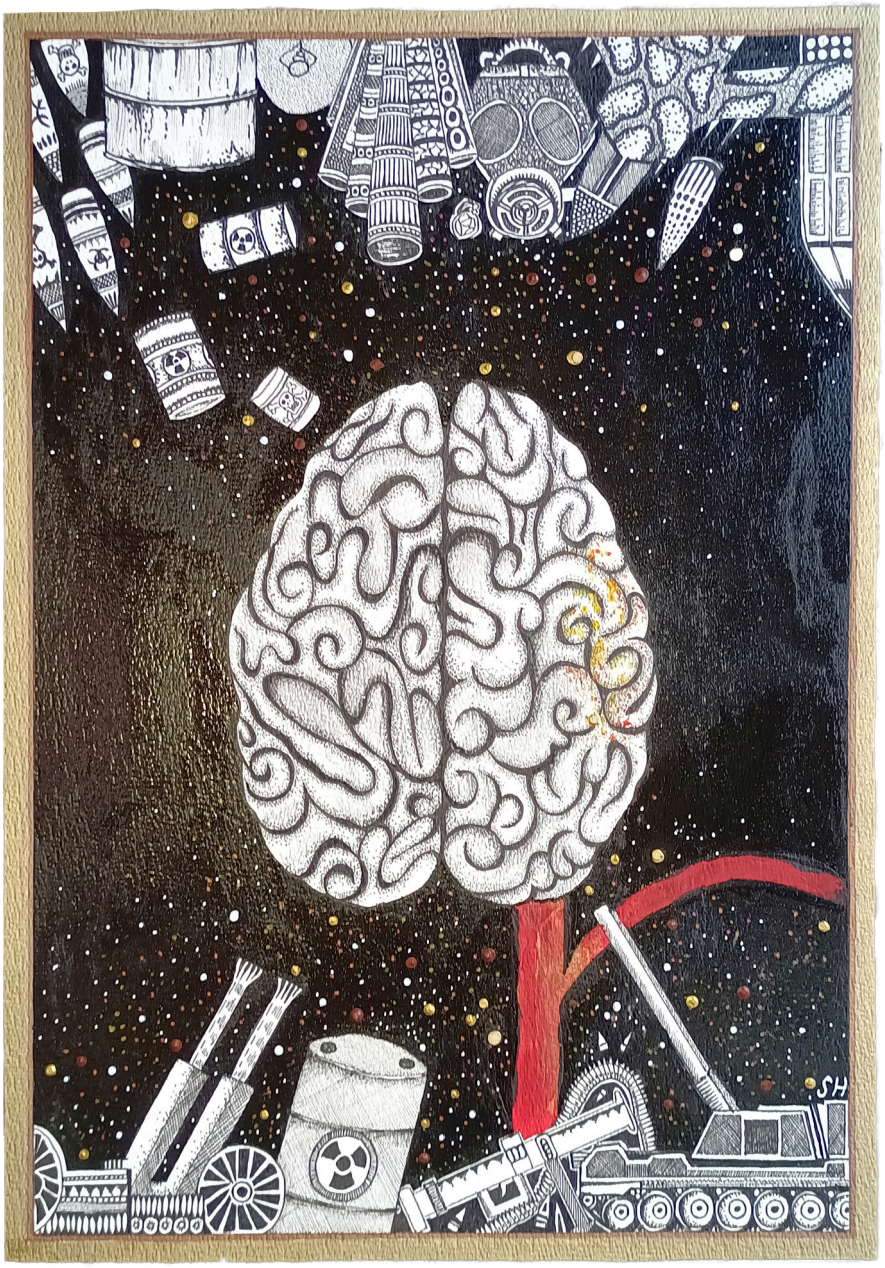
INTRAPLAQUE HEMORRHAGE ON CAROTID MRI IN STROKE PATIENTS:

on the Road Towards Clinical Application



Mohamed Kassem

Intraplaque Hemorrhage on carotid MRI in stroke patients: on the Road Towards Clinical Application

Mohamed Kassem

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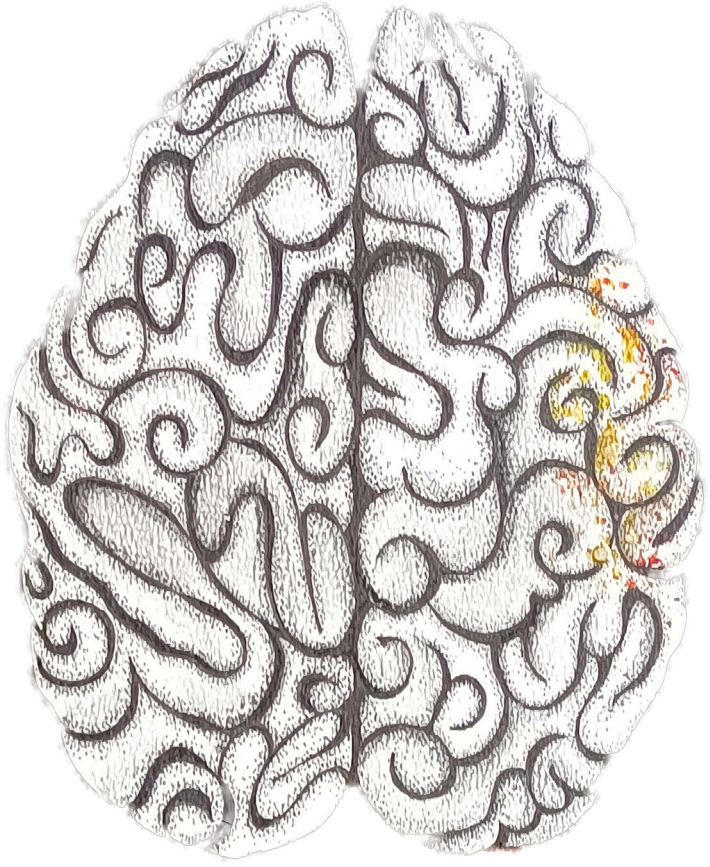
CHAPTER 1

General introduction



ISCHEMIC STROKE

Strokes can be caused by a blockage in an artery that supplies blood to the brain (ischemic stroke) or bleeding in the brain (hemorrhagic stroke). Ischemic stroke is a prevalent subtype, accounting for approximately 80% of all strokes [1]. Atherosclerotic carotid disease is one of the main underlying factors for ischemic stroke [2]. Atherosclerotic plaque formation in the carotid artery leads to vessel wall thickening and luminal narrowing (carotid artery stenosis). A carotid plaque can rupture due to a combination of local factors such as inflammation, the presence of a large lipid-rich necrotic core (LRNC), the presence of intraplaque hemorrhage (IPH), and rupture of the fibrous cap, and others [3]. Also, systematic factors such as high blood pressure, age, male sex, diabetes, and others can increase the likelihood of plaque rupture and forming a blood clot (thrombus) at the rupture site. If the clot is large enough, it can completely block blood flow, causing a thrombotic stroke, or a piece of the plaque or the clot itself can break off and travel through the bloodstream to a smaller artery in the brain, causing an embolic stroke.

MANAGING CAROTID ATHEROSCLEROTIC DISEASE TO PREVENT RECURRENT ISCHEMIC EVENTS

The rupture of atherosclerotic plaques in the carotid arteries that cause carotid stenosis is responsible for the thromboembolic occlusion of the cerebral vasculature in 15-20% of all ischemic strokes [4]. The North American Symptomatic Carotid Endarterectomy Trial (NASCET) and the European Carotid Surgery Trial (ECST) evaluated the benefit of carotid endarterectomy (CEA) compared to medical therapy[5, 6] in symptomatic patients with carotid artery stenosis [5, 6]. Both trials demonstrated a reduction in stroke risk in patients with severe symptomatic carotid stenosis of 70-99%. The number of CEAs to prevent one ipsilateral stroke during 5 years was 12 in ECST [6] and 5 CEAs in NASCET [5]. A subsequent re-analysis of the ECST data revealed that patients with 50-69% stenosis had a reduced risk of recurrent stroke by 5.7% (95% CI: 0-11.6), while those with 70-99% stenosis had a risk reduction of 21.2% (95% CI: 12.9-29.4) [7]. In contrast, surgery was not beneficial for patients with 30-49% stenosis and was even harmful for patients with stenosis less than 30% [7]. These findings were consistent with the North American trial [5, 6]. Moreover, reviewing 48 ECST (n= 16) and NASCET (n= 32) papers revealed that the simple assumption that all patients with symptomatic stenosis >70% benefit from CEA is untenable. Approximately 70-75% will not have a stroke if treated medically [8].

During the last three decades, several significant improvements in medical management have been introduced. The widespread use of high-potency statins, lower targets for blood pressure control, and improved antiplatelet therapy have contributed to the significant decline in global incidence, mortality, and disability-adjusted life years for ischemic stroke over the last two decades. Moreover, both carotid endarterectomy and carotid artery stenting (CAS) (a newer, less invasive alternative to carotid endarterectomy) can now be performed by experienced clinicians with a low perioperative risk of complications. Given these changes to medical practice, a growing uncertainty remains over which intervention (carotid revascularization with OMT or OMT only) provides the most durable long-term protection from recurrent stroke in patients, especially for mild-to-moderate (50-69%) symptomatic stenosis.

LOOKING BEYOND THE DEGREE OF CAROTID STENOSIS

Histopathological studies of carotid arteries have revealed substantial differences between plaques with identical degree of stenosis [9]. A stable plaque is characterized by a small LRNC and a thick and intact fibrous cap (FC). In contrast, a vulnerable plaque consists of a large LRNC, a thin or ruptured FC, and IPH [10]. Vulnerable plaques are at increased risk of rupture and, thereby, an increased risk for ischemic cerebrovascular events [11]. Plaque rupture occurs when a thin fibrous cap overlying the necrotic core breaks open. Subsequently, the surface of the LRNC comes in contact with platelets, leading to the formation of a blood clot in the vessel lumen due to the high thrombogenicity of the necrotic core [3, 12]. During the past decades, there has been abundant interest in imaging techniques to visualize vulnerable plaque components [13, 14]. The European Society for vascular surgery recently included plaque morphological characteristics in the new guidelines for treating asymptomatic patients [15].

Magnetic Resonance Imaging (MRI) is superior to other imaging modalities regarding noninvasively depicting carotid plaques for several reasons: 1) MRI provides excellent soft tissue contrast, allowing for the differentiation of different components of the plaque, including fibrous tissue, LRNC, IPH, and the discrimination between a thick and thin/ruptured fibrous cap. 2) MRI has the capability to provide multi-contrast imaging, meaning it can acquire different types of images using various MRI sequences that capture different aspects of the plaque, such as its composition and neovascularization. 3) MRI is a noninvasive imaging modality that does not use ionizing radiation. This makes it an attractive option for serial imaging in patients. Different plaque components can be distinguished using a combination of various MRI pulse

sequences (multisequence carotid MRI). Pre- and postcontrast T1 weighted (T1w) turbo spin echo (TSE), magnetization-prepared rapid acquisition gradient echo (MPRAGE), and time of flight (TOF) MRI are mainly used to depict the main components of the carotid plaque [16]. The validity and feasibility of MRI as a reliable imaging modality to delineate carotid plaque features have been extensively proven using histopathological specimens as a reference [9, 11]. Together with standard measures of luminal stenosis, the measurement of plaque features by MRI could provide more accurate phenotyping of atherosclerotic disease, thereby allowing clinicians to assess the risk of an ischemic event better and adjudicate the best form of therapy.

CAROTID IPH: THE KEY TO STROKE RISK ASSESSMENT

The presence of IPH in carotid plaques is considered a crucial feature of plaque vulnerability. IPH has been shown to promote plaque destabilization and rupture [17], leading to adverse cardiovascular events such as stroke [18]. MR imaging studies have shown that IPH is an important independent predictor of stroke, stronger than any clinical risk factors [19]. From the data of 560 symptomatic and 136 asymptomatic patients, the presence of IPH at baseline increased the risk of ipsilateral stroke both in symptomatic (HR: 10.2; 95% CI: 4.6 to 22.5) and asymptomatic (HR: 7.9; 95% CI: 1.3 to 47.6) patients [19]. Recently, the PARISK (Plaque At RISK) study showed that the presence of IPH was associated with recurrent ipsilateral ischemic stroke or TIA (HR: 2.12 [95% CI: 1.02-4.44] after 5.1 years of follow-up of 244 patients [20]. Despite compelling evidence that patients with IPH have an increased risk of stroke, the mechanisms contributing to the development of carotid IPH are not fully unraveled [17]. One prevailing hypothesis is that IPH arises from microvascular leakage within the plaque [21]. However, a recent study revealed a notable decrease in K^{trans}, which indicates a reduction in microvascular flow, density, and leakiness, within symptomatic carotid plaques exhibiting IPH [22]. IPH may also occur from the influx of blood from the lumen through fissures or ruptures in the fibrous cap. For instance, Ota et al. reported an association between IPH and TRFC (odds ratio (OR)=5.9; 95% CI, 2.6-13.1) [23]. Furthermore, a correlation between IPH and disrupted plaque surface (i.e., fissured or ulcerated plaque) on computed tomography angiography (CTA) (OR= 3.1; 95% CI, 1.2–7.8) has been demonstrated on both symptomatic and asymptomatic sides [24]. Exploring the link between TRFC and disrupted plaque surface at a specific time point and the subsequent development of IPH during follow-up has the potential to shed light on the underlying pathophysiological mechanisms of IPH occurrence.
On the other hand, it has been reported that IPH is associated with multiple cardiovascular risk factors, such as advanced age (OR= 1.1; 95% CI: [1.0-1.05]), male sex (OR= 2.5; 95% CI: [1.4-4.4]), and carotid stenosis (OR= 8.5; 95% CI: [4.6-15.3]) [25].

Previously also, a cross-sectional association between antiplatelet use before the index event and the presence of IPH in 100 TIA and stroke patients with mild to moderate symptomatic carotid stenosis was reported from the baseline data of the Plaque at RISK (PARISK) study [26]. Investigating the longitudinal association between the initiation of antiplatelet therapy subsequent to an ischemic event and the development of IPH during a follow-up period holds significant clinical relevance.

ROUTINE, DEDICATED, AND NOVEL PLAQUE MRI METHODS: FROM IPH ETIOLOGY TO CLINICAL APPLICATION

Previous studies that investigated the mechanisms that may contribute to the occurrence of IPH were cross-sectional studies; Thus, causal relations could not be studied. High-resolution MRI can be used to visualize IPH and offers the possibility of performing longitudinal studies instead of cross-sectional histological studies [27]. Additionally, MRI can be used to study symptomatic and asymptomatic patients regardless of the degree of carotid stenosis. In contrast, histological studies are typically performed in symptomatic patients with severe stenosis (>70%) who need to undergo revascularization. Therefore, we aim to investigate longitudinal changes in carotid IPH using carotid MR imaging.

A dedicated MRI sequence, MP-RAGE, also known as T1w inversion recovery turbo field echo (IR-TFE), is commonly used to identify IPH. In most centers, additional MR sequences beyond contrast-enhanced MR angiography (CE-MRA) or time-of-flight (TOF) angiography to measure the degree of stenosis are not acquired during a routine carotid MRA examination, primarily because of time constraints and other vulnerable carotid plaque features are currently not included in the treatment plan. Due to the magnetic properties of blood products, IPH can be recognized as an area of high signal intensity within the bulk of the plaque on hyper T1 weighted images. A small study reported that IPH could also be identified on the mask images of CE-MRA and TOF images [28]. Therefore, the ability to detect IPH in routine carotid CE-MRA or TOF images can be of clinical significance.

Expert consensus recommendations on using the multisequence carotid vessel wall imaging protocol have recently been published [16]. Despite its advantages, multisequence carotid MRI still poses some limitations, such as limited slice resolution caused by 2D sequences, long acquisition time, and potential image misalignment due to patient movement during scans, which can hinder clinical implementation. A few years ago, a new sequence, i.e., multicontrast atherosclerosis characterization

(MATCH), was introduced [29]. MATCH simultaneously provides three contrast weightings, hyper-T1w, grey-blood, and T2w, in a 5-min scan.

RESEARCH AIMS

The aims of this thesis were as follows:

1- To investigate the relationship between TRFC/disrupted plaque surface and the change in IPH volume in a longitudinal MRI study.
2- To provide further insight into the potential association between antiplatelet use and the incidence of IPH and changes in the volume of IPH in a longitudinal MRI study.
3- To determine the diagnostic accuracy of identifying IPH using mask images from CE-MRA and TOF images.
4- To compare the quantification of carotid plaque composition with MATCH and multisequence MRI.

OUTLINE OF THIS THESIS

Chapter 2 provides an overview of the current status and potential future clinical applications of carotid plaque MRI.

Chapter 3 investigates the relationship between fibrous cap status or plaque surface morphology and the volume of intraplaque hemorrhage over two years in symptomatic patients with mild to moderate carotid stenosis.

The association between antiplatelet therapy and changes in intraplaque hemorrhage during two years of follow-up in patients with mild to moderate symptomatic carotid stenosis has been described in Chapter 4.

Chapter 5 explores the application of mask CE-MRA and TOF images to detect intraplaque hemorrhage in patients with moderate to severe symptomatic and asymptomatic carotid stenosis.

We study the accuracy of the multicontrast atherosclerosis characterization (MATCH) MRI sequence to identify and quantify the composition of carotid plaques in Chapter 6.

