAMTS13 activity is associated with an increased ischemic stroke risk.[11, 12] The underlying mechanism of the association between ADAMTS13 and ischemic stroke risk, and the potential role of atherosclerosis, is also still unclear.

Novel imaging biomarkers of atherosclerosis assessed by MDCTA and MRI do not only quantify atherosclerotic burden, but can also indicate that characteristics of a vulnerable, i.e. rupture-prone, plaque are present. We hypothesized that this detailed atherosclerotic plaque information could help identify an association between atherosclerosis and blood biomarkers VWF and ADAMTS13. We found a strong correlation between calcification volume in the aortic arch and carotid arteries and VWF levels in patients with an ischemic stroke or TIA suggesting a positive association between atherosclerosis and VWF (**Chapter 2.1**). In accordance with current literature[2, 3], highest VWF levels were found in patients with large vessel disease compared with the other etiological subtypes (**Chapter 2.1**). However, if we look specifically in a group of patients with carotid artery atherosclerosis and a moderate carotid artery stenosis or if we look at vulnerable plaque characteristics like plaque ulceration, intraplaque hemorrhage and lipid, no associations were found between imaging biomarkers and the coagulation factors VWF and ADAMTS13 (**Chapter 2.2 and 2.3**). A previous study in acute coronary syndrome patients also showed that presence of atherosclerosis (measured with IVUS) was associated with VWF levels, but no associations were found between VWF levels and high risk, prone-to-rupture atherosclerotic lesions.[13]

Vulnerable plaque rupture is crucial in the pathophysiological cascade from atherosclerotic plaque development to ischemic stroke or TIA. Due to the role of VWF and ADAMTS13 in thrombus formation and the less clear role of VWF and ADAMTS13 in atherosclerotic plaque development, we expected that the association between plaque ulcerations (and other vulnerable plaque characteristics) and the blood biomarkers is more clear than the association between plaque volume and VWF and ADAMTS13. However, the opposite was found and our results do, unfortunately, not clarify the association between VWF and ADAMTS13 and atherosclerosis; more – probably basic or animal – studies are needed. It seems that not the local disturbance of blood flow or the disruptive plaque surface in the carotid bifurcation causes an increase in VWF or a decrease in ADAMTS13. The alteration of the endothelial layer due to widespread atherosclerotic disease may cause the change in coagulation markers. If this is true, blood coagulation markers are then a marker of a wide spread atherosclerotic disease and not a modifiable risk marker for secondary prevention. However, a complex role of VWF and ADAMTS13 in atherosclerotic plaque development can't be ruled out. Our results show that quantifying atherosclerotic burden and assessing vulnerable plaque characteristics indeed provides different information about the atherosclerotic plaque and that a clear distinction should be made in future studies between plaque volume measurements and (vulnerable) plaque characteristics.

Comparison of non-invasive plaque imaging techniques

US, MDCTA and MRI are all used in current clinical practice to assess degree of carotid stenosis, and to some extent plaque irregularity, and help identifying stroke patients who benefit most from a carotid-endarterectomy (CEA). The last decades have witnessed increased interest in imaging of the atherosclerotic plaque to characterize the plaque in other ways than only degree of stenosis and to gain knowledge on plaque pathophysiology. Plaque area, luminal border regularity, echolucency and

heterogeneity are all imaging biomarkers which can be assessed by US and can give information about plaque ulceration and plaque composition like lipid core, intraplaque hemorrhage and calcifications.[14, 15] However, sensitivity and specificity vary and are limited.[15] Fortunately, MDCTA is well capable to assess carotid plaque burden, plaque component volumes (especially calcifications) and plaque ulceration.[16-18] Nevertheless, radiation exposure is a drawback of MDCTA, especially in a screening setting, and soft tissue contrast is limited.[15, 19] MRI with excellent soft-tissue contrast and without ionizing radiation is, therefore, of great interest in plaque imaging research; and MRI can correctly evaluate carotid plaque burden, plaque component volumes (especially intraplaque hemorrhage) and fibrous cap status.[15, 19-22] However, MRI is less available, expensive and has relative long scan times.[20-22] Although advantages and limitations of the different non-invasive image modalities have been well studied individually, less is known about how the different image modalities relate to each other. In the clinical setting, it is impossible to perform all imaging techniques in one patient; it is time consuming, expensive and burdensome for the patients. We therefore do not know which imaging modality is best for application in the clinical setting. Many (prospective) studies on the clinical significance of plaque characteristics will be needed to answer this question. In my thesis, I performed three cross-sectional studies to investigate how US, MDCTA and MRI interrelate regarding plaque ulceration, intraplaque hemorrhage and calcifications.

Plaque irregularity (plaque ulceration and/or fissured fibrous cap; a sign of plaque rupture) is associated with an increased risk of ipsilateral ischemic stroke. Plaque irregularity and ulceration can – as mentioned before – be well assessed using MDCTA.[23, 24] However, some patients cannot undergo MDCTA due to contrast allergy and in addition, radiation exposure in MDCTA is a drawback in a screening setting. Perhaps US and MRI can be an alternative to visualize plaque ulceration. Assessment of plaque ulceration or fibrous cap status on MRI seems adequate, but multisequence, contrast-enhanced MRI protocols are necessary.[25-27] Sensitivity of regular US to show plaque ulceration is low, only 37,5%.[28] In contrast-enhanced ultrasound (CEUS) a microbubble contrast agent is administered and better delineation of the carotid lumen is achieved; CEUS might be a safe, cheaper and widely available alternative for MDCTA and MRI (**Chapter 3.3**).[29] I found, in accordance with the literature, indeed limited use for regular ultrasound in the detection of plaque ulcerations, but also that the use of microbubbles improves the accuracy of ultrasound with an increased agreement between ultrasound and MDCTA (κ=0.19 (US) to κ=0.42 (CEUS); sensitivity 29% (US) to 88% (CEUS); specificity 73% (US) to 59% (CEUS); Chapter 3.3). Nevertheless, acoustic shadowing of calcifications remains a limitation of CEUS and can potentially obscure plaque ulcerations. In addition, the 2-dimensional image data in CEUS can be a disadvantage compared to the 3-dimensional volumetric data in MDCTA.