In Chapter 7, the results of this thesis in relation to literature are discussed, and future perspectives are described.

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CHAPTER 2

Magnetic resonance imaging of carotid plaques: current status and clinical perspectives

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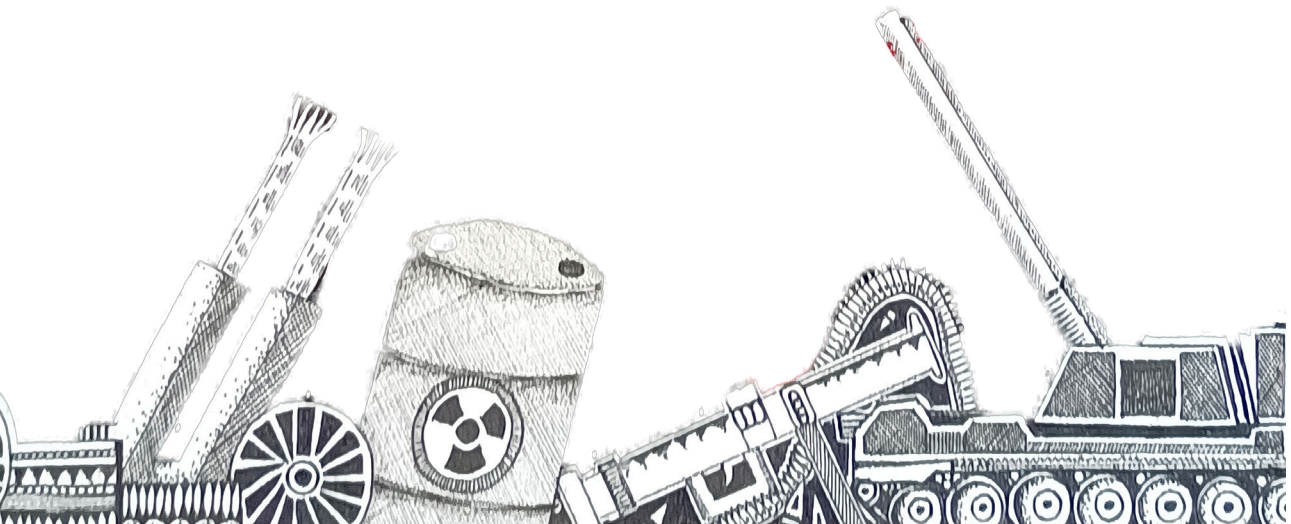

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ABSTRACT

Rupture of a vulnerable carotid plaque is one of the leading causes of stroke. Carotid magnetic resonance imaging (MRI) is able to visualize all the main hallmarks of plaque vulnerability. Various MRI sequences have been developed in the last two decades to quantify carotid plaque burden and composition. Often, a combination of multiple sequences is used. These MRI techniques have been extensively validated with histological analysis of carotid endarterectomy specimens. High agreement between the MRI and histological measures of plaque burden, intraplaque hemorrhage (IPH), lipid-rich necrotic core (LRNC), fibrous cap (FC) status, inflammation and neovascularization has been demonstrated. Novel MRI sequences allow to generate three-dimensional isotropic images with a large longitudinal coverage. Other new sequences can acquire multiple contrasts using a single sequence leading to a tremendous reduction in scan time. IPH can be easily identified as a hyperintense signal in the bulk of the plaque on strongly T_1-weighted images, such as magnetization-prepared rapid acquisition gradient echo images, acquired within a few minutes with a standard neurovascular coil. Carotid MRI can also be used to evaluate treatment effects. Several meta-analyses have demonstrated a strong predictive value of IPH, LRNC, thinning or rupture of the FC for ischemic cerebrovascular events. Recently, in a large meta-analysis based on individual patient data of asymptomatic and symptomatic individuals with carotid artery stenosis, it was shown that IPH on MRI is an independent risk predictor for stroke, stronger than any known clinical risk parameter. Expert recommendations on carotid plaque MRI protocols have recently been described in a white paper. The present review provides an overview of the current status and applications of carotid plaque MR imaging and its future potential in daily clinical practice.

INTRODUCTION

From numerous histopathological studies, it is well known that vulnerable plaque rupture, rather than perfusion defects due to luminal narrowing, is an important cause of stroke [1]. Atherosclerosis is a chronic inflammatory disease of the large arteries characterized by the accumulation of lipids in the vessel wall and the formation of fibrous tissue [2]. Bifurcations are preferential sites of plaque formation because of the locally reduced wall shear stress [3], which leads to an impaired endothelial function [4]. More advanced lesions are characterized by a lipid-rich necrotic core (LRNC), which is separated from the lumen by a fibrous cap (FC) [5]. Plaques can become increasingly complex, with calcifications, ulcerations, and intraplaque hemorrhage (IPH), thereby increasing the risk to rupture. It is well known that plaque characteristics such as IPH, a large LRNC, and a (TRFC) are associated with cerebrovascular symptoms. Therefore, diagnostic techniques providing information on the plaque vulnerability have been proposed as more accurate prognostic stratification methods compared to the simple measurement of luminal stenosis. Magnetic resonance imaging (MRI) provides excellent soft tissue contrast, no ionizing radiation, and is not subject to technical challenges, such as shadowing or blooming artefacts caused by calcium deposits. It is also uniquely suited to visualize IPH, which is a strong and independent predictor for stroke [6]. MRI is currently recognized as the optimal imaging modality for carotid plaque burden quantification and non-invasive assessment of plaque composition [7]. MRI is well validated, highly reproducible and can be used to predict stroke and evaluate treatment effects [8-10]. MRI studies also provided more insight in factors that contribute to plaque progression and clinical symptoms, since now, for the first time, the plaque could be followed in time, also before the occurrence of clinical symptoms.

In this review, the ability of MRI to quantify most the important plaque features will be discussed. We will provide an overview of pros and cons of different carotid MRI sequences. The associations of plaque components on MRI with stroke and the predictive value of carotid MRI for stroke will be reviewed. We will summarize the current status of carotid plaque MRI. Moreover, the capability of MRI to measure treatment effects will be elucidated. Additionally, we will discuss novel insights on carotid atherosclerosis that were derived from longitudinal MRI studies. Finally, we will describe new developments and clinical perspectives.

HISTOPATHOLOGICAL EVIDENCE ON PLAQUE VULNERABILITY

Numerous histopathological studies have contributed to the concept that rupture of the FC and subsequent thrombosis and embolization is the most important cause of stroke and myocardial infarction [11, 12]. It is well known that patients with symptomatic carotid artery disease have an enlarged LRNC, and a higher prevalence of a TRFC [13-15]. Inflammation, mainly represented by activated macrophages is another hallmark of vulnerable plaques [16-19]. Leaky angiogenic micro-vessels can be a port of entry for inflammatory cells and erythrocytes, leading to further plaque destabilization [20]. IPH is also considered as a key factor that is associated with neurological symptoms and is thought to stimulate plaque progression [3, 5, 21-25]. The carotid histological plaque composition in symptomatic patients that underwent CEA is an independent predictor of future cardiovascular events [26, 27]. Concluding, histopathological studies provided important clues for novel imaging targets, including macrophage-mediated inflammatory changes, neo-angiogenesis, IPH, a large LRNC, and the status of the FC.

MR IMAGING OF CAROTID PLAQUES

In the last two decades, MRI was established as the preferred imaging modality to study carotid plaque features [28]. High-resolution, multi-contrast carotid MRI can identify and quantify atherosclerotic plaque components [28]. The validity of these techniques has been extensively proven using histopathology as a reference standard (Table. 1) [7, 8, 29]. Large multicenter MRI studies demonstrated its feasibility [30]. Toussaint *et. al.* were the first to show that MRI allows *in vivo* discrimination of the LRNC, calcifications, and IPH [31]. A year later, Von Ingersleben *et. al.* confirmed that hemorrhagic regions, calcium, lipid deposits, and fibrous tissue within carotid plaques could be identified using MRI [32]. The different plaque components (*i.e.* LRNC, IPH, calcification, and TRFC) can be distinguished using a combination of various MRI pulse sequences, such as pre- and post-contrast T_1w turbo-spin echo [33], magnetization-prepared rapid acquisition gradient echo (MPRAGE), and time of flight (TOF) (Fig. 1) [34, 35]. Fat suppression is required to reduce signals from perivascular and subcutaneous adipose tissue. In addition to bright blood MR images to visualize juxtaluminal calcifications, black blood pre-pulses are crucial to optimize contrast between the vessel wall and the lumen. Initially, T_2-weighted MRI (T_2w) was used to identify the LRNC [36]. Later studies revealed that contrast-enhanced (CE)-MRI enabled improved discrimination of the FC and LRNC compared to conventional T_2w MRI [37]. Ultra-small superparamagnetic iron oxide particles (ferumoxtran-10) can be used to quantify plaque inflammation [38, 39].

However, this contrast medium is not widely available. Dynamic contrast-enhanced (DCE)-MRI allows to study plaque microvasculature [40, 41]. 2D black blood sequences are limited by the slice thickness, which hampers reproducible quantification due to partial volume effects. These challenges were overcome by recent advancements in three-dimensional (3D) sequences, which provide isotropic 3D images of the entire cervical carotid arteries [42-46] also enabling multi-planar reformatting.

Table 1. Validation of carotid MRI

Plaque component	MR sequence	Sensitivity/specificity or correlation with histology	Agreement
IPH	MPRAGE	84%/84% [47]	
	MPRAGE	93%/96% [7]	
	SNAP versus MPRAGE		κ = 0.82 [48]
	Meta-analysis	87%/92% [49]	
LRNC	Pre- and post-contrast T_1W	98%/100% [50] Strongly correlated with histology (Pearson's r= 0.84, P< 0.001) [29]	Inter-observer agreement (ICC, 0.89; 95% CI, 0.81 to 0.93) [29]
	T_2W 9if contrast injection is contraindicated)	85%/92% [36] 90%/84% [51] 95%/76% [52] Correlation with histology (r= 0.75; P<0.001) [52]	Inter-reader reproducibility for area measurements of LRNC (ICC:0.92, 95%CI: 0.82-0.97) [52]
TRFC	Pre- and post-contrast T_1W	Correlation with histology (r=0.80, P<0.001) [29]	Inter-observer agreement (ICC: 0.78; 95%CI, 0.68 to 0.86) [29]
	T_2W or TOF if contrast injection is contraindicated	90%/84% [51]	Agreement with histology κ= 0.87 [53]
Calcifications	Bright blood image and in addition at least one other weighting	Correlation with histology (*r*= 0.74; *P*< 0.001) [52]	Inter-observer agreement (ICC: 0.9; 95%CI, 0.77 to 0.96) Agreement with histology (κ= 0.75, 95%CI: 0.66-0.84) [52]
Ulceration	CE-MRA		Inter-observer agreement (κ= 0.86, 95%CI: 0.77– 0.95) [54]
	TOF (if contrast injection is contraindicated)	TOF: 81%/90% [55]	(κ= 0.72, 95%CI: 0.58– 0.86) [54]
	SNAP *vs* conventional multi-contrast		(κ= 0.82, 95%CI: 0.65-0.99) [56]

Initially, most studies were performed using 1.5 Tesla MRI. Later, 3.0 Tesla MRI studies were performed to enable improved spatial resolution or better signal-to-noise ratio (SNR) [57-59]. Dedicated multi-element carotid radiofrequency MRI coils can be used to acquire images with high SNR and/or high spatial resolution [60, 61]. This is especially important when visualizing small structures such as the FC. It has been shown that IPH can also be detected using a standard neurovascular coil [34]. Recently,

novel sequences such as multi-contrast Atherosclerosis Characterization (MATCH) and Simultaneous Non-contrast Angiography and IPH (SNAP), have been developed that allow the generation of multi-contrast imaging with a single sequence, leading to a tremendous reduction in scan time and resulting in inherent image co-registration [48, 62]. Recently, expert recommendations on vessel wall MR imaging protocol have been described in white paper [63].

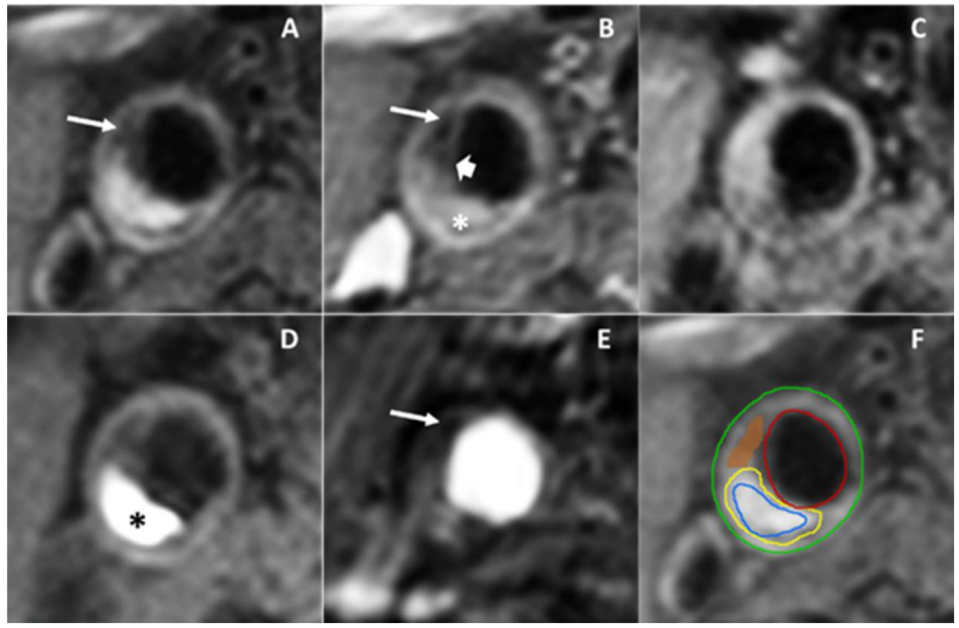

Figure 1. *Transversal magnetic resonance (MR) images of a carotid plaque in the right carotid artery with intraplaque hemorrhage (IPH). The following MR sequences were acquired (A) pre-contrast T_1w-weighted (T_1w) quadruple inversion recovery (QIR) turbo-spin echo [33] [33], (B) post-contrast T_1w QIR TSE, (C) T_2w TSE, (D) T_1w inversion recovery (IR) turbo-field echo (TFE) and (E)time of flight (TOF). A lipid-rich necrotic core LRNC was identified as a region within the bulk of the plaque that does not show contrast enhancement (* on B) with thin and/or ruptured fibrous cap (small arrow on panel B). On the T_1 IR-TFE image, a hyper-intense signal in the bulk of the plaque can be clearly observed, indicating the presence of intraplaque hemorrhage IPH within the area of LRNC (* on panel D). Calcification was identified as low signal intensity on TOF and at least two other weightings (long arrow on A, B and E). Panel (F) shows the plaque contours on the pre-contrast T_1w QIR TSE images (green = outer vessel wall, red = inner vessel wall, yellow = lipid-rich necrotic core, blue = IPH, orange/brown = calcifications).*

PLAQUE BURDEN

Luminal stenosis does not adequately represent plaque burden because of the compensatory enlargement as a response to plaque growth (Glagov remodelling) [64]. Therefore, imaging modalities that assess vessel wall dimensions, *i.e.* plaque burden,

provide a more accurate measure of plaque size and severity than current clinically used imaging modalities that only measure carotid artery stenosis. MRI is the most suited imaging technique to quantify plaque burden because its ability to obtain high-resolution three-dimensional images with high contrast between the vessel wall, the lumen and the perivascular tissue (Fig. 1).

Plaque burden measurements are commonly obtained by subtracting the luminal area from the area that encompasses the outer vessel wall and summing these areas from all MRI slices times the slice thickness, taking into account the slice gap [65]. The normalized wall index (NWI), defined as the wall area divided by the total vessel area, was proposed to overcome the different size of carotid arteries in the population [66]. It is a very accurate and reproducible measure [67, 68].

In the early days, it was shown that slow or turbulent flow can lead to plaque-mimicking artefacts, thereby overestimating the vessel wall [69]. Therefore, black blood pre-pulses are required. Early efforts of black blood MRI, using double inversion recovery prepulses in combination with 2D fast-spin echo imaging, revealed that MRI measurements of vessel wall dimensions (wall volume, maximum wall and minimum luminal area) are highly correlated with volumetric measurements of *ex vivo* CEA specimens (Pearson's $R \geq 0.90$) [70]. To obtain black blood images with the double inversion technique, different inversion times are required before and after contrast injection. Therefore, the quadruple inversion recovery technique was developed to acquire black blood MR images before and after contrast injection using the same sequence [42]. Inversion recovery techniques depend on outflow of blood from the imaging plane in the time period between the inversion pulse(s) and the acquisition of the MRI signal and are therefore problematic in combination with three-dimensional imaging, where thick imaging slabs are excited. Alternative black blood pre-pulses were developed such as multi-slice motion-sensitized driven-equilibrium pre-pulses that do not depend on outflow [69].

Novel 3D black blood sequences such as 3D-MERGE [71] and Delay Alternating with Nutation for Tailored Excitation (DANTE)-prepared 3D MRI [43], offer a high isotropic spatial resolution and the capability to cover the entire cervical carotid arteries. Its short imaging time, ease of use, and ability to accurately assess plaque burden makes these sequences well suited for clinical implementation.