A second important characteristic of the vulnerable plaque is intraplaque hemorrhage. Presence of intraplaque hemorrhage in the carotid artery is associated with an increased risk of ischemic stroke.[30-33] Common notion is that small leaky neovessels in the atherosclerotic plaque are the likely source of intraplaque hemorrhage.[34-36] Intraplaque hemorrhage can lead to plaque destabilization which can subsequently result in plaque rupture.[34-36] MDCTA is not capable to visualize intraplaque hemorrhage, but MRI can. In contrast, MRI is less suitable to detect plaque ulceration than MDCTA. By comparing MDCTA and MR images of the carotid artery in the same

patients, I found a strong association between intraplaque hemorrhage on MRI and disruption of the plaque surface (plaque ulceration and/or fissured fibrous cap) assessed at MDCTA (**Chapter 3.1**). This finding supports the notion that intraplaque hemorrhage increases the risk of plaque rupture. However, due to the cross-sectional design of our study, serial studies are needed to evaluate whether intraplaque hemorrhage indeed increases the risk for plaque rupture and subsequent symptoms.

Interobserver agreement for intraplaque hemorrhage was high (κ=0.95, **Chapter 3.1**), which makes assessment of presence of intraplaque hemorrhage on MRI a reliable prognostic marker in the clinical setting; probably more sound than the assessment of disruption of the plaque surface with MDCTA (interobserver agreement κ=0.41, **Chapter 3.1**).

Finally, a third interesting plaque characteristic is plaque calcification. Recently, interest in plaque calcification has renewed by arising evidence that plaque calcification is not just a passive process.[37] Carotid artery calcifications are associated with degree of carotid artery stenosis and are increasingly used as a marker of atherosclerosis.[38-40] (Macro)calcifications are considered to be plaque stabilizers; increased calcium density is associated with lower cardiovascular risk and the proportion of calcification in the atherosclerotic plaque is inversely associated with symptomatic plaques.[41, 42] Although MRI is increasingly used in plaque imaging studies, detection of calcifications with MRI is however a challenge.[37, 43] I found a good sensitivity, but a low specificity of MRI for the detection of plaque calcifications in comparison to MDCTA (**Chapter 3.2**). In addition, an overall agreement was found in calcification volume assessed with MDCTA and MRI; however, absolute individual differences were substantial. Methodological reasons like the use of a 600 HU threshold and an extended scan range (MDCTA), technical reason like insufficient image quality of MRI as well as wrong interpretation and delineation of low signal intensities on MRI were found as the most prevalent reasons for a mismatch in the detection of calcifications or a large difference in calcification volume measurements. Although the results for MRI are disappointing for now, we expect that, agreement between MRI and MDCTA calcifications can be improved by training in MRI interpretation and also by an increase in image quality.

In conclusion, (CE)US, MDCTA and MRI have their own potentials and limitations and are not interchangeable. No clear choice of one preferred or superior image modality can be made at this point. Additional cross-sectional and future prospective studies focusing on the evaluation of vulnerable plaque characteristics that increase the risk of ischemic stroke and TIA, should help clarify this question. However, the use of one or two short MRI series focusing on specific plaque characteristics, like intraplaque hemorrhage, in addition to a MDCTA to detect plaque ulcerations or calcifications, seems a likely possible scenario. Caution should be applied when interpreting calcifications on MRI.

Clinical studies

Numerous studies have investigated the opportunities and limitations of US, MDCTA and MRI to assess plaque characteristics and how these plaque characteristics relate with clinical characteristics or outcome. However, the question whether plaque imag-

ing can help clinical decision making is still unanswered.[44, 45] The multicenter **Plaque At RISK (PARISK)**, a large multicenter cohort study investigates if plaque imaging enables us to better identify patients in the 30-69% carotid stenosis group with an increased stroke risk, may help to answer this question (**Chapter 4.2**).

Study design of the PARISK-study is described in **Chapter 4.2**. Cross-sectional analyses of the data collected in the PARISK-study are used in several chapters of my thesis (**Chapter 2.2, 3.1 and 3.2**). Therefore, I will discuss some methodological considerations regarding the PARISK-study. The original goal of the PARISK-study was the inclusion of 300 patients in two years. However, a total of 240 patients was included in 3,5 years. After a slight delay in the start of the inclusion due to Institutional Review Board formalities, inclusion rate was lower than expected due to strict inclusion criteria and a higher refusal rate of patients than expected due to the intense study protocol (approximately 60% of eligible patients were willing to participate). By adding a fourth academic research center and addressing local peripheral hospitals, inclusion rate was increased. In addition, inclusion criteria were adapted. Amount of carotid atherosclerosis was a main inclusion criterion; the lower limit of amount of atherosclerosis was adjusted from NASCET > 30% to ECST > 30%.[46, 47] We observed considerable amounts of carotid atherosclerosis but without a 30% NASCET stenosis. Because of the interest in plaque characteristics rather than the degree of stenosis, adjusting the inclusion criteria was – in our opinion – valid.

The lower number of included patients and the new insights concerning the lower stroke recurrence rate due to improved secondary prevention have affected the statistical power of the PARISK-study. However, recent studies showed that 30-50% of the TIAs are accompanied by ischemic lesions on DWI-MRI in the acute phase and most of these lesions lead to permanent brain damage.[48, 49] In addition, silent brain infarcts are much more frequent than previously expected and have the same risk factors, pathophysiological mechanisms and prognostic value as symptomatic brain infarcts.[50] As a result, the primary endpoint of the study was changed into the composite of ischemic stroke, or TIA, or new ischemic brain lesions on the follow-up brain MRI (compared to baseline brain MRI).

The PARISK-study is among the first studies in which several different imaging techniques are compared where most previous studies have focused on only one image modality. As a result, a large set of possible imaging and blood biomarkers can be investigated. Based on the literature and cross-sectional studies, like the ones performed in my thesis, the most promising biomarkers will be selected for hypothesis-driven analysis. After the main analysis, other parameters can be investigated in hypothesis generating studies. Because of the multicenter design of the PARISK-study, different scanners are used and small differences in imaging protocols are inevitable. However, this reflects the normal clinical situation and increases the generalizability of the findings. Due to the amount of data, especially in the case of the MRI carotid artery data, the imaging data were scored by different observers. To ensure the quality of our data, all observers received training and were only allowed to analyze scans if an acceptable interobserver agreement was reached.