MRI can also be used to quantify the common carotid artery wall dimensions. It was shown that these dimensions correlate well ($r=0.89$, $P<0.001$) with common carotid intima-media thickness as measured with B-mode ultrasound, however with a much smaller measurement variability for MRI [72]. Therefore, when the thickness

of the common carotid artery wall is used as a surrogate measure in cardiovascular prevention trials smaller sample sizes and potentially shorter study duration may be possible when using MRI instead of B-mode ultrasound. Recently, it was pointed out by Paraskevas et al, that there is confusion in literature on intima-media thickness [73]. Some ultrasound studies have measured the thickness of the far wall of the distal common carotid artery, at a site where there is no plaque, while others included plaque thickness in the measurement of intima-media thickness. The latter method results in an invalid comparison since the vessel wall thickness of patients with and without plaque is a very distinct feature [73].

IPH

To date, IPH is the most widely described predictor of stroke from carotid plaque MRI [6, 35, 74].The depiction of IPH as a hyper-intense signal compared to surrounding muscle tissue using an MPRAGE sequence *(Fig. 1)* was first presented by Moody *et. al.* [47]. Due to relatively short T_1 relaxation time of methemoglobin, IPH is hyper-intense on all T_1w images [75, 76]. Cappendijk *et. al.* showed a high detection rate (>80%) of IPH on MPRAGE images, also known as T_1w inversion recovery turbo-field echo (IR-TFE) MRI, using histology as a reference standard [77]. The IR-TFE sequence performed superior for the detection of IPH compared to a black blood T_1w TSE sequence. The inter-observer agreement was high for the IR-TFE (κ=0.73), while it was low for the T1w TSE sequence (κ= 0.35). Later, Ota *et al.* confirmed that MPRAGE has a higher specificity (97%) and sensitivity (80%) for the detection of IPH compared to fast-spin echo and TOF sequences [78]. Semi-automatic quantification of IPH volume on MRI has shown to correlate well with histology [75, 76, 79, 80]. IPH can also be identified on contrast-enhanced MR angiography (CE-MRA) mask images [81]. Recently, a multi-contrast sequence, 3D-SNAP, was developed to detect lumen stenosis and IPH with a single sequence with inherent image co-registration. Its performance for the identification of IPH was comparable with MPRAGE (κ= 0.82) [48]. Alternatively, another multi-contrast sequence, MATCH simultaneously obtains 3 different contrast weightings (hyper-T_1w, T_1w, and gray blood) in a 5-minute scan to image IPH, the LRNC and calcifications [62]. These novel multi-contrast sequences may represent alternatives for multi-sequence MR imaging. However, larger studies to validate MATCH are required. A meta-analysis on the diagnostic performance of MRI for detecting IPH in the carotid arteries revealed excellent specificity (92%) and good sensitivity (87%) [49]. Moreover, carotid plaque T_1 mapping has been developed to obtain more quantitative, reproducible measurements of IPH [82, 83].

LRNC

The LRNC has a short transverse relaxation time (T_2) compared to the surrounding fibrous tissue [84]. Therefore, the LRNC is detected as hypo-intense on T_2w images. Later, it was shown that the contrast between the LRNC and fibrous tissue increases

after contrast injection [45, 52, 85]. The LRNC can be identified as a focal non-enhancing region on CE- T_1w MR images (Fig. 1). The LRNC area can be measured more reproducibly on the contrast-enhanced than the pre-contrast-enhanced images [45, 46]. The coefficient of variation decreased from 33.5% to 17.6% for interreader measurements [86].

FIBROUS CAP (FC) STATUS

FC rupture or ulceration exposes the thrombogenic interior of the plaque to platelets and coagulation factors, which can lead to thrombus formation and distal embolization with clinical consequences. Hatsukami *et. al.* [53] described for the first time that the FC status can be determined with MRI using a 3D-TOF sequence. High sensitivity (0.81) and specificity (0.90) has been revealed for identifying the status of the FC by using multi-sequence (TOF, T_1w, proton density , and T_2w) MRI [55]. Contrast-enhanced MRI (using a gadolinium-based contrast medium) enables to measure the dimensions of the FC [29]. After gadolinium administration, the FC strongly enhances, whereas the LRNC enhances only slightly [29, 37, 50]. The inter-observer agreement for assessment of FC status using pre-and post-contrast T_1w TSE MRI is good (κ = 0.64-0.74) [87].

ULCERATION

Computed Tomography Angiography is considered the best noninvasive imaging modality to evaluate carotid plaque ulceration with a sensitivity and specificity of 94 and 99%, respectively [54, 88, 89]. MRA can identify the presence of carotid ulcerations with a sensitivity similar to computed tomography angiography [54, 90]. CE-MRA was superior (sensitivity: 82%) to TOF-MRA (sensitivity: 55%) for the detection of carotid ulcerations [54]. In addition, CE-MRA can identify ulcerations in calcified plaques, which is considered as a limitation of CTA [91]. The addition of a longitudinal black blood MRA to a cross-sectional multi-sequence vessel wall MR imaging protocol increases the accuracy of detecting carotid atherosclerotic plaque ulcerations [92].

INFLAMMATION AND NEOVASCULARIZATION

DCE-MRI can be used to quantify plaque microvasculature [40, 93]. K^{trans} (volume transfer coefficient) as derived from pharmacokinetic modeling of DCE-MRI showed a significant correlation (with excised plaque neo-vascularity (R= 0.41-0.7) [41, 94, 95]. This technique has been shown to be highly reproducible and reliable (coefficient of variation: 16% for K^{trans}) [41]. Alternatively, gadofosveset-enhanced MRI can be used to visualize plaque microvasculature without the need to use pharmacokinetic modeling. [96]. Van Hoof *et. al.* reviewed the current status and future potential of DCE-MRI in the evaluation of plaque microvasculature. [93]. Recently, Juan *et. al.* demonstrated the feasibility of using a single sequence to acquire both high-resolution 4D CE-MRA and DCE-MRI to evaluate both plaque surface morphology and function [97].

Ultrasmall superparamagnetic particles of iron oxide (ferumoxtran-10)-enhanced MRI has been shown to be strongly associated with carotid plaque macrophage infiltration on histology as these particles are taken up by macrophages [39, 98, 99]. Ferumoxtran-10, however, is not broadly available. Alternatively, PET/MRI can be used to study plaque inflammation and composition with a single examination [100-102].

ASSOCIATIONS BETWEEN PLAQUE COMPOSITION ON MRI AND STROKE

CROSS-SECTIONAL STUDIES

The ability of MRI to distinguish high-risk and low-risk plaques was demonstrated in several proof-of-concept studies. It has been demonstrated that enlarged plaque burden, IPH, LRNC, TRFC, inflammation and neovascularization are more common in symptomatic lesions as summarized below.

The relationship between carotid plaque burden and stroke risk has been demonstrated in various studies (Table. 2). Carotid plaque burden was found to be greater in patients with recurrent stroke than that in those with first-time stroke [103]. Carotid plaque burden was significantly associated with ipsilateral acute cerebral infarction volume independent of the degree of carotid stenosis [104]. Recently Liu *et. al.* showed that carotid plaque burden in patients with ≥1.5 mm carotid plaques was associated with the presence of acute stroke [105]. Based on numerous ultrasound studies, it is well known that plaque burden is a better parameter for risk prediction than measurement of the common carotid artery intima-media thickness [73].

It is well known that carotid IPH is associated with ipsilateral stroke in patients with ≥50% carotid stenosis [35, 104, 106-109]. IPH is more prevalent in the ipsilateral carotid artery compared with the contralateral, asymptomatic, side (60% *vs.* 36%) [110] (Table. 2). Interestingly, in patients that were diagnosed with cryptogenic stroke and a non-stenotic (<50%) carotid plaque, a higher prevalence of IPH was also reported by several studies on the ipsilateral side in several studies [111-114], indicating that in a subgroup of these patients IPH may have been the underlying cause of the patients that were diagnosed with cryptogenic stroke. The presence of IPH also increased the risk for ipsilateral abnormalities on diffusion-weighted imaging (OR: 6.2, 95%CI: 1.7-21.8, p<0.05) [115].

The need to assess the LRNC is justified by two important clinical needs: a large LRNC can lead to plaque rupture and LRNC size can be used to monitor treatment effects of lipid-lowering medication [116]. The LRNC volume was shown to be significantly associated with the severity of cerebral infarction on DWI MRI [104] . The multicenter CARE-II study of 687 symptomatic patients with an intima-media thickness ≥1.5 mm showed that the volume of the LRNC was significantly associated with TIA/stroke [105, 117].

Several studies showed that there is a strong association between FC rupture on MRI and recent stroke or transit ischemic attack (TIA) [118-120] (Table. 2). In symptomatic patients, the volume of IPH was associated with fibrous cap disruption in carotid arteries (OR: 2.867, 95%CI, 1.505–5.461; P=0.001) after adjusting for clinical confounding factors and plaque burden [121]. The irregular plaque surface as determined by MRI was an independent indicator for ipsilateral stroke in patients with carotid plaque(≥1.5mm) [122].

PROSPECTIVE LONGITUDINAL STUDIES

A large number of studies have reported the ability of MRI to identify patients at higher (or lower) risk of stroke [6, 35, 74, 120, 123, 124]. Prevention of primary stroke in asymptomatic patients is very challenging since the degree of stenosis is not an adequate predictor for stroke [125, 126]. Prospective studies in asymptomatic patients with carotid artery stenosis (50-79%) showed that an increase in vessel wall thickness is associated with a larger risk for subsequent ischemic cerebrovascular symptoms (HR for 1mm increase: 1.6; 95%CI, 1.1-2.3) [123]. The predictive value of IPH, LRNC, and TRFC [35, 127] as assessed by MRI for cerebrovascular events was determined in several studies. There were considerable differences between these studies regarding the degree of stenosis as well as the symptomatic status [35, 127]. Meta-analyses showed a significant positive relationship between IPH and the risk of future ischemic events (HR:5.69, 95%CI:2.98-10.87) [127], (HR: 4.59, 95%CI: 2.92–7.24) [35]. Over a median follow-up of 19.6 months, the presence of IPH was associated with a 6-fold higher risk for cerebrovascular ischemic events [127] (Table. 2). HRs for LRNC and TRFC as predictors of subsequent stroke/transient ischemic attack were (3.00, 95% CI: 1.51–5.95) and (5.93, 95% CI: 2.65–13.20), respectively [35]. Lately, Schindler *et. al.* performed a large meta-analysis of individual patient data from 7 cohort studies including 560 and 136 patients with symptomatic and asymptomatic carotid stenosis, respectively, with ipsilateral stroke as the primary endpoint [6]. IPH was present in 51.6% and 29.4% of the patients with symptomatic and asymptomatic carotid stenosis, respectively. Presence of IPH at baseline increased the risk of ipsilateral stroke both in symptomatic (HR: 10.2, 95%CI: 4.6-22.5) and asymptomatic (HR: 7.9, 95%CI: 1.3-47.6) patients (Table. 2). A multivariate analysis in symptomatic patients showed that the risk of ipsilateral stroke was independently increased by the presence of IPH (HR: 11.0, 95%CI: 4.8-25.1) and severe degree of stenosis (HR: 3.3, 95%CI:1.4–7.8) [6]. Among symptomatic patients, carotid IPH was a stronger predictor of stroke risk than any known clinical risk factors. Among patients with symptomatic carotid stenosis, annualized event rates of ipsilateral stroke in those with IPH *vs.* those without were

9.0% *vs.* 0.7% (<50% stenosis), 18.1% *vs.* 2.1% (50% to 69% stenosis), and 29.3% *vs.* 1.5% (70% to 99% stenosis). This also confirms that also cryptogenic stroke patients with a non-stenotic (<50% stenosis) plaque with IPH are at increased risk for stroke. Annualized event rates among patients with asymptomatic carotid stenosis were 5.4% *vs.* 0.8% in those with and without IPH, respectively.

Table 2. Relation between carotid plaque MRI parameters and cerebrovascular symptoms.

plaque component	association with cerebrovascular symptoms	predictive value for cerebrovascular events
IPH	60% symptomatic vs 36% asymptomatic [110]; 37.5% vs 0% [111, 113]; (HR:3.5; 95%CI: 1.05-11.87; P= 0.040) [120]	5-6-fold higher risk for cerebrovascular events (HR:5.69; 95%CI: 2.98-10.87) [74] (HR:4.59, 95%CI: 2.92–7.24) [35] IPH at baseline predicts ipsilateral stroke in symptomatic (HR:10.2, 95%CI: 4.6-22.5) and asymptomatic patients (HR:7.9, 95%CI: 1.3-47.6) [6]
LRNC	(HR:3.2001; 95%CI, 1.078-9.504; P= 0.036) [120]	(HR:3.00, 95%CI: 1.51–5.95) [35] presence of LRNC predicts cardiovascular events (hazard ratio of one standard deviation increase in percent lipid core volume: (HR:1.57 95%CI: 1.22-2.01) [128]
TRFC	Patients with ruptured fibrous caps were 23 times more likely to have had recent ischemic neurological symptoms (95%CI: 3-210) [118] (HR:5.756; 95%CI: 1.9-17.3; P= 0.002) [120]	The hazard ratio for TRFC as predictor of stroke / TIA (HR:5.93, 95%CI: 2.65–13.29) [35] The hazard ratio for TRFC as predictor of cardiovascular events (HR:4.31; 95%CI: 1.67-11.1) [128]
Calcifications	Calcified plaques were found to be 21 times less likely to be symptomatic than non-calcified plaques [129]	
Ulceration	86% Symptomatic vs 36% asymptomatic; P=.039 [130-134]	

RELATION BETWEEN PLAQUE COMPOSITION AND CLINICAL RISK FACTORS

Noninvasive MRI can also be used to study the relation between plaque composition and clinical risk factors, also in lower risk populations, for which carotid endarterectomy specimens for histological studies are not available.

Carotid IPH is associated with clinical parameters that are known to affect stroke risk, i.e. positively with male sex, blood pressure, age and negatively with statin use [135-137]. IPH and LRNC were more prevalent in men compared with women (28.8% *vs.* 18.3% and 28.9% *vs.* 21.7%, respectively) [138]. In fact, for the same degree of carotid stenosis men have a larger LRNC than women [139]. No association was found between total testosterone and LRNC in either sex. Higher total estradiol (OR: 1.58, 95%CI: 1.03–2.40) and lower total testosterone (OR: 0.82, 95%CI: 0.68–0.98) were associated with IPH in women but not in men [140].

In the Rotterdam study, a large population study of individuals with ≥2.5 mm plaque, it was shown that systolic blood pressure and pulse pressure were significantly positively associated with IPH (OR:1.13, 95%CI: 0.99–1.28; OR:1.22, 95%CI: 1.07–1.40, respectively) after adjustment for age and sex [141]. The serum insulin levels are also associated with IPH (OR: 1.42, 95%CI: 1.12-1.7), while they were not associated with the presence of calcifications or a LRNC [142]. Recently, Pletsch-Borba *et. al.* [143] showed in a sub-analysis of 198 participants of the Rotterdam study, that hypertension is significantly associated with new IPH and new calcifications over a 4 year period (OR: 3.87, 95%CI:1.90-7.90;OR: 2.20, 95%CI: 1.07-4.40, respectively), while higher levels of cholesterol were associated with LRNC progression (OR: 1.40, 95%CI: 1.10-1.70).

Although statin therapy leads to a reduction in plaque progression [135], patients with IPH may be less sensitive to statin therapy [144]. Moreover, statin treatment with any dosage was associated with a higher presence of calcifications (OR: 1.73, 95%CI: 1.22-2.44), while a high dosage of statin treatment was also associated with a lower prevalence of LRNC (OR: 0.66, 95%CI: 0.42-1.04) [145]. In another large population study, the Multi-Ethnic Study of Atherosclerosis (MESA), of individuals with thickened carotid vessel wall ≥1 mm, total plasma cholesterol, but not other established clinical risk factors, was strongly associated with the presence of LRNC on MRI.

DETERMINANTS OF PLAQUE PROGRESSION

Progression of atherosclerosis is a complex phenomenon. Several studies have shown that IPH leads to enlargement of the lipid core, leading to plaque progression and destabilization [144, 146]. Kwee *et. al*. showed that the LRNC, IPH and FC status changed only in a minority of TIA/stroke patients with mild to moderate carotid stenosis over a one year period [147].

ABILITY OF MRI TO MEASURE TREATMENT EFFECTS

High resolution MRI studies on the effects of different statin therapies and different dosages on plaque composition were summarized in a systematic review and meta-analysis [116]. Although there was no significant difference in LRNC size at 1-6 months and at 7-12 months following initiation of statin therapy, at >12 months, there was a significant decrease in LRNC volume.

In the ORION trial, the mean percentage of the vessel wall composed of LRNC on MRI decreased by >40% (P=0.005) in both low and high dose of rosuvastatin therapy [148]. T2-mapping demonstrated a depleted lipid content of the carotid plaque after 3 months of high-intensity statin treatment (atorvastatin 80 mg) from 10.3% (7.2-14.2) to 7.4% (5.4-10.0), P=0.002 [149].