In addition to the potential role of plaque imaging in risk stratification, imaging biomarkers may help expand our knowledge on plaque pathophysiology and ischemic stroke subtypes. A frequently used division of ischemic strokes are the lacunar versus the nonlacunar infarcts. Approximately 25% of all ischemic strokes are

supposed to be lacunar infarcts; caused by occlusion of small perforating arteries of the brain (small vessel disease).[51] Whereas nonlacunar infarcts are supposed to be caused by thromboembolism from the heart or large extracranial arteries (large vessel disease), Jackson et al. already found a higher prevalence of significant carotid artery stenosis in nonlacunar infarcts compared to lacunar infarcts.[51, 52] In addition, Homburg et al. showed that plaque ulceration on MDCTA is associated with nonlacunar ischemic stroke as compared to lacunar strokes; supporting the notion that nonlacunar and lacunar infarcts have different types of atherosclerotic disease.[53] However, I found no difference in extracranial and intracranial carotid artery calcifications between nonlacunar and lacunar ischemic strokes, but I did find that a higher calcification volume in the aortic arch was associated with nonlacunar strokes (**Chapter 4.1**). These findings only partially confirm the notion of distinct etiologies for nonlacunar and lacunar ischemic strokes and suggest that the potential role of other plaque components, plaque morphology, and aortic arch calcifications in ischemic stroke subtypes needs further evaluation; a research question which we can also address in the PARISK-study or other future prospective plaque imaging studies.

The PARISK consortium consists of several technical and clinical partners. The multimodality approach and addition of blood biomarkers and clinical follow-up makes it a multi-disciplinary effort as well. Reaching beyond the borders of one owns expertise will become more and more important to achieve scientific and clinical progress. From the perspective of personalized medicine, characterization of the atherosclerotic plaque by CEUS, MDCTA and MRI is an important feature to identify plaque characteristics in an individual patient and to assess patient specific risks. The PARISK study will assess which imaging biomarkers increase the risk of recurrent stroke and this could mean a considerable step forward in personalized medicine in ischemic stroke and TIA patients. However, the translation of plaque imaging from research setting to the clinical setting still has to be effectuated. CEUS is better equipped to detect plaque ulcerations compared to regular US and has the potential to detect neovascularization, however it is still very operator dependable and currently only 2-dimensional data is acquired. MDCTA is already used on a large scale in the clinical setting to assess degree of stenosis and can detect plaque ulceration and calcifications. However, ionizing radiation and the use of contrast material makes it less suitable for a screening setting. Plaque characterization with MRI is promising due to its excellent soft tissue contrast and reliable assessment of vulnerable plaque characteristics like intraplaque hemorrhage, but it is not a straightforward process. Duration of MRI sequences is long, plaque analysis needs elaborate training, is difficult and time consuming. However, the use of a few short MRI sequences focused on a specific plaque features, automated analysis and integration of different image modalities will be supportive in the transfer of plaque imaging from the research setting to the clinical setting.

Finally, performing a clinical multicenter study comes with a lot of challenges. Acquisition protocols need to be synchronized as much as possible, which is hampered by different equipment and expertise. Observers need to be well trained. Inclusion of patients for a study with an elaborate study protocol soon after an ischemic stroke or TIA with often significant implications for the patient, can be an arduous task. Fortunately, many patients are eager to help expanding knowledge on their disease.

Conclusions and future perspectives

Characterization of the atherosclerotic plaque by advanced imaging techniques gives unique and valuable insights in the association between atherosclerotic plaque composition and blood coagulation; between plaque composition and plaque pathophysiology; and between plaque composition and ischemic stroke subtypes. The association between VWF and ADAMTS13 and atherosclerosis remains unclear. In addition, CEUS, MDCTA and MRI each have their own advantages and limitations and do not allow an evidence based preference for one particular image modality at this point. The multicenter **Plaque At RISK (PARISK) study** will hopefully help answer the question whether plaque imaging indeed can help clinical decision making. The PARISK study is an example for future studies; cooperation between different academic and technical partners and reaching beyond the borders of your own expertise to achieve scientific and clinical progress.

Current plaque imaging techniques and analyses are elaborate and complicated. Future studies should focus on simplifying and standardization of plaque imaging protocols and analyses to enable the transition to the clinical situation. In addition, automated plaque analysis tools need to be further improved and simplified. A future prospective multicenter study is needed to compare (1) the elaborate plaque imaging protocols and analyses currently used in the research setting (a multisequence contrast-enhanced MRI combined with a MDCTA and/or US and complete plaque delineation and assessment of the plaque surface), with (2) a more clinical feasible plaque imaging protocol (a few short MRI sequences, focusing on for example total plaque volume, presence (and volume) of intraplaque hemorrhage/(lipid), in combination with MDCTA, to detect degree of stenosis, plaque ulceration and calcifications), and with (3) current clinical practice (assessment of degree of carotid artery stenosis). This may provide further insight into optimal plaque imaging that is feasible in the normal clinical setting.

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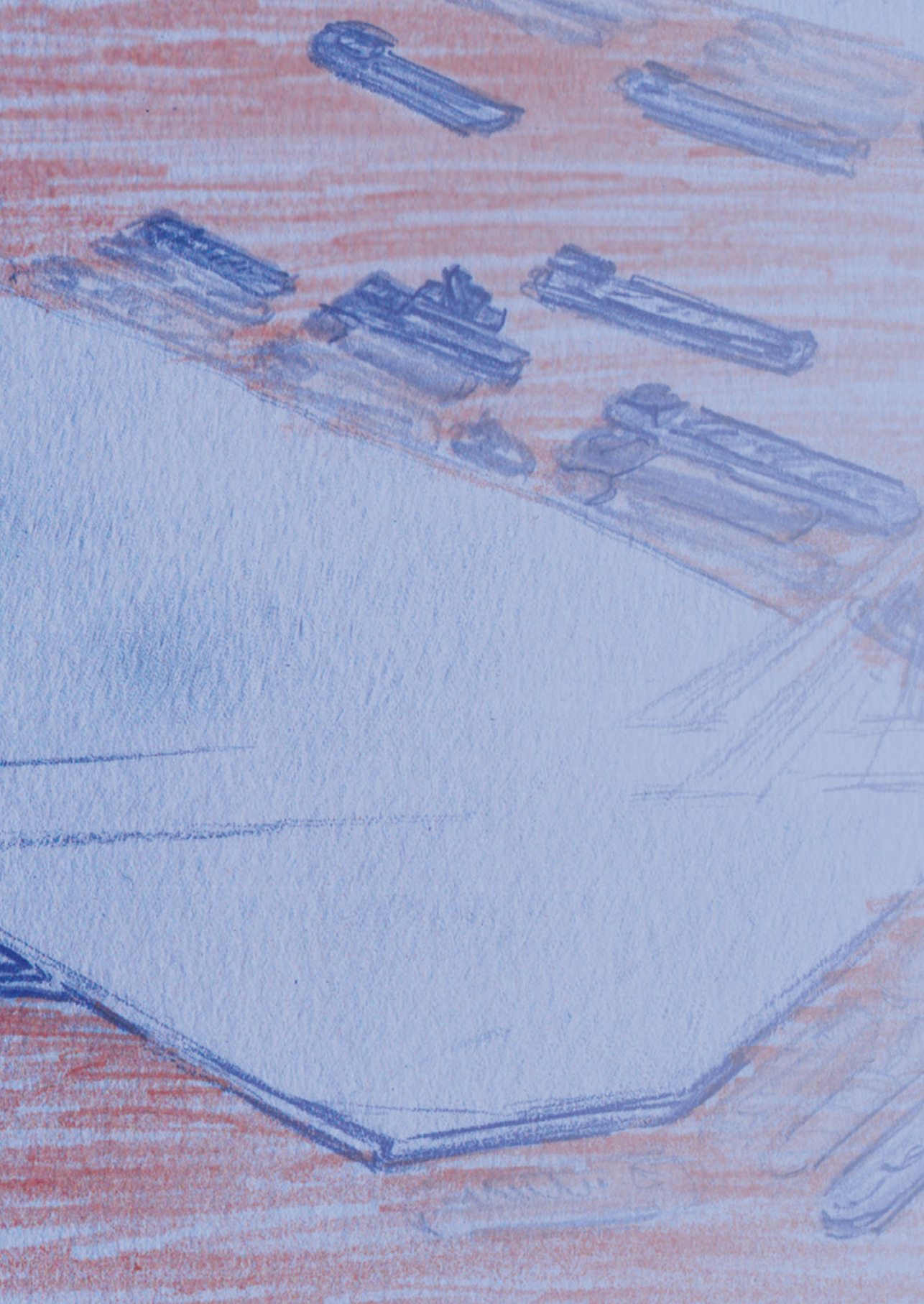

Chapter **6**

Summary / Samenvatting

Summary

Cardiovascular disease is the second-leading cause of death in the Netherlands with approximately 38.000 deaths a year.[1] In addition, stroke is the leading cause of disability. The majority of strokes is of ischemic origin (87%)[2] in which a blood clot occludes one of the arteries of the brain. In a transient ischemic attack (TIA), this interruption and the subsequent neurological symptoms are only temporary, mostly hours, and do not lead to a permanent deficit.[3] Ischemic stroke and TIA have similar causes and their clinical management is identical as atherosclerosis plays an important causal role in both.[2]

Atherosclerosis is a chronic inflammatory disease of especially large arteries with thickening of the arterial vessel wall which progresses into plaque development and stenosis of the vessel lumen. One of the major hazards of the atherosclerotic plaque is that it can rupture. Thrombogenic components of the plaque then come into contact with blood and a thrombus develops. This thrombus may generate distal emboli that occlude an artery of the brain, leading to an ischemic stroke. However, not all plaque ruptures lead to a clinical event (**Chapter 1**).[4-6]

Imaging techniques like contrast-enhanced ultrasound (CEUS), multidetector-row computed tomography angiography (MDCTA) and magnetic resonance imaging (MRI) have made it possible to quantify atherosclerotic burden by degree of stenosis, maximum vessel wall area or calcification volume. In addition, characteristics of the vulnerable, i.e.rupture-prone, plaque can be visualized as plaque ulceration, intraplaque hemorrhage (IPH) and lipid core. In this thesis, I focused on extracted imaging biomarkers of the atherosclerotic plaque in the carotid artery acquired using CEUS, MDCTA and MRI for a better understanding of plaque pathophysiology and the role of blood coagulation. In addition, I evaluated which image modalities and which imaging biomarkers can be valuable in the clinical setting.

Blood coagulation plays a role in atherosclerosis and the development of ischemic events. We found a strong correlation between calcification volume in the aortic arch and carotid arteries and VWF levels in patients with an ischemic stroke or TIA suggesting a positive association between atherosclerosis and VWF (**Chapter 2.1**). In accordance with current literature[7, 8], highest VWF levels were found in patients with large vessel disease compared with the other etiological subtypes (**Chapter 2.1**). However, if we look specifically in a group of patients with carotid artery atherosclerosis and a moderate carotid artery stenosis or if we look at vulnerable plaque characteristics like plaque ulceration, intraplaque hemorrhage and lipid, no associations were found between imaging biomarkers and the coagulation factors VWF and ADAMTS13 (**Chapter 2.2 and 2.3**). The precise mechanism of the association between coagulation factors VWF and ADAMTS13 and cardiovascular disease risk remains unclear; blood biomarkers could mark widespread atherosclerosis or have a more complex role in atherosclerotic plaque development.