2 CLINICAL PERSPECTIVES AND FUTURE OUTLOOK

The continued high incidence of stroke despite advances in optimized medical therapy strongly indicates that individual response to therapy may vary widely. Numerous histopathological studies performed on CEA specimens have shown that specific plaque features are associated with an increased stroke incidence [26, 27]. High-resolution, multi-contrast carotid MRI can identify and quantify atherosclerotic components within carotid plaques [7, 10, 36, 63]. Multi-contrast sequences enable to obtain images with different contrast with a short single sequence [48, 62]. MRI is currently recognized as the most valuable imaging modality for carotid plaque burden quantification and non-invasive assessment of plaque composition [7, 8]. Furthermore, MRI is well validated, highly reproducible and can be used to predict stroke and evaluate treatment effects [8, 10, 52]. Carotid MRI has increased our knowledge on carotid atherosclerotic disease, since MRI studies allowed, for the first time, to follow changes in plaque composition over time, also before clinical symptoms occur [51, 150-152]. It provides an excellent opportunity of improved stroke risk assessment, noninvasive monitoring of disease progression and evaluation of therapeutic efficacy[6, 35, 74, 107, 123].

MRI may be used to identify a subgroup of patients that may benefit from expensive new treatments, such as anti-inflammatory therapy or intensified lipid-lowering therapy. IPH on MRI is currently the most promising approach for implementation in daily clinical practice in the near future, since IPH is easy to recognize on MR images, it can be visualized with a standard MRI sequence with a scan time of a few minutes using a standard neurovascular coil [34]. A recent meta-analysis showed that IPH is a strong independent predictor for stroke in symptomatic as well as asymptomatic patients with carotid stenosis, independent of degree of stenosis and stronger than any known clinical risk factors [6].

In asymptomatic patients with a significant carotid artery stenosis performance of carotid endarterectomy is highly controversial [153]. The number needed to treat to prevent one stroke within 5 years in this asymptomatic population is very high and annual stroke rates due to best medical treatment may be declining [154-156]. Therefore, the recent guidelines of the European Society for Vascular Surgery (ESVS)

state that, while waiting for results of large clinical trials and the development of validated algorithms for patient selection, the presence of one or more imaging features, such as large plaque burden of intraplaque hemorrhage on MRI may be useful to select higher risk for stroke patients [157]. In these clinical guidelines, the advantage of MRA and CTA compared to Duplex ultrasound to visualise simultaneously the aortic arch, supra-aortic trunks, carotid bifurcation, distal internal carotid artery, and the intracranial circulation is also highlighted [157]. They state that such an examination is mandatory if a patient is considered for carotid artery stenting [157]. A short additional MRI sequence of a few minutes to quantify plaque burden and to identify intraplaque hemorrhage can be easily added to an MRA examination.

IPH and LRNC are observed not only in patients with significant stenosis, but also in patients with non-significant (<50%) stenosis [111-114]. These features have been acknowledged as critical factors for clinical disease assessment [35]. IPH has been linked with accelerated plaque progression, luminal narrowing, and clinical events [144, 146]. The LRNC volume can be used to monitor therapeutic effects of lipid lowering treatment [116]. Carotid plaque MRI can also be helpful in borderline clinical cases. Expert consensus recommendations on carotid vessel wall MRI have recently been published [63]. In this white paper detailed recommendations on MRI protocols are provided. For identification of the LRNC and the fibrous cap status, currently contrast injection is still preferred [45, 52, 85]. Recently, fast three-dimensional sequences have been developed with a large longitudinal coverage [42, 43, 158]. Artificial intelligence may lead to further improvements in MR image quality, shorter scan times and it can provide tools for automated image analysis [159]. The development and validation of risk prediction models that include carotid plaque MRI findings can further aid in improved risk stratification.

Large clinical trials are warranted to study whether carotid plaque MRI can be used to select patients that benefit from carotid revascularization, but also to identify patients that can be safely treated with best medical treatment alone.

Further studies might be needed to investigate the relation between MRI features and the most suitable type of therapy. The introduction of hybrid PET/MRI systems allows for the combination of anatomical imaging with MRI, and metabolic/functional imaging with PET [102]. Together these advances are expected to lead to a reduction in stroke and the substantial economic burden associated with stroke mortality, morbidity and long-term disability.

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CHAPTER 3

The relationship between fibrous cap status and plaque surface morphology and the volume of intraplaque hemorrhage over two years in symptomatic patients with mild to moderate carotid stenosis: The Plaque at RISK (PARISK) Study

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ABSTRACT

BACKGROUND AND PURPOSE

Carotid intraplaque hemorrhage (IPH) is a strong predictor of stroke, but factors contributing to IPH development are incompletely understood. Cross-sectional studies have demonstrated an association between IPH and a thin/ruptured fibrous cap (TRFC)/disrupted plaque surface. We aim to investigate the longitudinal relationship between a TRFC/disrupted plaque surface and the IPH volume in symptomatic patients with mild-to-moderate carotid stenosis.

METHODS

116 ischemic TIA/stroke patients with ipsilateral carotid plaques underwent baseline and two-year follow-up MRI. IPH and fibrous cap status (thick versus TRFC) on MRI and disruption of the plaque surface (smooth versus fissure/ulceration) on CTA were assessed. The median changes in IPH volume were calculated and the risk of IPH progression was compared between groups. Multivariate logistic regression was used to evaluate the association between TRFC/disrupted plaque and the risk of IPH being present at follow-up after adjustment for baseline IPH volume and potential confounders.

RESULTS

In the TRFC and disrupted plaque surface groups, the median IPH volume tended to decrease during follow-up (baseline IPH volume: 97.3 mm^3, IQR [3.2-193.3 mm^3] versus follow-up IPH volume: 29.7 mm^3, IQR [0.0-115.1 mm^3], p=0.09, and baseline IPH volume: 25.1 mm^3, IQR [0.0-166.2 mm^3] versus follow-up IPH volume: 11.2 mm^3, IQR [0.0-68.3 mm^3], p=0.04, respectively). On the other hand, in the group with a thick fibrous cap and the group with a smooth plaque surface the median IPH volumes were zero at baseline and remained zero at follow-up. The risk of IPH progression was higher in the TRFC/disrupted plaque groups (risk ratio (RR): 2.9 and 2.0, respectively) than in patients with a thick fibrous cap/smooth plaque surface. Additionally, patients with TRFC were at increased risk of having IPH at follow-up (OR=6.32, 95% CI: 2.4-16.9; p<0.05). Similar results were obtained for patients with disrupted plaque surface, although these results were non-significant (OR=1.8, 95% CI: 0.6-4.8; p=0.3).

DISCUSSION AND CONCLUSION

For stroke patients with a TRFC/disrupted plaque, with a larger median baseline IPH volume, the net decrease in IPH volume indicates plaque healing in some of these patients over time. Nevertheless, patients with a TRFC/disrupted plaque are still at increased risk for IPH progression.

BACKGROUND

Stroke is a major global health problem that causes disability or death [1]. Over 80% of all strokes are of ischemic origin following occlusion of a cerebral artery [2]. Carotid atherosclerosis is a major cause of ischemic stroke, contributing to approximately 20% of all ischemic strokes [3].

The best management of patients with mild or moderate carotid stenosis (30-69%) is unclear [4, 5]. There is increasing interest in assessing the composition of the atherosclerotic carotid plaque instead of measuring only the degree of stenosis to assess plaque vulnerability [6, 7]. Vulnerable plaques are more prone to rupture, which can lead to stroke [8]. Many characteristics of vulnerable plaques can be detected non-invasively with magnetic resonance imaging (MRI) [6], such as intraplaque hemorrhage (IPH) and thin or ruptured fibrous cap (TRFC), with good histological correlation [9, 10]. On the other hand, computed tomography angiography (CTA), in addition to its ability to determine the degree of stenosis, can be used to assess plaque surface morphology [11].

Carotid plaques with IPH, TRFC, and plaque surface disruption are associated with an increased risk for new and recurrent cerebrovascular events [6, 12, 13]. IPH on MRI increases the risk of future stroke in both symptomatic (hazard ratio (HR): 10.2, 95% confidence interval (CI): 4.6-22.5) and asymptomatic patients (HR: 7.9, 95% CI: 1.3-47.6) [12]. Furthermore, a meta-analysis showed that TRFC on MRI also increases the risk for TIA/stroke (HR: 5.9, 95% CI: 2.6-13.2) [13]. Carotid plaque ulceration/fissuring, visible as plaque surface disruption, is also associated with recurrent ischemic stroke with hazard ratios ranging from 1.24 (95% CI: 0.61-2.52) to 3.43 (1.49-7.88) [14].

Although there is strong evidence that IPH increases stroke risk, the factors contributing to the development of IPH are incompletely understood. It has often been suggested that IPH originates from leaky plaque microvasculature [15, 16]. However, in a recent study, we demonstrated a decreased K^{trans}, indicating less leaky microvasculature, in symptomatic carotid plaques with IPH [17]. On the other hand, IPH can originate from the entry of luminal blood [18, 19] due to fissuring or rupture of the fibrous cap. Indeed, Ota *et al.* [20] observed a strong association between the presence of IPH and TRFC (odds ratio (OR)=5.9; 95% CI, 2.6-13.1), and IPH was also shown to be linked with a disrupted plaque surface (fissured or ulcerated plaque) on CTA (OR, 3.1; 95% CI, 1.2–7.8) in both symptomatic and contralateral asymptomatic vessels [21]. However, due to the cross-sectional study design, these studies could not conclude on causality.

The present study aimed to investigate the relationship between TRFC/disrupted plaque surface and IPH in a longitudinal study. At baseline and after two years of follow-up, the

fibrous cap status and IPH volume were assessed with MRI, while the plaque surface (i.e., fissured/ulcerated plaque) was examined with CTA. We hypothesized that patients with a TRFC/disrupted plaque surface are more likely to demonstrate progression of IPH. Finally, we explored to what extent the fibrous cap or plaque surface morphology changed during the two-year follow-up and whether these changes had an impact on IPH volume.

MATERIALS AND METHODS

SUBJECT CHARACTERISTICS

Patients were derived from the Plaque At Risk (PARISK) study. The PARISK study (clinical trials.gov NCT01208025) is a prospective multicenter cohort study that investigates whether advanced carotid plaque imaging beyond the degree of stenosis can be used to identify a subgroup of patients with increased stroke risk [22]. Eligible for the study were patients with a transient ischemic attack (TIA), amaurosis fugax, or minor stroke (modified Rankin scale ≤3) of the carotid artery territory, and < 70% stenosis of the ipsilateral internal carotid artery (ICA) who were not scheduled for a revascularization procedure. The 70% stenosis was the upper cut-off according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria. The lower cut-off 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%. Carotid stenosis was measured by CTA or duplex ultrasound. TIA was defined as an episode of temporary and focal cerebral dysfunction of vascular origin, lasting for a maximum of 24 hours and leaving no persistent neurological deficit. Minor stroke was defined as an episode of focal cerebral dysfunction of vascular origin lasting for more than 24 hours or a nondisabling stroke with a modified Rankin score ≤ 3. Monocular ischemia was defined as a sudden loss of vision of presumed vascular origin and confined to one eye. Exclusion criteria were a probable cardiac source of embolism, a clotting disorder, severe comorbidity, and standard contraindications for MRI. A more detailed description of the study design can be found elsewhere [23]. At baseline, clinical data such as age, sex, the occurrence of last symptoms, medication use, and cardiovascular risk factors were collected. A total of 244 patients were included. Two years after inclusion, noninvasive imaging of the carotid artery was repeated in the first 150 patients [22]. The current study included patients with baseline carotid MR imaging and a two-year follow-up MRI (Figure 1). The study was approved by the Institutional Review Board, and all patients gave written informed consent.

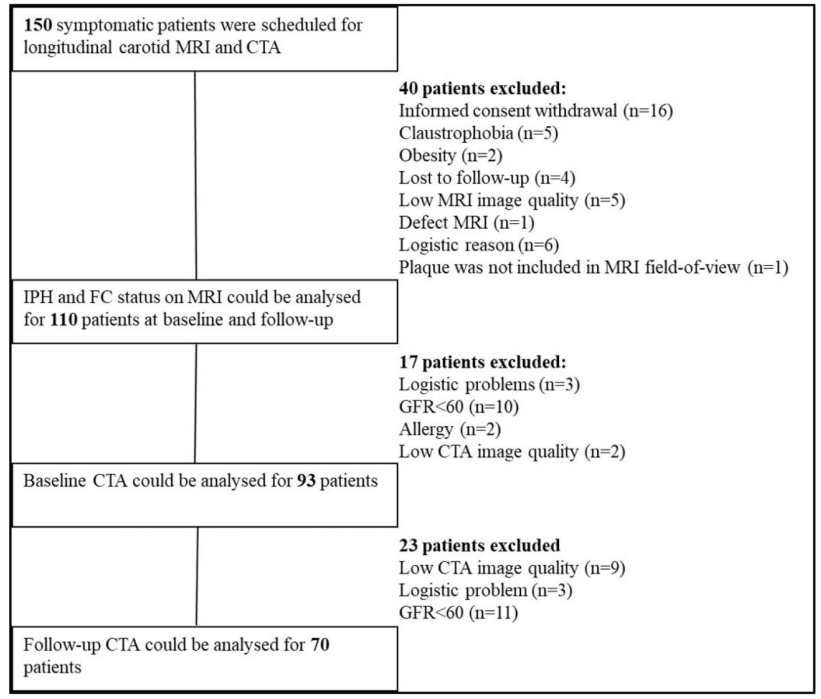

Figure 1. Flow chart of the study
MRI= magnetic resonance imaging, CTA= computed tomographic angiography, GFR= glomerular filtration rate, IPH= intraplaque hemorrhage, FC = fibrous cap

ASSESSMENT OF PLAQUE SURFACE MORPHOLOGY ON CTA

Contrast-enhanced carotid CTA was performed at baseline and at the two-year follow-up using a standardized protocol as described previously [23]. Image reconstructions were made with a field-of-view of 120-160 mm, matrix size 512 x512, and 1.0 mm slice thickness [23]. All CTA images were transferred to a dedicated 3D image analysis software package (Leonardo and syngo.via; Siemens, Erlangen, Germany). The CTA images of the ipsilateral carotid plaques were evaluated by two independent trained readers blinded to the clinical data and other imaging examinations. Discrepancies were solved by an experienced third observer (10 years of experience). Images were analyzed in three different planes (coronal, oblique, and sagittal). Plaque surface morphology was classified as smooth, fissured, or ulcerated [24]. Plaque ulceration was defined as an extension of contrast material of more than 1 mm into the atherosclerotic plaque on at least two slices, being visible in at least two perpendicular planes [24, 25]. An extension ≤ 1 mm in depth with an angle of 230° or more was considered plaque fissuring [26]. Within the present study, fissured and ulcerated plaque surface morphologies were grouped as disrupted plaque surface. Plaque surface morphology was classified as smooth when there was no disrupted plaque surface. Image quality was rated on a

previously described 3-point scale: poor (1), moderate (2), and good (3) [21]. Patients with a mean quality score >1 were included.

ASSESSMENT OF IPH AND FIBROUS CAP STATUS ON MR IMAGING

The carotid MRI protocol has been previously described [23]. In brief, multisequence MRI of the ipsilateral carotid plaque was performed on a 3.0 Tesla system. A dedicated 3-dimensional (3D) hyper T_1-weighted (T1W) sequence was used to visualize IPH (3D T1W inversion recovery turbo field echo (IR-TFE) at Maastricht University Medical Center, Amsterdam University Medical Center, and Utrecht University Medical Center, or 3D fast spoiled gradient echo (FSPGR) at Erasmus Medical Center). 2D T1W quadruple inversion recovery turbo field spin echo (T1W QIR TSE) or 2D T1 weighted double inversion recovery fast spin echo (T1W DIR FSE) images were acquired pre-contrast and six minutes after injection of 0.1 mmol/kg body weight of a gadolinium-based contrast agent. Fifteen transverse adjoining slices with a thickness of two millimeters each were obtained, covering the entire plaque. The acquired and reconstructed resolutions were 0.62 mm × 0.62–0.67 mm and 0.30 mm × 0.24–0.30 mm, respectively [23]. The follow-up carotid MRI was always performed on the same scanner using the same sequences as the baseline carotid MRI.

Dedicated vessel wall imaging analysis software (VesselMass, Department of Radiology, Leiden University Medical Centre, Leiden) was used to analyze the MR images of the ipsilateral carotid plaque by trained observers blinded to clinical data and CT results and supervised by a single experienced observer (20 years of experience). After an extensive training period and demonstrating good interobserver agreement (ICC for IPH volume and κ-value for fibrous cap status > 0.7 and no statistically significant bias in IPH volume according to Bland-Altman analysis) in a validation set that was previously delineated in consensus by 2 experts (with >7 and >10 years of experience, respectively), the observers scored and delineated the MR images [27]. Follow-up MR images were scored additionally blinded to the baseline MRI findings. Image quality was scored based on a 5-point scale that was previously proposed [28]. Patients with a mean quality <2 were excluded. The lipid-rich necrotic core (LRNC) was defined as an area in the bulk of the plaque that does not show contrast enhancement on postcontrast T1w images [10]. Intraplaque hemorrhage was delineated and defined as a hyperintense signal in the bulk of the plaque compared with the adjacent sternocleidomastoid muscle on 3D T1W IR-TFE or 3D FSPGR images that were acquired before contrast injection. Intraplaque hemorrhage is usually diffusely distributed in the lipid core [27, 29]. Therefore, any region that was delineated as IPH was also considered to be part of the LRNC, in line with previous studies (26, 27). Note that the IPH volume was defined as the pure volume of IPH and only included hyperintense regions on IR-TFE or FSPGR images. Hyperintensity was not quantified by a certain threshold in signal intensity, but the presence was scored qualitatively by trained observers. If there were separate IPH regions within the same carotid plaque, the volumes of these regions were summed. Fibrous cap status was dichotomized according to the previously published categorization [30] and was classified as thick/intact when no LRNC was scored or when a region with signal enhancement between the LRNC and the lumen was identified on postcontrast images. It was classified as TRFC when no signal enhancement or an interrupted region of signal enhancement was identified between the LRNC and the lumen.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Statistics version 25. A p-value <0.05 was considered statistically significant. For all data, normality (i.e., Gaussian Distribution) was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Continuous data with a normal distribution were presented as the mean ± standard deviation (SD) [31]. Non-normally distributed variables were reported as medians with interquartile ranges (IQRs). The median is the same as the 50th percentile value, with half of the patients having a value less than the median and half of the patients having a value larger than the median.