US, MDCTA and MRI are all used in current clinical practice to assess degree of carotid stenosis, and to some extent plaque irregularity, and help identifying stroke patients who benefit most from a carotid-endarterectomy (CEA). The last decades have witnessed increased interest in imaging of the atherosclerotic plaque to charac-

terize the plaque in other ways than only degree of stenosis and to gain knowledge on plaque pathophysiology. We found that intraplaque hemorrhage on MRI is associated with disruption of the plaque surface (plaque ulceration and/or fissured fibrous cap), a manifestation of plaque rupture, on MDCTA (**Chapter 3.1**). Because the cross-sectional design of our study, serial studies are needed to confirm a causal relationship between intraplaque hemorrhage, plaque rupture and subsequent ischemic stroke or TIA. In addition, we showed that MRI has good sensitivity, but low specificity for the detection of plaque calcifications compared to MDCTA. There was an overall agreement in calcification volume assessed with MRI and MDCTA. However, due to substantial individual differences, MRI plaque imaging techniques presently cannot be recommended to estimate volume of plaque calcifications in individual patients with symptomatic carotid artery stenosis (**Chapter 3.2**). Finally, we found limited use for regular ultrasound in the detection of plaque ulcerations, however the addition of contrast-enhanced ultrasound (CEUS) can have additional value in the detection of ulcerations (**Chapter 3.3**). In conclusion, (CE)US, MDCTA and MRI have their own potentials and limitations and are not interchangeable. No clear choice of one preferred or superior image modality can be made at this point. Additional cross-sectional and future prospective studies focusing on the evaluation of vulnerable plaque characteristics that increase the risk of ischemic stroke and TIA, should help clarify this question.

An example of a study which hopefully help answer this question is the **Plaque At RISK (PARISK);** a large multicenter cohort study investigating if plaque imaging enables us to better identify patients in the 30-69% carotid stenosis group with an increased stroke risk (**Chapter 4.2**). In addition to the potential role of plaque imaging in risk stratification, imaging biomarkers may help expand our knowledge on plaque pathophysiology and ischemic stroke subtypes. We found no difference in extracranial and intracranial carotid artery calcifications between nonlacunar and lacunar ischemic strokes. However, a higher calcification volume in the aortic arch was associated with nonlacunar strokes (**Chapter 4.1**). These findings only partially confirm the notion of distinct etiologies for nonlacunar and lacunar ischemic strokes. They suggest that the potential role of other plaque components, plaque morphology, and aortic arch calcifications in ischemic stroke subtypes needs further evaluation; a research question which we can also address in the PARISK-study or other future prospective plaque imaging studies.

In the final chapter (**Chapter 5**) the main findings, their implications, methodological considerations and future perspectives are discussed. In conclusion, characterization of the atherosclerotic plaque by advanced imaging techniques gives unique and valuable insights in the association between atherosclerotic plaque composition and blood coagulation; between plaque composition and plaque pathophysiology; and between plaque composition and ischemic stroke subtypes. The association between VWF and ADAMTS13 and atherosclerosis remains unclear. In addition, CEUS, MDCTA and MRI each have their own advantages and limitations and do not allow an evidence based preference for one particular image modality at this point. The multicenter **Plaque At RISK (PARISK) study** will hopefully help answer the question whether plaque imaging indeed can help clinical decision making. The PARISK

study is an example for future studies; cooperation between different academic and technical partners and reaching beyond the borders of your own expertise to achieve scientific and clinical progress. Future studies should focus on simplifying and standardization of plaque imaging protocols and analyses to enable the transition to the clinical situation.

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Samenvatting

Hart- en vaatziekten, waaronder beroerte, zijn de tweede doodsoorzaak in Nederland met ongeveer 38.000 sterfgevallen per jaar.[1] Daarnaast is beroerte de belangrijkste oorzaak van invaliditeit. De meeste beroertes zijn herseninfarcten (87%)[2]; er is sprake van een zuurstoftekort in de hersenen doordat een bloedstolsel een bloedvat in de hersenen heeft afgesloten. Bij een TIA (Transient Ischemic Attack) zijn deze onderbreking en de daaropvolgende neurologische symptomen tijdelijk, meestal uren, en is er geen permanente schade.[3] Bij zowel herseninfarcten als TIA's speelt slagaderverkalking (atherosclerose) een belangrijke rol en is de klinische behandeling vergelijkbaar.[2]

Atherosclerose is een chronische ontstekingsziekte van vooral de grote slagaders. In eerste instantie verdikt de vaatwand, deze verdikking ontwikkelt zich tot een atherosclerotische plaque en kan een vernauwing (stenose) geven van het bloedvat. Eén van de grote gevaren van de atherosclerotische plaque is het scheuren van de plaque (plaque ruptuur) waardoor de inhoud van de plaque in contact komt met het bloed en er een stolsel kan ontstaan. Dit stolsel kan embolieën veroorzaken die een slagader van de hersenen kunnen afsluiten met als gevolg een herseninfarct. Echter, niet elke plaque ruptuur leidt tot klinisch symptomen (**Hoofdstuk 1**).[4-6]

Beeldvormende technieken zoals contrast-enhanced echografie (CEUS), multidetector-row computed tomografie angiografie (MDCTA) en magnetic resonance imaging (MRI) hebben het mogelijk gemaakt om de hoeveelheid atherosclerose te kwantificeren door middel van de stenosegraad, maximale vaatwand oppervlak of kalkvolume. Bovendien kunnen kenmerken van de kwetsbare, i.e. ruptuur gevoelige, plaque worden afgebeeld zoals plaque ulceratie, intraplaque bloeding (IPH) en de focale necrose en vetophoping. In dit proefschrift heb ik me geconcentreerd op de atherosclerotische plaque in de halsslagader afgebeeld met CEUS, MDCTA en MRI. De hoeveelheid atherosclerose en specifieke plaque kenmerken, ook wel beeldvormende biomarkers genoemd, heb ik gebruikt om te proberen een beter begrip te krijgen van de plaque pathofysiologie en de rol van de bloedstolling. Daarnaast heb ik onderzocht welke beeldvormende technieken en biomarkers waardevol kunnen zijn in de klinische praktijk.

Bloedstolling speelt een rol bij atherosclerose en het ontstaan van herseninfarcten. We hebben een sterke correlatie gevonden tussen kalkvolume in de aortaboog en de halsslagaders en Von Willebrand Factor (VWF) levels bij patiënten met een herseninfarct of TIA; dit suggereert een positieve associatie tussen atherosclerose en VWF (**Hoofdstuk 2.1**). Net zoals in de huidige literatuur[7,8] werden de hoogste VWF levels gevonden bij patiënten met atherosclerose in vergelijking met patiënten met een andere oorzaak van het herseninfarct of TIA (bijvoorbeeld een stolsel uit het hart of afwijkingen van de kleine vaatjes in de hersenen) (**Hoofdstuk 2.1**). Echter, als we specifiek kijken in een groep patiënten met atherosclerose van de halsslagaders of als we kijken naar kenmerken van de ruptuur gevoelige plaque zoals plaque ulceraties, intraplaque bloedingen en de focale necrose en vetophoping, vinden we geen associaties tussen de beeldvormende biomarkers en de stollingsfactoren VWF en ADAMTS13 (**Hoofdstuk 2.2 en 2.3**). Het precieze mechanisme van de associatie tussen de stollingsfactoren VWF en ADAMTS13 en het risico op hart- en vaatziekten is nog

onduidelijk; bloed biomarkers kunnen een marker zijn van wijdverspreide atherosclerose of een meer complexe rol hebben in atherosclerotische plaque ontwikkeling.

US, MDCTA en MRI worden gebruikt in de huidige klinische praktijk om de stenosegraad van de halsslagader te beoordelen. De stenosegraad wordt gebruikt om patiënten met een herseninfarct of TIA te identificeren die het meest profijt hebben van een carotis-endarterectomie (CEA). De laatste decennia is er veel belangstelling voor het gedetailleerd afbeelden van de atherosclerotische plaque, zodat we op een andere manier naar de plaque kunnen kijken dan alleen met de stenosegraad, en meer kunnen leren over de plaque pathofysiologie. We hebben gevonden dat intraplaque bloedingen op MRI geassocieerd worden met onderbrekingen van het plaque oppervlak op MDCTA (**Hoofdstuk 3.1**). Omdat we voor dit onderzoek een dwarsdoorsnede hebben gebruikt, zijn er follow-up studies nodig om een oorzakelijk verband tussen de intraplaque bloeding, plaque ruptuur en het herseninfarct en/of TIA te bevestigen. Verder hebben we aangetoond dat MRI een goede sensitiviteit, maar lage specificiteit heeft voor de detectie van kalk in de plaque vergeleken met MDCTA. Kalkvolume gemeten met MRI kwam over het algemeen overeen met kalkvolume gemeten met MDCTA. Er waren echter grote individuele verschillen, zodat MRI momenteel niet kan worden aanbevolen om kalkvolume in de atherosclerotische plaque te bepalen in individuele patiënten met een symptomatische vernauwing van de halsslagader (**Hoofdstuk 3.2**). Op de laatste plaats hebben we een beperkt nut gevonden voor het gebruik van "normale" echografie bij de detectie van plaque ulceraties. Het gebruik van contrastmiddel (CEUS) kan echter toegevoegde waarde hebben bij de detectie van ulceraties (**Hoofdstuk 3.3**). Concluderend, (CE)US, MDCTA en MRI hebben ieder hun eigen mogelijkheden en beperkingen en zijn niet uitwisselbaar. Op dit moment is er geen duidelijke voorkeur voor een bepaalde beeldvormende techniek. Extra studies gericht op de evaluatie van kenmerken van de ruptuur gevoelige plaque die het risico op het krijgen van een herseninfarct of TIA verhogen, moeten helpen om deze vraag te beantwoorden.

Een voorbeeld van een studie die hopelijk helpt bij het beantwoorden van deze vraag, is de Plaque at Risk (PARISK) studie; een grote multicenter cohortstudie die onderzoekt of gedetailleerde beeldvorming van de atherosclerotische plaque gebruikt kan worden om beter te identificeren welke patiënten met een 30-69% symptomatische stenose in de halsslagader een verhoogd risico hebben op het krijgen van een nieuw herseninfarct of TIA (**Hoofdstuk 4.2**). Naast de mogelijke rol van beeldvorming van de atherosclerotische plaque in risicoschatting, kunnen beeldvormende biomarkers ook helpen om onze kennis van plaque pathofysiologie en de oorzaken van herseninfarcten te vergroten. We vonden geen verschil in kalk in de halsslagader tussen twee soorten herseninfarcten (niet-lacunaire en lacunaire herseninfarcten). Echter, een groter kalkvolume in de aortaboog was geassocieerd met niet-lacunaire infarcten (**Hoofdstuk 4.1**). Dit bevestigt slechts gedeeltelijk de veronderstelde verschillende oorzaken van niet-lacunaire en lacunaire herseninfarcten. Het suggereert dat de rol van andere plaque kenmerken en aortaboog calcificaties in oorzakelijke subtypen van het herseninfarct verder moeten worden onderzocht. Een onderzoeksvraag die ook zal worden beantwoord in de PARISK-studie of andere toekomstige studies naar beeldvorming van de atherosclerotische plaque.

In het laatste hoofdstuk (**Hoofdstuk 5**) worden de belangrijkste bevindingen, de implicaties, methodologische overwegingen en toekomstperspectieven besproken. Concluderend, karakterisering van de atherosclerotische plaque door middel van geavanceerde beeldvormende technieken geeft unieke en waardevolle inzichten in de relatie tussen atherosclerotische plaque samenstelling en de bloedstolling; tussen plaque samenstelling en plaque pathofysiologie; en tussen de plaque samenstelling en herseninfarct subtypen. De associatie tussen VWF en ADAMTS13 en atherosclerose blijft onduidelijk. CEUS, MDCTA en MRI hebben elk hun eigen voor- en nadelen en er is op dit moment geen duidelijke voorkeur voor een bepaalde beeldvormende techniek. De multicenter Plaque at Risk (PARISK) studie zal hopelijk helpen bij het beantwoorden van de vraag of beeldvorming van de atherosclerotische plaque inderdaad kan helpen bij de klinische besluitvorming. De PARISK studie is een voorbeeld voor toekomstige studies; een nauwe samenwerking tussen verschillende academische en technische partners en het integreren van verschillende expertisegebieden om wetenschappelijke en klinische vooruitgang te boeken. Toekomstig onderzoek moet zich richten op de vereenvoudiging en standaardisatie van protocollen en beeldanalyses van de atherosclerotische plaque om de overgang van wetenschappelijk onderzoek naar de dagelijkse klinische praktijk mogelijk te maken.