Differences in baseline clinical characteristics between patient groups classified according to fibrous cap and plaque surface status were tested for significance using the Chi-square test for categorical variables. For comparison of continuous variables, the independent-samples t-test or Mann-Whitney test was used, as appropriate. Fisher's exact test was used to test the association between the FC status on MRI and plaque surface morphology on CTA. For comparison of IPH change during follow-up between patients with and without a TRFC and with and without a disrupted plaque surface, the nonparametric Mann-Whitney U test was used. To investigate changes in IPH volume over time in the same group, a Wilcoxon signed-rank test was performed. The measurement error is the uncertainty of a parameter that is not attributed to true changes in the measurement [32]. The measurement error was determined by assessing differences in IPH volumes by independent delineation of IPH by 5 trained readers blinded to the other readers in 17 patients. A threshold for IPH progression or regression was defined as two times the mean coefficient of variation. The coefficient of variation is defined as 100% * standard deviation (SD)/mean [33]. A threshold of twice the mean coefficient of variation was chosen, thus corresponding to 2SDs, to ensure that 95% of the differences in IPH volume, that are caused by the measurement error, will fall within the threshold. The mean coefficient of variation for the volume of IPH was 11%. Thus, progression of IPH was defined as a volumetric increase compared to the baseline value larger than 22% or new IPH. Regression was defined as an IPH volume decrease of more than 22% or disappearance of IPH. A change between -22%

and +22% was classified as no change in IPH volume.

The probability of IPH progression and regression in the TRFC versus thick FC group and disrupted versus smooth plaque surface group was determined by calculating the relative risk ratios (RRs).

Multivariate logistic regression analysis was used to evaluate the association between baseline fibrous cap/plaque surface status and risk of IPH still being present at the end of follow-up after adjustment for baseline IPH volume and potential confounders. Confounders were defined as clinical risk factors that were different between groups (i.e., p-value <0.1). The magnitude of the association was expressed as the odds ratio with 95% confidence interval.

We also explored to what extent the change in fibrous cap status on MRI or plaque surface morphology on CTA during the two-year follow-up affected IPH volume. We used the relative risk to determine the probability of IPH progression and regression in these subgroups.

RESULTS

PATIENT CHARACTERISTICS

A total of 150 patients out of the 244 patients were scheduled for baseline and two years of follow-up imaging. A total of 116 of these 150 patients eventually underwent baseline and follow-up carotid MRI. The MR images of 5 patients could not be analyzed due to low image quality. One patient needed to be excluded because the MR images were acquired below the location of the plaque. Therefore, MR images could be analyzed for 110 patients. Plaque surface morphology on CTA could be scored in 93/110 and 70/93 patients at baseline and follow-up, respectively (Figure 1). The distribution of baseline clinical characteristics of these 110 patients is presented in Table 1. The median age was 70 (64-76) years, and 77 (70%) patients were male. Differences between groups stratified for fibrous cap status and status of the plaque surface were non-significant except for the difference in the presence of diabetes mellitus and the use of antidiabetic medication before the index event for disrupted plaque surface versus smooth surface as scored on CTA.

Patients with a TRFC more frequently had a disrupted plaque surface status than patients with a thick cap (61.5% versus 29.6%). According to Fisher's exact test, there was a significant association between TRFC on MRI and disrupted plaque on CTA (odds ratio: 3.8, 95% CI: 1.6-9.1; two-sided, p=0.003).

Table 1. Clinical characteristics at baseline and the medication use before the index event, at baseline (after the index event) and at two years of follow-up.

	Total (n=110)	Baseline fibrous cap status (n=110)			Total (n=93)	Baseline plaque surface morphology		
		TRFC (n=45)	thick FC (n=65)	p-value		disrupted plaque surface (n=40)	smooth (n=53)	p-value
Age, years median (IQR)	70(64-76)	71(67-75)	70(62-76)	0.6	70(64-76)	68(64-73)	71(63-76)	0.4
BMI, kg/m2 (mean ± SD)*	26.3±3.8	25(24-29)	25(24-28)	0.8	25.5(24.1-28.4)	25(23-27)	25(24-28)	0.5
Height, cm (mean ± SD)	172±8.9	174±8.3	172±9.2	0.2	171±8.8	170±8.5	173±8.9	0.2
Sex, male (number, %)	77(70%)	34(75.5%)	43(66.1%)	0.3	68(73.1%)	31(77.5%)	37(69.8%)	0.4
Hypertension, yes (number, %)	62(56.4%)	26(57.7%)	36(55.4%)	0.9	49(52.7%)	21(52.5%)	28(52.8%)	0.9
Hypercholesterolemia, yes (number, %)	57(51.8%)	21(46.7%)	36(55.3%)	0.4	51(54.8%)	23(57.5%)	28(52.8%)	0.6
Smoking (number, %)								
- No	26(23.6%)	11(24.4%)	15(23.1%)	0.9	20(21.5%)	7(17.5%)	13(24.5%)	0.4
- Current	23(20.9%)	10(22.2%)	13(21.0%)		22(23.7%)	8(20.0%)	14(26.4%)	
- Former	61(55.5%)	24(53.3%)	37(56.9%)		51(54.8%)	25(62.5%)	26(49.1%)	
Diabetes mellitus, yes (number, %)	25(22.7%)	12(26.6%)	13(20.0%)	0.4	20(21.5%)	4(10.0%)	16(30.2%)	**0.02**
Classification event (number, %)								
- TIA	49(44.5%)	17(37.7%)	32(49.2%)	0.13	40(43.0%)	13(31.7%)	27(51.9%)	0.16
- Stroke	46(41.8%)	24(53.3%)	22(33.8%)		42(45.2%)	22(53.7%)	20(38.5%)	
- Amaurosis Fugax	15(13.6%)	4(8.8%)	11(16.9%)		11(11.8%)	6(14.6%)	5(9.6%)	
History (number, %)								
- History of ischemic stroke	22(20%)	8(17.7%)	14(21.5%)	0.5	20(21.5%)	9(22.0%)	11(21.2%)	0.9
- History of heart disease	21(19.1%)	9(20.0%)	12(18.5%)	0.9	16(17.2%)	9(22.0%)	7(13.5%)	0.2
- History of peripheral artery disease	16(14.5%)	7(15.5%)	9(13.8%)	0.6	13(13.9%)	7(17.1%)	6(11.5%)	0.5
Rankin score (number, %)	40(36.4%)	14(31%)	26(40%)		36	15(37.5%)	21(39.6%)	
0= no symptoms	44(40%)	17(37.7%)	27(41.5%)		37	15(37.5%)	22(41.5%)	
1= minor symptoms	15(13.6%)	8(17.7%)	7(10.7%)	0.6	12	5(12.5%)	7(13.2%)	0.6
2= minor handicap	11(10%)	6(13.3%)	5(7.6%)		8	5(12.5%)	3(5.6%)	
3= moderate handicap	0	0	0		0	0	0	
4= moderately severe handicap					0	0	0	
5= severe handicap	0	0	0					
Medication (number, %)								

Table 1. Continued.

	Total (n=110)	Baseline fibrous cap status (n=110)			Total (n=93)	Baseline plaque surface morphology		
		TRFC (n=45)	thick FC (n=65)	p-value		disrupted plaque surface (n=40)	smooth (n=53)	p-value
- Antihypertensive								
Before the index event	60(54.5%)	24(53.3%)	36(55.4%)	0.8	48(51.6%)	22(55.0%)	26(49.1%)	0.6
At baseline	68(61.8%)	26(57.8%)	42(64.6%)	0.5	55 (59.1%)	22 (55.0%)	33(62.3%)	0.5
At 2 years follow-up	73(66.4%)	27(60.0%)	46(70.1%)	0.4	62 (66.7%)	28(70.0%)	34(64.2%)	0.6
- Antidiabetic								
Before the index event	20(18.2%)	9(20.0%)	11(16.9%)	0.7	15(16.1%)	2(5.0%)	13(24.5%)	**0.01**
At baseline	20(18.2%)	9(20.0%)	11(16.9%)	0.7	15(16.1%)	2(5.0%)	13(24.5%)	**0.01**
At 2 years follow-up	22(20%)	10(22.2%)	12(18.5%)	0.6	17(18.3%)	4(10.0%)	13(24.5%)	**0.07**
-Statins								
Before the index event	53(48.2%)	18(40%)	35(53.8%)	0.1	42(45.2%)	17(42.5%%)	25(47.2%)	0.6
At baseline	99(90%)	40(88.9%)	59(90.8%)	0.7	82(88.2)	35(87.5%)	47(88.7%)	0.9
At 2 years follow-up	102(92.3%)	42(93.3%)	60(92.3%)	0.8	85(91.4%)	36(90.0%)	49(92.5%)	0.8
-Antiplatelet & Anticoagulants								
Before the index event	41(37.3%)	17(37.8%)	24(36.9%)	0.9	34(36.6%)	17(42.5%)	17(32.1%)	0.3
At baseline	110(100%)	45(100%)	65(100%)	na	93(100%)	40(100%)	53(100%)	na
At 2 years follow-up	110(100%)	45(100%)	65(100%)	na	93(100%)	40(100%)	53(100%)	na
Time between index event and MRI (days) (mean ± SD)	47±12	48±15	45±10	0.9	41±16	42±16	41±16	0.9
Time MRI baseline-MRI FU (weeks) (mean ± SD)	110 ± 10	110 ± 9	110 ± 10	0.6	111±11	111± 12	110±12	0.8
Median IPH volume at baseline (IQR) mm3	0.0(0.0-69.5)	97.3(3.2-193.3)	0.0(0.0-0.0)	**<0.05**	0.0(0.0-91.8)	25.1(0.0-166.2)	0.0(0.0-4.9)	**<0.05**

*Continuous data are presented as median (IQR) or mean ± standard deviation (SD), and categorical data are presented as the number of patients (%). TRFC indicates thin or ruptured fibrous cap, FC indicates fibrous cap, BMI indicates body mass index, TIA indicates transient ischemic attack, p-value < 0.05. * The data of one patient is missing*

IPH VOLUME AT BASELINE AND AT THE TWO-YEAR FOLLOW-UP

A total of 43/110 (39.1%) and 47/110 (42.7%) patients had IPH at baseline and at follow-up, respectively. The IPH volumes at baseline and follow-up were not normally distributed because of many zero values. Patients with a TRFC at baseline had a higher median baseline IPH volume than patients with a thick fibrous cap (97.3 mm^3, IQR [3.2-193.3 mm^3] vs. 0.0 mm^3, p<0.05).

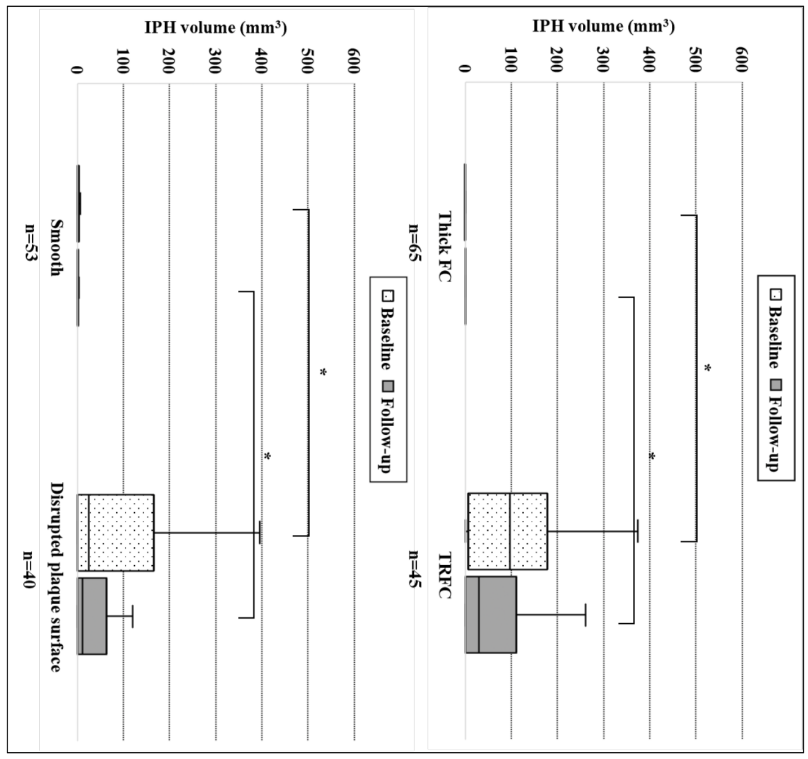

Figure 2. Median intraplaque volume at baseline and at the two-year follow-up.
** p<0.05, IPH indicates intraplaque hemorrhage; FC indicates fibrous cap; TRFC indicates thin or ruptured fibrous cap.*

In line, patients with disrupted plaque surface on CTA at baseline had a larger median baseline IPH volume than patients with smooth plaque surface (25.1 mm^3, IQR: [0.0-166.2 mm^3] vs. 0.0 mm^3; p<0.05) (Figure 2). In the TRFC and disrupted plaque surface groups, the median IPH volume (tended to) decrease during follow-up (baseline IPH volume: 97.3 mm^3, IQR [3.2-193.3 mm^3] versus follow-up IPH volume: 29.7 mm^3, IQR [0.0-115.1 mm^3], p=0.09, and baseline IPH volume: 25.1 mm^3, IQR [0.0-166.2 mm^3]

versus follow-up IPH volume: 11.2 mm^3, IQR [0.0-68.3 mm^3], p=0.04, respectively). On the other hand, the median IPH volumes in the group with a thick fibrous cap and in the group with a smooth plaque surface were zero at baseline and remained zero at follow-up (baseline IPH volume: 0.0 mm^3, IQR [0.0-0.0 mm^3] versus follow-up IPH volume: 0.0 mm^3, IQR [0.0-0.0 mm^3], p=0.4, and baseline IPH volume: 0.0 mm^3 IQR [0.0-4.9 mm^3] versus follow-up IPH volume: 0.0 mm^3 IQR [0.0-2.7 mm^3], p=0.8, respectively),. As a result, patients with a TRFC tended to have a larger median IPH volume regression than those with a thick fibrous cap (-2.1 mm^3, IQR: [-83.1-24.2 mm^3] vs. 0.0 mm^3 [0.0-0.0], p=0.052). The median difference in IPH volume change after two years between patients with a disrupted plaque surface and those with a smooth plaque surface was not significant (0.0 mm^3, [-104.5-4.5] vs. 0.0 mm^3, [0.0-0.0], p=0.25). Table 2 shows the percentage of patients with progression, regression, or no change in IPH volume stratified according to baseline fibrous cap and plaque surface status. The risk of IPH progression was higher in the TRFC group than in the thick FC group (risk ratio (RR)=2.9, 95% CI: 1.5-5.5). An example of IPH progression is shown in Figure 3. In the TRFC group, with a much larger baseline IPH volume, the likelihood of regression was also higher (RR=5.4, CI: 2.6-11.1). The results were similar for the comparison of the plaque surface groups. In the group with a disrupted plaque surface, both progression (RR=2.0, CI: 0.9-4.5) and regression (RR=2.3, CI: 1.2-4.4) occurred more frequently than in the group with a smooth surface. Patients with TRFC versus thick fibrous cap at baseline, after adjustment for baseline IPH volume, were at increased risk of having IPH at the end of the two-year follow-up (OR=6.3, 95% CI: 2.4-16.9; p<0.05). In line, patients with a baseline disrupted versus smooth plaque surface also had a higher prevalence of IPH at the end of follow-up after adjustment for baseline IPH volume, although this result was not statistically significant (OR=2.0, CI: 0.7-5.4; p=0.2). After correction for potential confounders (i.e., the presence of diabetes mellitus), the result remained similar (OR=1.8 (95% CI: 0.6-4.8; p=0.3).