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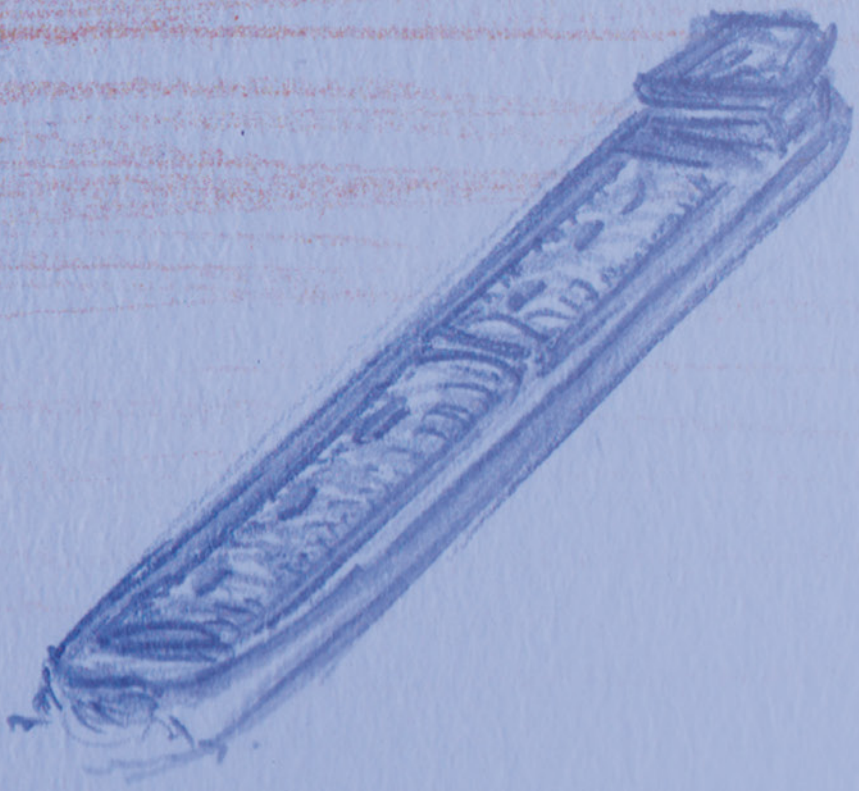

Chapter 7

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List of publications

M.J. van Gils, D. Vukadinovic, **A.C. van Dijk**, D.W.J. Dippel, W.J. Niessen, A. van der Lugt. Carotid atherosclerotic plaque progression and change in plaque composition over time; a 5-year follow-up study using serial CT angiography. *AJNR Am J Neuroradiol* 2012;33:1267-1273.

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PhD portfolio

Courses	Year	Workload ECTS
Cardiovascular Pharmacology (COEUR, Rotterdam, The Netherlands)	2010	1.0
Basic MRI Course (EMRIN, Bunnik, The Netherlands)	2010	2.5
Introduction to Clinical Research (NIHES, Rotterdam, The Netherlands)	2011	0.9
Biostatistics for Clinicians (NIHES, Rotterdam, The Netherlands)	2011	1.0
Genome Wide Association Analysis (NIHES, Rotterdam, The Netherlands)	2011	1.4
Vascular Biology (Dutch Heart Foundation, Papendal, The Netherlands)	2011	2.0
Basiscursus Regelgeving en Organisatie Klinische Onderzoekers (Erasmus MC, Rotterdam, The Netherlands)	2011	0.9
Biostatistical Methods I: Basic Principles (NIHES, Rotterdam, The Netherlands)	2012	5.7
Basiscursus Regelgeving en Organisatie Klinische Onderzoekers Hercertificering (Erasmus MC, Rotterdam, The Netherlands)	2016	0.1

(Inter)national conferences and presentations	Year	Workload ECTS
Center for Translational Molecular Medicine Annual Meeting (Utrecht, The Netherlands). *Poster.*	2011	0.3
European Society for Magnetic Resonance in Medicine and Biology Annual Scientific Meeting (Leipzig, Germany).	2011	1.2
European Stroke Conference (Lisbon, Portugal). *Poster (2x).*	2012	1.2
Center for Translational Molecular Medicine Annual Meeting (Utrecht, The Netherlands). *Poster.*	2012	0.3
Radiologendagen (Den Bosch, The Netherlands). *Oral presentation.*	2012	1.0
Radiological Society of North America Annual Meeting (Chicago, United States). *Poster.*	2012	1.8
European Congress of Radiology (Vienna, Austria). *Poster.*	2013	1.5
European Stroke Conference (London, England). *Poster.*	2013	1.2
European Society for Magnetic Resonance in Medicine and Biology Annual Scientific Meeting (Toulouse, France). *Oral presentation.*	2013	1.3
European Stroke Organisation Conference (Barcelona, Spain). *Poster (2x).*	2016	0.9

Other	Year	Workload ECTS
Research seminar: Biomarkers for risk prediction (COEUR, Rotterdam, The Netherlands)	2010	0.4
Research seminar: Imaging of atherosclerosis (COEUR, Rotterdam, The Netherlands)	2010	0.4
CPO mini course (Consultation center for Patient Oriented research, Rotterdam, The Netherlands)	2011	0.3
Research seminar: Imaging carotid arteries: structure and function (COEUR, Rotterdam, The Netherlands). *Oral presentation*.	2011	0.8
Research seminar: Carotid atherosclerotic plaques: biomechanics and imaging (COEUR, Rotterdam, The Netherlands). *Oral presentation*.	2013	0.8

Presentations and lectures	Year	Workload ECTS
Experiences OpenClinica in clinical study (PARISK semi-annual meeting, Eindhoven, The Netherlands)	2012	0.4
Characteristics of the carotid atherosclerotic plaque. Role of calcifications and association between plaque ulcerations and intraplaque hemorrhage (Department of Radiology, Erasmus MC)	2013	0.4
PARISK-study: Non-invasive plaque imaging in TIA and stroke patients with a non-significant carotid artery stenosis (Department of Neurology, Erasmus MC)	2016	0.4

Teaching activities	Year	Workload ECTS
Supervising research projects of 3 medical students (Taihra Zadi, Martijn van Hattem, Burhan Hussain)	2010-2013	1.2
Teaching "Neurovascular imaging in clinical practice" to technical students	2011, 2013	0.8

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Als promovendus mocht ik ook samen werken met een aantal enthousiaste studenten waaronder Burhan, Nawid en Karlijn. Met jullie hulp zijn er mooie papers tot stand gekomen en ik wens jullie een mooie toekomst toe.