Table 2. IPH volume change during two years of follow-up

	TRFC	Thick FC	Total
No change of IPH	10 (22.2%)	47 (72.3%)	57 (51.8%)
Progression of IPH	12 (26.7%)	11 (16.9%)	23 (20.9%)
Regression of IPH	23 (51.1%)	7 (10.8%)	30 (27.3%)
Total	45 (40.9%)	65 (59.1%)	110
	Disrupted plaque surface	**Smooth plaque surface**	**Total**
No change of IPH	15 (37.5%)	35 (66.0%)	50 (53.8%)
Progression of IPH	9 (22.5%)	8 (15.1%)	17 (18.3%)
Regression of IPH	16 (40.0%)	10 (18.9%)	26 (27.9%)
Total	40 (43.0%)	53 (57.0%)	93

Cross table for the number of patients who showed progression, regression, and no change in IPH volume on MRI after two years of follow-up stratified for fibrous cap status on MRI or plaque surface morphology on CTA at baseline. Progression of IPH: IPH volume increased by more than 22% or new IPH. Regression of IPH: IPH volume decreased by more than 22% or disappearance of IPH. IPH: intraplaque hemorrhage; TRFC: thin or ruptured fibrous cap; FC: fibrous cap.

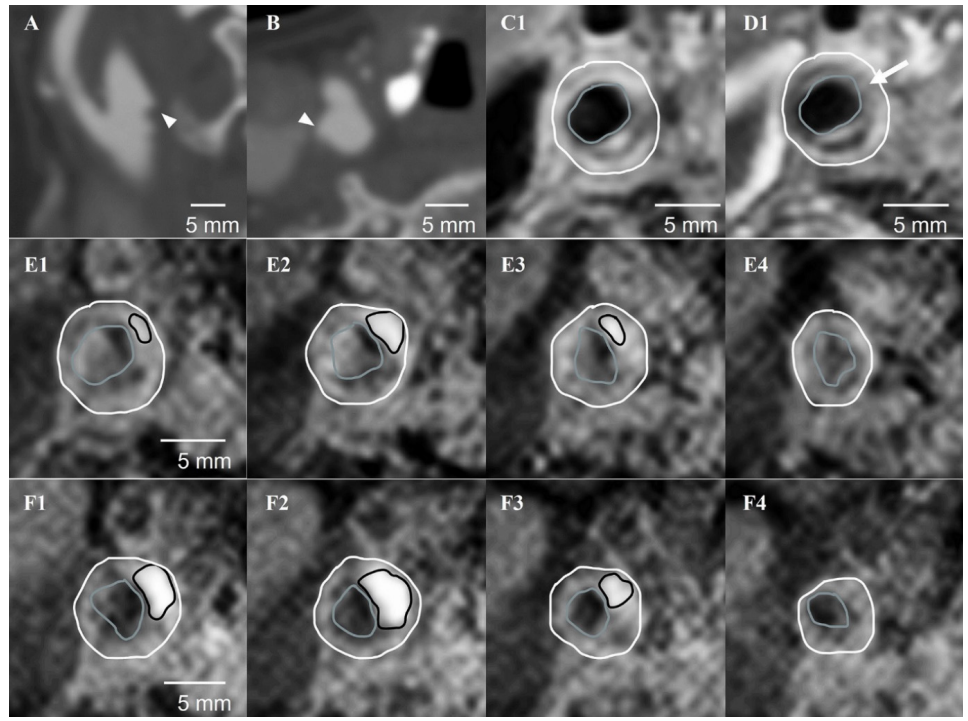

Figure 3. Example of a patient with IPH progression after two years of follow-up.

A) and B) Baseline sagittal and transversal computed tomography angiography (CTA) images, respectively. The arrowhead indicates a disrupted plaque surface at baseline. C1) and D1) Baseline pre- and postcontrast transversal T1-weighted quadruple inversion recovery TSE images, respectively. The arrow indicates a thin or ruptured fibrous cap (TRFC). The gray and white contours indicate the lumen and outer vessel wall delineation. E1-4) and F1-4) show consecutive 3D T1-weighted inversion recovery turbo field echo (IR-TFE) images of the internal carotid artery of the same patient with IPH at baseline and follow-up, respectively. Images E1 and F2 correspond to the same cross-sectional position as images C1 and D1. The black contours indicate the delineations of intraplaque hemorrhage (IPH) on 3D T1w IR-TFE at baseline (E1-E4) and follow-up (F1-F4). The total IPH volume increased by 62% after two years of follow-up. Note that after two years of follow-up, the fibrous cap status remained TRFC. At follow-up, no CTA could be performed since the glomerular filtration rate (GFR) was smaller than 60 mL/min/1.73 m^2.

Figure 4 shows that in most patients, fibrous cap (85/110) and plaque surface (56/70) status remained the same during follow-up. Patients with a TRFC at both time points (33/110) or disrupted plaque surface at both time points (20/70) had the largest median IPH baseline volume, and during follow-up a net nonsignificant decrease in IPH volume was observed In patients with a thick fibrous cap changing to TRFC (n=13) or a smooth plaque surface changing to disrupted (n=2), there was an increase in IPH volume (Figure 4). The opposite trend, i.e. a decrease in IPH volume, was observed in patients where TRFC changed to thick or the disrupted plaque surface changed to smooth. Note that for these exploratory analyses with a relatively small number of patients,

differences were non-significant. Our explorative analysis showed that the relative risk of IPH progression is larger, although not statistically significant, for patients with a baseline TRFC that remained TRFC at follow-up (RR=1.5, 95% CI: 0.4-5.4) compared to patients where the fibrous cap status changed from TRFC to thick. In contrast, the probability of IPH regression was smaller in the TRFC/TRFC group (RR=0.84, 95% CI: 0.51-1.37) with respect to the TRFC/thick group, although the difference was not statistically significant. The probability of IPH progression and IPH regression in the group of patients with a baseline disrupted plaque surface that remained disrupted at follow-up was not different compared to patients with a baseline disrupted plaque surface that changed to a smooth surface (RR=0.96, 95% CI: 0.3-3.4 and RR=1.0, 95% CI: 0.4-2.4, respectively). Note that for these exploratory analyses with a relatively small number of patients, differences were non-significant.

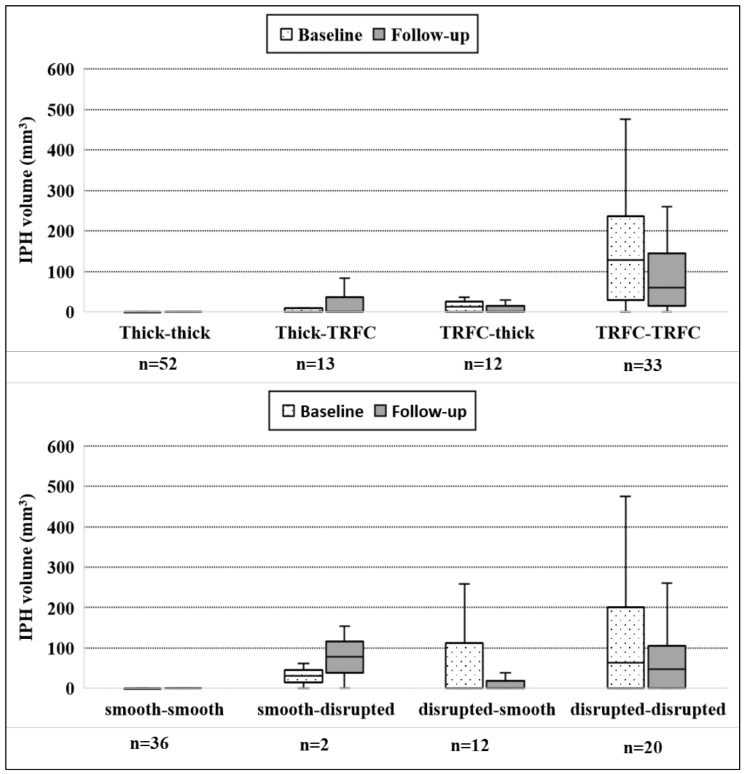

Figure 4. Median IPH volumes stratified by fibrous cap or plaque surface morphology at baseline and follow-up.
For instance, thick-thin indicates a thick fibrous cap at baseline and a TRFC at follow-up. TRFC indicates thin or ruptured fibrous cap; IPH indicates intraplaque hemorrhage.

DISCUSSION

In this prospective study, we demonstrated that symptomatic patients with a TRFC or disrupted plaque surface show a significantly larger median IPH volume at baseline than patients with a thick fibrous cap/smooth plaque surface. In these patients with a TRFC/disrupted plaque surface, a (tendency) towards a median decrease in IPH volume was observed during a two-year period, suggesting that the plaque healed in some of these patients. However, despite this median regression, we showed that patients with a baseline TRFC/disrupted plaque surface are still at increased risk for IPH progression. Together, this suggests that rupture of the fibrous cap may contribute to IPH. In contrast, patients with a thick FC/smooth plaque surface show hardly any IPH at baseline as well as at follow-up. Therefore, these patients have a more stable plaque phenotype over the two-year time period after the TIA or stroke.

Several studies have investigated the relationship between the volume of IPH and the risk of stroke [34, 35]. A previous cross-sectional study reported an association between the volume of carotid IPH and the presence of ipsilateral cerebrovascular (OR=2.5-2.9, $p<0.05$) events in 687 patients with carotid atherosclerotic plaque [35]. Presently, we showed that symptomatic patients with TRFC or a disrupted plaque surface have a larger median volume of IPH at baseline as well as at follow-up. In patients with a thick FC, with hardly any IPH at baseline, no volume changes in IPH were observed over two years of follow-up. In contrast, an overall decrease in IPH volume was observed in patients with a TRFC during this period, indicating that in some of these patients healing of the plaque seems to occur in the two years after the stroke. Similar findings were observed for patients with a smooth versus disrupted plaque surface. However, the median decrease in IPH volume in the patients with a disrupted plaque surface was not statistically significant, possibly due to the smaller sample size since not all patients were eligible for contrast-enhanced CTA.

Strikingly, despite the median decrease in IPH volume in the two years after the stroke in patients with a TRFC, we demonstrated that these patients are still at increased risk for IPH progression compared to patients with a thick fibrous cap. This finding, which may be contradictory, could be related to plaque healing in a subgroup of these patients in the two years after the TIA or stroke. An additional explorative analysis in the present study indicated that patients with a TRFC at baseline that healed during the follow-up period (i.e., thick at follow-up) had a lower probability of IPH progression and a higher probability of IPH regression than patients with a TRFC at baseline and at follow-up. The sample size of this explorative analysis was underpowered, so this should be further investigated in larger studies.

Our present study did not have the power to investigate whether patients with IPH progression are at increased risk for future stroke. An explorative analysis (supplementary data) indicated that patients with IPH progression may be at increased risk. Future studies with a larger sample size are warranted to investigate this further. Regardless of the volume of IPH, the presence of IPH in a carotid plaque increases the risk of stroke in symptomatic and asymptomatic patients with carotid artery stenosis and is a stronger predictor for stroke than any of the known clinical risk factors [6, 12]. In a recent consensus paper by an expert panel on vessel wall imaging biomarkers of carotid plaque vulnerability, it was concluded that IPH might be the most promising of all new imaging biomarkers [36]. However, the factors that contribute to IPH are incompletely understood. The results of the present study demonstrate that patients with TRFC are still at higher risk of having IPH after two years of follow-up. These new results complement Van Dijk et al. (21), who showed using baseline data from the PARISK study that IPH on MRI was positively associated with a disrupted plaque surface (ulceration and fissure) on CTA in both symptomatic and contralateral asymptomatic plaques.

We also explored to what extent the fibrous cap or plaque surface morphology changed during the two-year follow-up and whether these changes had an impact on IPH volume. In a previous study by Kwee et al, the status of IPH and the fibrous cap did not change in the majority of patients between baseline and one year of follow-up in symptomatic patients with <70% carotid stenosis (77% and 74%, respectively). Pletsch-Borba et al. studied changes in the incidence of IPH over a four-year follow-up period using serial MR imaging of 346 carotid arteries with a wall thickness ≥2.5 mm. They showed that the persistence of IPH during the follow-up period occurred in 64% of the patients. In conclusion, the presence of IPH and TRFC is not altered over time in most patients, which is in line with the findings of our present study. Interestingly, in addition to these findings, in our exploratory analysis, we also found that in patients with a thick fibrous cap or smooth plaque surface at both time points, the volume of IPH was small at baseline and follow-up, while the opposite was the case for patients with a TRFC or disrupted plaque surface at both time points. In patients where the fibrous cap status changed from thick to thin or the plaque surface changed from smooth to disrupted, the volume of IPH seemed to increase, and the opposite was the case when the status changed to thick/smooth. The probability of IPH progression was higher and the probability of regression was lower in the group of patients with a TRFC at both time points compared to patients where the TRFC healed and became thick after two years. However, possibly due to the small size of these subgroups, these findings were not statistically significant. In addition, future studies could investigate the relationship between FC status or plaque surface morphology and IPH volume change on the contralateral side. In the present study, as expected, we demonstrated a strong association between a TRFC on MRI and a disrupted plaque surface on CTA. It is important to keep in mind that the presence of a thin fibrous cap does not necessarily indicate that the plaque surface is disrupted and vice versa. These two parameters are not completely interchangeable.

In our study, we identified clinical risk factors that were different between groups to select those factors that needed to be included as potential confounders in the multivariate analysis. Type II diabetes mellitus (T2DM) was more prevalent in patients with a smooth plaque surface. However, only 25 patients had T2DM in our population, so the relationship between DM and plaque surface morphology is underpowered. In the future, the relationship between T2DM and plaque surface morphology could be explored in studies with a larger sample size.

A strength of our study was that instead of only investigating IPH presence and absence, we studied the volume of IPH over a period of two years. Another strength was investigating the status change of fibrous cap or plaque surface morphology over two years' follow-up and its relation with IPH volume change. In this way, we were able to obtain more insight into the relationship between TRFC and surface disruption and IPH in a longitudinal study. It has been suggested that some medications such as statins [37], angiotensin-converting enzyme inhibitors, calcium channel blockers [38], and antiplatelet agents [39], stabilize and slow the progression of vulnerable atherosclerotic plaques. Note that after a TIA or stroke, patients in the Netherlands receive optimized medical treatment to adjust the risk factors for long-term secondary prevention after stroke. In the present study, overall, no significant difference was observed between groups (thick vs TRFC or smooth surface vs disrupted). A limitation of our study is that we were unable to determine whether the carotid plaque was the culprit since our MRI examination did not include the aortic arch and the intracranial arteries. Another limitation is that we did not include dynamic contrast-enhanced MRI to investigate the relationship between plaque microvasculature and IPH volume change over time. Future studies are warranted to investigate whether there is a contribution of leaky plaque microvasculature to IPH development. In addition, scanners from two different manufacturers and two different MRI sequences to delineate IPH can also be considered a limitation. To mitigate this, repeated measurements at baseline and follow-up of each patient were performed on the same scanner using the same sequence. Moreover, we demonstrated very good interscan and intrareader reproducibility in a study in which patients were scanned twice within a mean of 4 days for the assessment of FC status (κ=1.00)[40]. The interobserver reproducibility for the assessment of the presence of ulcerations on 100 consecutive CTA images was also very good, as previously reported by us (κ=1.0; 95% CI, 0.86 to 1.00) [41]. In addition, the intraplatform, intrareader, and interreader agreement of quantifying carotid plaque composition, including intraplaque hemorrhage on different MR scanners from different manufacturers, is

strong (intraclass correlation coefficients: 0.83 to 0.99), as has been demonstrated by Saam et al. We did not use a quantitative threshold for defining hyperintensities within the carotid plaque. In the present study, the measurement error (coefficient of variation) for the IPH volume was 22%. Future studies could investigate whether such a threshold could reduce the measurement error.

In conclusion, in stroke patients with a TRFC and disrupted carotid plaque surface, with a larger median baseline IPH volume, the net decrease in IPH volume indicates a tendency towards healing in some of these patients over two years. Nevertheless, patients with a TRFC/disrupted plaque surface at baseline are still at increased risk for IPH progression compared to those with a thick fibrous cap/smooth plaque surface. As a result, patients with a TRFC/disrupted plaque surface may benefit from more frequent monitoring. Next, the predictive value of IPH progression for future events needs to be investigated in future studies.

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SUPPLEMENTAL FILE

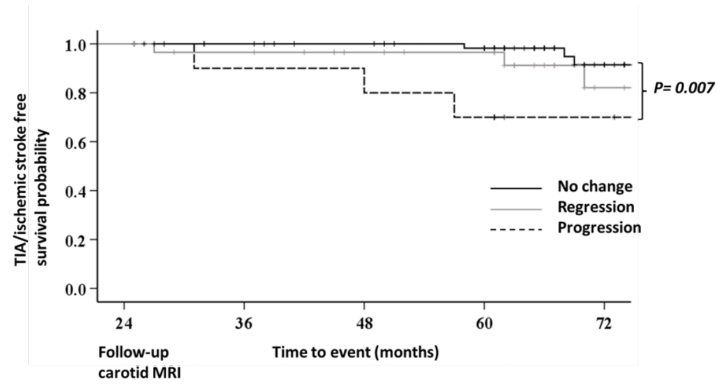
THE PREDICTIVE VALUE OF IPH PROGRESSION FOR RECURRENT ISCHEMIC EVENTS

Supplementary methods

In the 110 patients with baseline and follow-up carotid MRI, we analyzed how many (recurrent) cerebrovascular ischemic events occurred after the 2-year carotid MRI examination to investigate in an explorative analysis the predictive value of IPH progression for future ischemic cerebrovascular events. The endpoint was the composite of a clinical ipsilateral recurrent cerebral or monocular ischemic stroke or TIA. The cumulative incidence of the study endpoints over time was analyzed using the Kaplan-Meier method. The Cox proportional hazards ratio was calculated to determine the association of IPH progression with cerebrovascular ipsilateral ischemic events.

Supplementary results

After the two-year carotid MRI scan, the 110 patients were clinically followed-up for a mean period of 38±16 months. During this follow-up period, nine clinical endpoints occurred: 2 cerebral TIAs and 7 cerebral ischemic strokes. The explorative analysis of the Kaplan-Meier curves (Supplementary Figure 1) and the explorative Cox proportional hazard analysis showed that the cumulative incidence of the clinical endpoint was higher for patients with IPH progression in the ipsilateral carotid plaque than for patients without an IPH change (HR: 9.4 [95% CI: 1.8-47.3]; p=0.007).



Supplementary Figure 1.
Kaplan-Meier Curves of Event-Free Survival for progression, regression, and no volume change of IPH. The Kaplan-Meier curves show the survival probability after the follow-up carotid MRI examination for the clinical endpoint, defined as a clinical ipsilateral recurrent ischemic stroke or transient ischemic attack (TIA), for patients with intraplaque hemorrhage (IPH) progression (dashed line), regression (gray line), and no change (solid line).

Supplementary discussion

The explorative analysis indicates that patients with IPH progression may be at increased risk for future (recurrent) ipsilateral ischemic cerebrovascular events. However, our study had a small sample size, and only nine events occurred after the two-year carotid MRI, while typically, at least ten events are required for each degree of freedom. Therefore, the predictive value of IPH progression for ischemic cerebrovascular events needs to be investigated in future studies.

CHAPTER 4

The association between antiplatelet therapy and changes in intraplaque hemorrhage in patients with mild to moderate symptomatic carotid stenosis: a longitudinal MRI study

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ABSTRACT

INTRODUCTION

Carotid atherosclerotic intraplaque hemorrhage (IPH) predicts stroke. Patients with a history of stroke are treated with antiplatelet agents to prevent secondary cardiovascular events. A positive association between previous antiplatelet use and IPH was reported in a cross-sectional analysis. We investigated changes in IPH over two years in patients who recently started versus those with continued antiplatelet use.

METHODS

In the Plaque at Risk (PARISK) study, symptomatic patients with <70% ipsilateral carotid stenosis underwent carotid plaque MRI at baseline and after two years to determine IPH presence and volume. Participants were categorized into new users (starting antiplatelet therapy following the index event) and continued users (previous use of antiplatelet therapy before the index event). The association between previous antiplatelet therapy and the presence of IPH at baseline MRI was investigated using multivariable logistic regression analysis. IPH volume change over a period of two years, defined as the difference in volume between follow-up and baseline, was investigated in each group with a Wilcoxon signed-rank test. The IPH volume change was categorized as progression, regression, or no change. Using multivariable logistic regression, we investigated the association between new antiplatelet use and 1) newly developed ipsilateral or contralateral IPH and 2) IPH volume progression.

RESULTS

A total of 108 patients underwent carotid MRI at baseline and follow-up. At baseline, previous antiplatelet therapy was associated with any IPH (OR=5.6, 95% CI: 1.3–23.1; p=0.02). Ipsilateral IPH volume did not change significantly during the two years in patients who continued receiving antiplatelet agents (86.4 mm^3 [18.2-235.9] vs. 59.3 mm^3 [11.4-260.3]; p=0.6) nor in the new antiplatelet users (n=31) (61.5 mm^3 [0.0-166.9] vs. 27.7 mm^3 [9.5-106.4]; p=0.4). Similar results of a nonsignificant change in contralateral IPH volume during those two years were observed in both groups (p>0.05). No significant associations were found between new antiplatelet use and newly developed IPH at two years (odds ratio (OR)=1.0, 95% CI:0.1-7.4) or the progression of IPH (ipsilateral: OR=2.4, 95% CI:0.3-19.1; contralateral: OR=0.3, 95% CI:0.01-8.5).

CONCLUSION

Although the baseline association between IPH and previous antiplatelet therapy was confirmed in this larger cohort, the new onset of antiplatelet therapy after TIA/stroke was not associated with newly developed IPH or progression of IPH volume over the subsequent two years.

INTRODUCTION

Approximately 20% of ischemic strokes are caused by a vulnerable atherosclerotic carotid plaque rupture [1]. The term vulnerable plaque refers to rupture-prone plaques. Intraplaque hemorrhage (IPH) is an important feature of plaque vulnerability. A large meta-analysis showed that IPH is a strong and independent predictor for ipsilateral stroke [2-4]. Magnetic Resonance Imaging (MRI) is the optimal noninvasive imaging modality to determine the presence and volume of IPH [5].

As part of routine treatment, platelet aggregation inhibitors are prescribed to prevent further ischemic events in symptomatic patients [6]. However, although antiplatelet agents have a favorable effect in reducing thrombus formation, they also increase the risk of bleeding complications [7]. Previously, in a cross-sectional study, we reported an association between antiplatelet use before the index event and the presence of IPH in transient ischemic attack (TIA) and stroke patients with <70% symptomatic carotid stenosis [8]. We suggested that the use of antiplatelet medication might contribute to the formation of IPH, but since this was a cross-sectional study, causality could not be demonstrated. In the current longitudinal study, we hypothesize that patients who started antiplatelet therapy after the index event (new antiplatelet users) are at higher risk of developing new IPH and IPH progression over a two-year period than patients who already used antiplatelet agents before the index event.

MATERIALS AND METHODS

STUDY POPULATION

The baseline and follow-up MRI and clinical data from patients included in the PARISK study were analyzed (clinicaltrials.gov NCT1208025) [9]. Patients were eligible for inclusion when they had a recent (<3 months) cerebral or monocular TIA or minor ischemic stroke (modified Rankin scale ≤3) in the anterior circulation, as diagnosed by a neurologist through a comprehensive evaluation involving medical history, physical examination, and neuroimaging (CT or MRI), and an ipsilateral carotid plaque of at least 2-3 mm thick with <70% carotid artery stenosis (NASCET) and were not scheduled for carotid revascularization. Individuals suspected of having embolic origins from cardiac sources or patients with clotting disorders were excluded. Additionally, cases of hemorrhagic stroke were not eligible for inclusion. Approval from the local Institutional Ethical Review Board was obtained, and each patient gave written informed consent.

CLINICAL INFORMATION

At baseline, information was gathered regarding the index event, history of other

symptomatic arterial diseases, and cardiovascular risk factors. Medication prescribed to participants after the index event (baseline) and at two years (follow-up) was also collected. Patients who received antiplatelet therapy following the index event were classified as new antiplatelet users, whereas patients who had already used antiplatelet therapy before the index event were classified as continued users. In those patients, the duration of antiplatelet use prior to the index event was recorded.

MRI ACQUISITION AND ANALYSIS

Carotid MRI was scheduled at baseline and after two years in the first 150 patients of the PARISK study using 3.0 Tesla scanners with dedicated carotid coils [9]. Evaluation of the MR images was performed as previously described using dedicated vessel wall analysis software (VesselMass, Leiden University Medical Centre, Leiden, The Netherlands) [10]. IPH, defined as a hyperintense region in the bulk of the plaque compared to the surrounding muscle, was manually delineated on T1-weighted (T1w) inversion recovery turbo field echo (IR-TFE) images (three centers), also known as magnetization prepared-rapid gradient echo (MP-RAGE) or on spoiled gradient echo (SPGR) images (one center). Ipsilateral and contralateral IPH at baseline and after two years were delineated by independent, trained observers, who were blinded to the clinical data [11]. The follow-up MR images were delineated blinded to the baseline MRI.

STATISTICAL ANALYSIS

For all statistical analyses, SPSS version 25 (IBM Co., Armonk, NY, United States) was used. Differences in patient characteristics between groups were examined by a χ^2 test for categorical values and a Mann-Whitney U test or unpaired t-test for continuous values. A potential difference in the prevalence of IPH at baseline and follow-up was determined using a McNemar test. Any IPH was defined as the presence of IPH in the ipsilateral, contralateral or both carotid arteries. IPH is categorized as "new" if it is identified on a follow-up carotid MRI in patients who did not display IPH in that particular artery at baseline. The IPH volume change over a period of two years was investigated in each group with a Wilcoxon signed-rank test. A threshold for IPH progression or regression was defined by calculating the mean coefficient of variation (measurement error), defined as 100% * standard deviation (SD)/mean [12]. The coefficient of variation was calculated based on IPH volumes that were calculated by independent delineation of IPH by five trained readers. The measurement error for the IPH volume was 11%. Therefore, the progression of IPH was defined as a volumetric increase >11% or new IPH. Regression was defined as an IPH volume decrease of >11% or the disappearance of IPH. A change of ≤11% was classified as no change. Multivariable logistic regression analysis was used to evaluate the association between new antiplatelet therapy use and the presence of IPH at baseline or the progression in IPH volume at the end of follow-up (progression versus regression/no change) after adjusting for potential

confounders. Clinical confounders were selected based on biological plausibility [13, 14]. Therefore, based on our sample size and the biological effects, age, sex, smoking, hypercholesterolemia, history of cardiovascular disease (CVD), the type of baseline event (Amaurosis fugax, TIA or stroke) and time from index event to baseline MRI were considered potential confounders. The magnitude of the association was expressed as the odds ratio (OR) with a 95% confidence interval (CI).

RESULTS

Of the 150 patients who were invited to undergo baseline and follow-up carotid MRI, 108 patients were included for further analysis. Forty-two patients were excluded for different reasons, including anticoagulant use (=7), (supplemental Fig. 1). At baseline, 42 (39%) patients were receiving antiplatelet therapy prior to the index event, and they all continued to use antiplatelet therapy after the index event (continued usersreferred to as 'continued users'), while 66 (61%) patients started with antiplatelet treatment immediately following the index event (new antiplatelet users).referred to as 'new antiplatelet users'). Twenty-three of the 42 continued patients used antiplatelet therapy for a median period of 6 years (IQR: 3-12 years) before the index event. However, data regarding this period could not be retrieved for 19 patients. All 108 (100%) patients were still on antiplatelet therapy at the two-year follow-up.

BASELINE ANALYSIS

At baseline, IPH was present on the ipsilateral, contralateral, or bilateral side in 44/108 (41%), 12/108 (11%), or 6/108 (6%) of the patients, respectively. Patients with any IPH (ipsilateral, contralateral or both) were older, more often male, and more often used antiplatelet agents before the index event (27 (54%) vs. 15 (26%) p=0.003) (Table 1).

Table 1. Univariable analysis of clinical variables that are associated with the presence of any IPH at baseline.

Variable	All patients (N=108)	[a] IPH – N=58 (54%)	[a] IPH + N=50 (46%)	P-value
Age, years mean (± SD)	68 (±8)	66 (±9)	70 (±6)	**0.02**
Sex, male n (%)	76 (70%)	35 (60%)	41 (82%)	**0.01**
BMI, kg/m^2 median (IQR)	26 (24-28)	26 (23-28)	25 (24-27)	0.5
Currently smoking n (%)	23 (21%)	16 (28%)	7 (14%)	0.08
Diabetes mellitus n (%)	25 (23%)	12 (21%)	13 (26%)	0.5
Hypertension n (%)	61 (56%)	31 (53%)	30 (60%)	0.5
Hypercholesterolemia n (%) *	54 (50%)	24 (41%)	30 (60%)	0.08
Previous antiplatelet use n (%)	42 (39%)	15 (26%)	27 (54%)	**0.003**
Baseline statins use n (%)	52 (48%)	25 (43%)	27 (54%)	0.3

Table 1. Continued.

Variable	All patients (N=108)	[a] IPH – N=58 (54%)	[a] IPH + N=50 (46%)	P-value
Baseline antihypertensive Medication use n (%)	60 (55%)	29 (50%)	31 (62%)	0.2
Baseline antidiabetic medication use n (%)	20 (18%)	9 (15%)	11 (22%)	0.3
History of cardiovascular disease n (%)	44 (41%)	21 (36%)	23 (46%)	0.2
Type ischemic event, stroke n (%)	47 (44%)	22 (38%)	25 (50%)	0.2
Time index event to baseline MRI, days; median (IQR)	49 (34-65)	45 (33-62)	52(35-68)	0.2

*[a]Any intraplaque hemorrhage (IPH) at baseline MRI (ipsilateral, contralateral, or bilateral); *two missing data; IPH= intraplaque hemorrhage; SD= standard deviation; IQR= interquartile range; BMI= body mass index; MRI= magnetic resonance imaging*

In the multivariable analysis, after adjusting for covariates, only previous antiplatelet therapy was associated with any IPH at baseline MRI (OR=5.6, 95% CI: 1.3–23.1; p=0.02) (Table 2).

Table 2. Logistic regression analysis of variables associated with any IPH at baseline.

	Univariable, OR (95% CI)	p-value	Multivariable, *OR (95% CI)	p-value
Age	1.1 (1.0-1.1)	0.03	1.0 (1.0-1.1)	0.3
Sex	3.0 (1.2-7.3)	0.02	2.0 (0.7-5.4)	0.2
Smoking	2.3 (0.9-6.2)	0.09	1.5 (0.4-5.3)	0.5
Hypercholesterolemia	2.0 (0.9-4.3)	0.08	1.5 (0.7-11.1)	0.4
[a] Previous antiplatelet therapy	3.4 (1.5-7.5)	0.003	5.6 (1.3-23.1)	**0.02**
History of CVD	1.7 (0.8-3.8)	0.2	2.8 (0.7-11.1)	0.1
Time index event to baseline MRI	1.0 (1.0-1.0)	0.3	1.0 (1.0-1.0)	0.8

*[a] Before the index event; OR= odds ratio; CI= confidence interval; IPH= intraplaque hemorrhage and CVD= cardiovascular disease; MRI = magnetic resonance imaging; BMI= body mass index; DM= diabetes mellites. *Variables in the model: age, sex, current smoking, hypercholesterolemia, antiplatelet agent use, history of CVD and time index event to baseline MRI.*

THE ASSOCIATION BETWEEN NEW DEVELOPMENT OF IPH DURING TWO YEARS OF FOLLOW-UP AND NEW ANTIPLATELET USERS

The percentage of patients with IPH did not change significantly between baseline and two-year follow-up in the new antiplatelet users (ipsilateral: 32% vs. 39%; p=0.3, contralateral: 5% vs. 9%; p=0.4, respectively), nor did it change in the continued antiplatelet users (ipsilateral: 55% vs 57%; p=1.0, contralateral: 21% vs 29%; p=0.4, respectively). Fifteen and eight percent of new antiplatelet users developed new ipsilateral and contralateral IPH, respectively, while 9% and 9% of patients who were already using antiplatelets prior to the index event developed new ipsilateral and contralateral IPH, respectively, but these changes were not statistically significantly different (Table 3).

Univariable analysis showed no significant associations between the start of antiplatelet agent use and the formation of newly developed ipsilateral or contralateral IPH at the two-year follow-up (OR=2.1, 95% CI: 0.5-8.1; p=0.3). After correcting for potential confounders, the association between new antiplatelet use and newly developed IPH during two years of follow-up remained nonsignificant (OR=1.0, 95% CI: 0.1-7.4;

p=0.9). Furthermore, no significant associations were observed between age, sex, current smoking status, hypercholesterolemia, the history of cardiovascular diseases, the type of baseline event (stroke vs TIA/ Amaurosis fugax) and the time from index event to the baseline MRI and the development of new ipsilateral or contralateral IPH.

Table 3. Cross table between the status of antiplatelet agent use and changes in prevalence of IPH during two-year follow-up.

	Presence of ipsilateral IPH during two-year follow-up			
	IPH did not change	**New IPH developed**	**IPH disappeared**	**Total**
New antiplatelet user	51 (77%)	10 (15%)	5 (8%)	66
Continued antiplatelet user	35 (83%)	4 (9%)	3 (7%)	42
Total	86 (80%)	14 (13%)	8 (7%)	108
	Presence of contralateral IPH during two-year follow-up			
	IPH did not change	**New IPH developed**	**IPH disappeared**	**Total**
New antiplatelet user	59 (89%)	5 (8%)	2 (3%)	66
Continued antiplatelet user	37 (88%)	4 (9%)	1 (2%)	42
Total	96 (89%)	9 (8%)	3 (3%)	108

IPH= intraplaque hemorrhage

THE VOLUME CHANGE OF IPH

The IPH volumes in the 58 ipsilateral and 20 contralateral arteries that showed IPH at baseline, at follow-up, or both were analyzed. The median ipsilateral IPH volume did not change after two years of follow-up in patients who continued receiving antiplatelets therapy (n=27) (86.4 mm^3 [18.2-235.9] vs. 59.3 mm^3 [11.4-260.3]; p=0.6) nor in the new antiplatelet users (n=31) (61.5 mm^3 [0.0-166.9] vs. 27.7 mm^3 [9.5-106.4]; p=0.4). Similar results of a nonsignificant change in contralateral IPH volume during those two years were observed in the continued antiplatelet therapy (n=12) (26.6 mm^3 [1.0-111.8] vs. 12.8 mm^3 [6.0-144.4]; p=0.8), as well as in the new antiplatelet users (n=8) (0.0 mm^3 [0.0-8.6] vs. 11.8 mm^3 [1.4-77.0]; p=0.2) (Fig. 1). Nevertheless, even though the overall median volume did not significantly change, 26 out of 58 (45%) ipsilateral carotid arteries and 12 out of 20 (60%) contralateral carotid arteries showed progression of IPH volume (defined as volumetric increase>11% or new IPH) after two years (Table 4 and Fig 2). While 29 out of 58 (52%) on the ipsilateral side and 6 out of 20 (30%) on the contralateral side exhibited regression.