Dear Piotr, it has been a while since we were scanning the PARISK patients in room 10. Sometimes the discrepancy between the interest of the patients and the interest of the research lead to a small discussion, but we always made it work. It was a real pleasure to work with you. Sylvia, Hanneke en Sita, dank voor jullie hulp bij de praktische zaken rondom de MRI.

Voor de praktische zaken die komen kijken bij het opzetten en laten lopen van een studie zoals de PARISK en een promotietraject in het algemeen, was ik erg blij met de hulp van Laurens, Mart, Stefan, Naziha, Ton en Jolanda. Laurens, samen hebben we volgens mij één van de eerste "afdelings OpenClinica" databases opgezet (al was het met veel uitzoekwerk). En wat had, en heb, je toch altijd mooie vakantiefoto's. Mart en Stefan, als ik het allemaal niet begreep qua servers, DICOM bestanden en andere te "technische" zaken, stonden jullie altijd klaar om het uit te leggen en me te helpen, dank daarvoor. Naziha, jij kreeg er zomaar even de PARISK follow-up bij… Voor mij was het wel een hele opluchting dat iemand, zo ervaren als jij, de follow-up gesprekken op zich wilde nemen. Ton, met vragen over posters, figuren en photoshop, kon ik altijd even bij je binnenlopen. Bovendien heb je me geholpen met het maken van een mooi proefschrift, dank voor al je hulp. Jolanda, in de periode dat het even iets minder ging, heb jij me geholpen bij de zaken waar ik tegenaan liep en soms gewoon alleen geluisterd. Zonder die steun, stond ik hier nu niet.

Multicenter studies zoals de PARISK kunnen tot veel problemen leiden onder de verschillende centra. Gelukkig was dit in de PARISK-studie niet zo, we hebben altijd met veel plezier samen gewerkt, getuige de verschillende "PARISK-etentjes" welke we hebben gehad met de promovendi. Martine, Floris, Anja, Alexandra, Madieke, Geneviève, Remko, Jos, Jeire, Raf, Eline en Werner bedankt voor al die jaren aan mooie samenwerking. Ook de perifere centra in de regio mag ik natuurlijk niet vergeten. De afdelingen neurologie en radiologie van het Maasstad ziekenhuis (Ritu Saxena), Vlietland ziekenhuis (Hans Verhey), SFG (Stef Bakker) en IJsselland ziekenhuis (Annemarie Wijnhoud) wil ik graag bedanken voor hun deelname aan de PARISK-studie. In het bijzonder het Maasstad ziekenhuis, waar ik elke week welkom was om te zien of er nog potentiële deelnemers waren langs geweest.

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spannende tijden voor je aan, eerst een klein wondertje op de wereld zetten en dan je eigen promotie- en opleidingstraject afronden. Veel succes en plezier toegewenst. Maaike en Dorien, we leerden elkaar kennen op de stroke preventie poli waar ik altijd jullie advies kon vragen, vervolgens een hoop gezelligheid op de congressen en nu werken we ook weer samen in het Stroke Center. Leuk om zo samen te blijven werken! Boudewijn, jou heb ik al leren kennen toen ik mijn keuzeonderzoek nog deed op de KNF. Toen ik terugkwam voor mijn promotie traject op de radiologie, zijn we altijd contact blijven houden. Bij onze lunches kwamen geregeld de hoogtepunten en tegenslagen van het promoveren voorbij… Veel succes in Utrecht.

Buiten de samenwerking met andere centra, was en is het ook heel prettig samenwerken met de afdelingen in huis zoals de hematologie en cardiologie. Michelle, Moniek, dankzij jullie is de stolling veel meer voor mij gaan leven. Niet voor niks beslaat het een aanzienlijk deel van mijn proefschrift. Michelle, veel succes met je opleiding tot internist. Moniek, onze samenwerking houdt nog niet op, er liggen nog een paar mooie projecten in het verschiet. Anouchska, Heleen, het is helaas nog niet gelukt om ons gezamenlijk project af te ronden, maar daar zijn we druk mee bezig. Hopelijk kun jij dan ook toewerken naar de afronding van je promotietraject, Anouchska.

In januari 2016 ben ik gestart als coördinator van het Erasmus MC Stroke Center. Ik wil de directie (Peter Sillevis-Smit, Clemens Dirven, Peter Koudstaal, Diederik Dippel, Aad van der Lugt) en het dagelijks bestuur (Diederik Dippel, Aad van der Lugt, Ruben Dammer, Tilly van den Bout) van het Stroke Center bedanken voor de kans welke ik heb gekregen. Zeker het internationaal congres wat er aan zit te komen in maart, is een mooie kans om het Stroke Center nog meer op de kaart te zetten. Ik kijk uit naar alle mooie dingen die we nog mogen doen samen. Diederik, met de jaren hebben onze paden al diverse keren gekruist, binnen het Stroke Center werken we nu intensiever samen en met veel plezier. Dank voor alles wat ik hier nog kan en mag leren.

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About the author

Anouk Catharina van Dijk was born on January 20th, 1986 in Naaldwijk, The Netherlands. After finishing secondary school at the Interconfessionele Scholengemeenschap het Westland in Naaldwijk in 2004, she started Medical School at the Erasmus University, Rotterdam. During her clinical training and research master, her interest in clinical research was piqued by participating in the research projects "Inventarisation of cognitive complaints and impairments in patients with multiple sclerosis" (Department of Neurology, Sint Elisabeth ziekenhuis, Tilburg. Supervised by prof. dr. L.H. Visser) and "The excitability of peripheral nerves in acute ischemic stroke" (Department of Clinical Neurophysiology, Erasmus Medical Center, Rotterdam. Supervised by dr. J.H. Blok and dr. M.H. den Hertog).

She obtained her medical degree in 2010 and started as a PhD candidate at the Department of Radiology and Neurology of the Erasmus Medical Center, Rotterdam under supervision of prof. dr. A. van der Lugt and prof. dr. P.J. Koudstaal. Coordinating and executing "The assessment of the Plaque At RISK by non-invasive (molecular) imaging and modelling (ParisK): Prospective clinical study for diagnosis efficacy for high risk plaque and stroke" was an important part of her PhD project. Some results of this study are described in her thesis.

In January 2016, Anouk started as the coordinator of the Erasmus MC Stroke Center in Rotterdam. She lives in Waalwijk with her husband Marcel and son Bram.