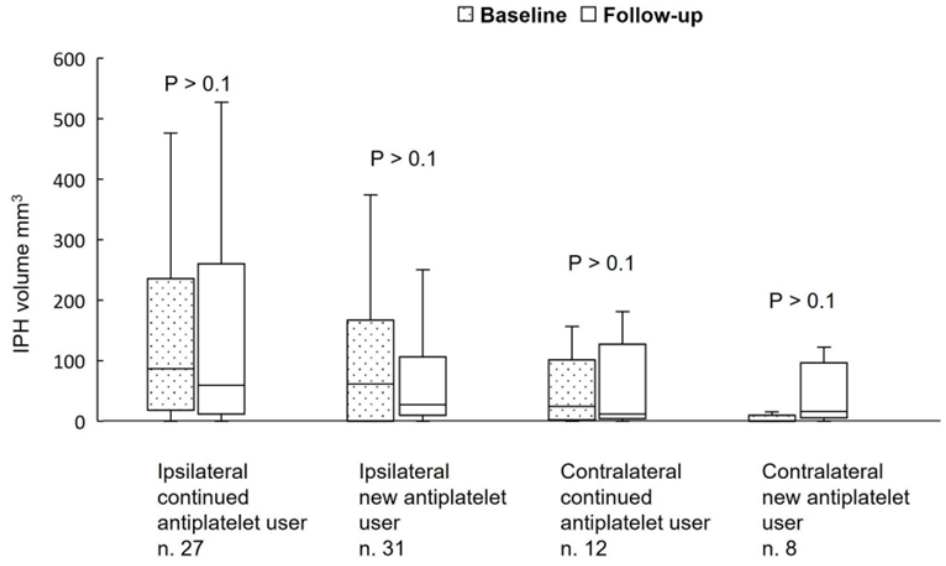

Figure 1. *The volume of intraplaque hemorrhage (IPH) at baseline and after the two-year follow-up was categorized by using antiplatelet therapy before the index event.*

Table 4. The prevalence of IPH progression in relation to antiplatelet therapy after the index event in patients with IPH at baseline, at follow-up or at both time points.

	Ipsilateral IPH during two-year follow-up			
	Progression	**Regression**	**No change**	**Total**
New antiplatelet user	15 (48%)	15 (48%)	1 (3%)	31
Continued antiplatelet user	11 (41%)	15 (56%)	1 (4%)	27
Total	26 (45%)	29 (52%)	2 (3%)	58
	Contralateral IPH during two-year follow-up			
	Progression	**Regression**	**No change**	**Total**
New antiplatelet user	6 (75%)	1 (13%)	1 (13%)	8
Continued antiplatelet user	6 (50%)	5 (42%)	1 (8%)	12
Total	12 (60%)	6 (30%)	2 (10%)	20

IPH= intraplaque hemorrhage

No association was found between new antiplatelet use after the index event and the progression of ipsilateral or contralateral IPH volume after two years (OR=1.4, 95% CI: 0.5-3.9; p=0.6 and OR=1.2, 95% CI: 0.2-7.5; p=0.8, respectively). In the multivariable analysis, there was also no significant association between starting antiplatelet therapy after the index event and IPH volume progression on the ipsilateral (OR=2.4, 95% CI: 0.3-19.1; p=0.4) or on the contralateral side after two years (OR=0.3, 95% CI: 0.01-8.5; p=0.5). Furthermore, there was no significant association between age, sex, current

smoking status, hypercholesterolemia, the history of cardiovascular diseases, the type of baseline event (stroke vs TIA/ Amaurosis fugax) and the time from index event to the baseline MRI and the progression of ipsilateral and contralateral IPH after two years follow-up.

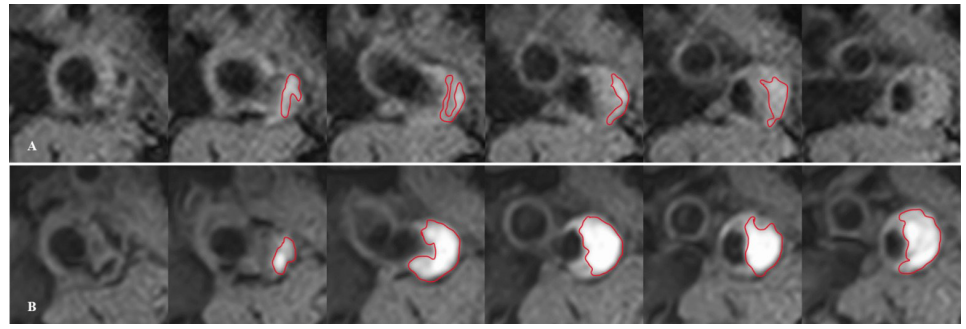

Figure 2. *IPH progression after two 2 years' follow-up in a new antiplatelet user. 3D T1-weighted inversion recovery turbo field echo (IR-TFE) images of carotid atherosclerotic plaque with IPH at baseline (A) and follow-up (B). IPH volume (red contours) increased from 121.0 mm³ at baseline to 250.6 mm3 after 2 years of follow-up (107% increase).*
IPH= intraplaque hemorrhage

DISCUSSION

In the present study, we reported on longitudinal changes in IPH prevalence and volume in relation to continued versus new antiplatelet agent use. Our study confirmed the association between the presence of IPH at baseline and previous use of antiplatelet therapy in symptomatic patients with ipsilateral nonsignificant (<70%) carotid artery stenosis. However, the frequency and volume of IPH did not increase during the two years of follow-up in the new antiplatelet users or in the continued users. Additionally, although we showed a slightly higher prevalence of new IPH in the new antiplatelet users, no significant association was found between new antiplatelet agent use and the formation of new IPH or IPH volume progression during the two-year follow-up.

Most of the previous studies focused on a cross-sectional analysis on the association between IPH and antiplatelet use. A histopathological study showed a significantly higher amount of multiple carotid IPH in 154 endarterectomy specimens from patients who used antiplatelet therapy (80.1% vs. 19.7%; p<0.001) [15]. Another histopathology study failed to identify an association between antiplatelet use and the prevalence of IPH in 1070 patients [16]. Current and past use of antiplatelet agents was (non-significantly) associated with a higher prevalence of carotid IPH in a subpopulation

of the Rotterdam Study with a carotid plaque larger than 2.5 mm in at least one of the carotid arteries [17]. Furthermore, a recent study in 34 symptomatic patients with carotid IPH and at least six months of follow-up showed that atherosclerotic plaques were significantly more likely to be in the progressed IPH group if the patient used antiplatelet therapy at baseline (86.7 vs. 53.3%, p=0.046) [19]. In that study, the use of antiplatelet therapy at follow-up was not reported. In our study, all patients were on antiplatelet therapy during the follow-up period.

Although the baseline association between IPH and previous antiplatelet therapy was confirmed in our study, against our hypothesis, the new onset of antiplatelet therapy after TIA/stroke was not associated with newly developed IPH or progression of IPH volume over the subsequent two years. Possibly, the baseline association between IPH and previous antiplatelet therapy is affected by the fact that previous antiplatelet users may have had a more severe disease stage, since usually antiplatelet therapy is prescribed in high risk patients, although we did correct for previous cardiovascular disease in the multivariable analysis.

Another explanation may be that the present study lacks a control group consisting of patients who did not use antiplatelet agents at follow-up. The current clinical guideline states that the prescription of antiplatelet therapy after stroke has to be continued for a lifetime; therefore, we could not include such a control group.Our Following a stroke, a significant number of patients previously treated with a single antiplatelet agent often have a transition to dual antiplatelet therapy, typically involving both aspirin and clopidogrel, for a few weeks. Nevertheless, in this current study, we opted not to distinguish between mono- and dual therapy among continued users due to the limited sample size. Moreover, in the present study, the follow-up MRI was performed after 2 years and we cannot exclude that this period may not have been long enough to show an association between new onset of antiplatelet therapy and new IPH or progression of IPH. In the previous cross-sectional studies that demonstrated an association between antiplatelet use and IPH, the median interval between the initiation of antithrombotic medication and the subsequent MRI was 6 years (with a range of 1-28 years) and 72 months (interquartile range: 30-123 months), respectively [8, 17]. One study reported no association between the duration of antiplatelet therapy and IPH (p=0.94) [8], while in the other study, a longer duration of the use for antiplatelet agents showed a positive, but statistically non-significant, trend of IPH (OR: 1.21, 95% CI: 0.88–1.67) [17].

Finally, our our population was limited to patients with ipsilateral carotid artery stenosis <70% who were not scheduled for revascularization surgery or stenting. Symptomatic patients with severe stenosis (>70%) will be operated on in the Netherlands, which

means follow-up of the plaque and IPH is not possible. Future studies could be performed in asymptomatic individuals with and without IPH in the carotid plaque. If that group consists of both subjects using antiplatelet agents and subjects who do not use antiplatelet agents, the relationship between (no) use of antiplatelet agents and the presence/volume of IPH can be studied.

CONCLUSION

Stroke patients with mild to moderate carotid artery stenosis who previously used antiplatelet therapy show a significantly higher prevalence of IPH at baseline. However, no association was found between starting antiplatelet agents and either newly developed IPH or progression of IPH volume during two years of follow-up. Additional studies are warranted to further investigate the relationship between antiplatelet agent use and IPH progression in asymptomatic individuals with carotid stenosis.

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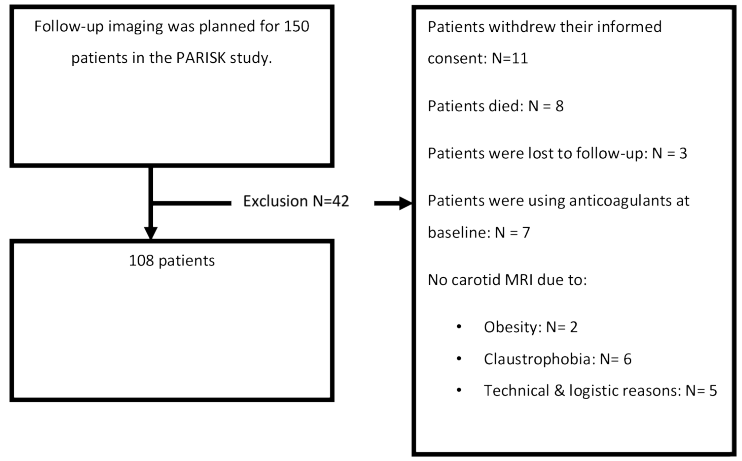
SUPPLEMENTARY FILE

Supplementary Figure 1. Flow chart of the study



CHAPTER 5

Application of mask images of contrast-enhanced MR angiography to detect carotid intraplaque hemorrhage in patients with moderate to severe symptomatic and asymptomatic carotid stenosis

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ABSTRACT

Purpose

Carotid intraplaque hemorrhage (IPH) on MRI predicts stroke. Magnetization-prepared rapid acquisition gradient (MP-RAGE) is widely used to detect IPH. CE-MRA is used routinely to assess stenosis. Initial studies indicated that IPH can be identified on mask images of CE-MRA, while Time-of-Flight (TOF) images were reported to have high specificity but lower sensitivity. We investigated the diagnostic accuracy of detecting IPH on mask images of CE-MRA and TOF.

Methods

Thirty-six patients with ≥50% stenosis enrolled in the ongoing 2nd European Carotid Surgery Trial underwent carotid MRI. A 5-point quality score was used. Inter-observer agreement between two independent readers was determined. The sensitivity and specificity of IPH detection on mask MRA and TOF were calculated with MP-RAGE as a reference standard.

Results

Of the 36 patients included in the current analysis, 66/72 carotid arteries could be scored. The inter-observer agreements for identifying IPH on MP-RAGE, mask, and TOF were outstanding (κ: 0.93, 0.96, and 0.85). The image quality of mask (1.42±0.66) and TOF (2.42±0.66) was significantly lower than MP-RAGE (3.47±0.61). When T1w images were used to delineate the outer carotid wall, very high specificities (>95%) of IPH detection on mask and TOF images were found, while the sensitivity was high for mask images (>81%) and poor for TOF (50-60%). Without these images, the specificity was still high (>97%), while the sensitivity reduced to 62-71%.

Conclusion

Despite the lower image quality, routinely acquired mask images from CE-MRA, but not TOF, can be used as an alternative to MP-RAGE images to visualize IPH.

Keywords

Intraplaque hemorrhage; carotid, CE-MRA, mask, MP-RAGE, stroke

INTRODUCTION

It has been estimated that about 15% of TIAs or ischemic strokes are related to carotid atherosclerotic plaques [1]. Currently, clinical decisions concerning managing patients with carotid artery plaques are still mainly based on degree of internal carotid artery stenosis [2].

Recently, it has been recognized that plaque composition and specifically intraplaque hemorrhage (IPH) detected using Magnetic Resonance Imaging (MRI) are important predictors of patients at high risk of stroke [3-7]. Schindler et al showed that IPH has a strong predictive value for ipsilateral ischemic stroke in symptomatic patients (hazard ratio : 10.2, 95% confidence interval (CI): 4.6-22.5) and asymptomatic patients (hazard ratio: 7.9, 95% CI: 1.3-47.6) [3]. However, additional specialized advanced MR sequences are used to identify IPH. Most commonly a magnetization-prepared rapid acquisition gradient (MP-RAGE) sequence, also known as T1w "inversion recovery turbo-field echo" (IR-TFE) is used for this purpose. MP-RAGE is well validated to visualize IPH with high sensitivity and specificity (80% and 97%, respectively) [8] and high inter-observer agreement (κ=0.73, 95% CI=0.53-0.92) [9]. However, in most centers, additional MR sequences beyond the contrast-enhanced MR angiography (CE-MRA) to measure degree of stenosis are not acquired during a routine carotid MRA examination, mostly because of time constraints and because they are currently not included in imaging guidelines.

CE-MRA is a standard element of a carotid MRI examination [10]. A Time-of-flight (TOF) MRA is also acquired in most centers during carotid MRI examination. Due to the magnetic properties of blood products, IPH can be recognized as an area of high signal intensity within the bulk of the plaque on CE-MRA and TOF [11]. The usefulness of pre-contrast source images of CE-MRA (mask images) to identify high signal intensity in the vessel wall, to determine IPH was shown in a case report [12]. Later, , the sensitivity and specificity to detect IPH on mask images was shown to be 87% and 99% and on TOF images it was 79% and 87% in a single center study of 15 patients [11].

The objective of the present study is to determine the diagnostic accuracy of identifying IPH using mask images from CE-MRA and TOF images in patients with ≥ 50% carotid stenosis by using MP-RAGE as reference standard in a multi-center study.

MATERIAL AND METHODS

STUDY POPULATION

This study involved patients enrolled at participating centers in the ongoing 2nd European Carotid Surgery Trial (ECST-2; ISRCTN 97744893). Eligible patients were adult (>18 years) with symptomatic or asymptomatic atherosclerotic carotid artery stenosis (> 50%, NASCET criteria), a 5-year risk of ipsilateral stroke < 20% estimated by the Carotid Artery Risk score. The score predicts the 5-year risk of ipsilateral stroke on the side of the stenotic carotid artery in patients treated with optimized medical therapy alone. The model was derived from the results of a Cox regression model in ECST-1 and validated in the NASCET trial [13]. In the current analysis, we included only patients that underwent a baseline carotid MRI examination including an MP-RAGE, TOF and CE-MRA (with mask images available) sequences. Ethical and NHS approval from the National Research Ethics Service in the UK after review by the NRES Committee East of England, Cambridge Central was obtained. Outside of UK, approval was obtained from the local medical ethical committees. Written informed consent was obtained from all subjects. The study protocol conforms to the ethical guidelines of Declaration of Helsinki.

CAROTID MRI ACQUISITION AND IMAGE ANALYSIS

The carotid MR imaging examinations were carried out on 3T MRI systems (Achieva; Philips Healthcare, The Netherlands or Prisma; Siemens Healthineers, Erlangen, Germany) using a neurovascular or dedicated carotid radio-frequency coil. The parameters of the carotid MR sequences are presented in Table 1.

For the CE-MRA series, a 3D fast field echo fast field echo sequence was acquired before (i.e., the mask images) and after intravenous injection of 0.1 mmol/kg body weight of gadobutrol (Gadovist, Bayer Schering, Leverkusen, Germany)).

Table 1. Scan parameters of the carotid MRI examination

Pulse sequence	CE-MRA				MP-RAGE				TOF				T1w TSE	T1w BLADE
Center	1	2	3	4	1	2	3	4	1	2	3	4	1 2 3	4
Vendor	Philips			Siemens	Philips			Siemens	Philips			Siemens	Philips	Siemens
Scanner type	Achieva			Prisma	Achieva			Prisma	Achieva					Prisma
Acquisition format	3D				3D				3D				2D	2D
Acquisition plane	Coronal				Coronal				Transversal				Transversal	
Sequence name	T1-FFE	T1-FFE	T1-FFE	FLASH	T1-FFE	T1-FFE	T1-FFE	MP-RAGE	T1-FFE	T1-FFE	T1-FFE	T1-TFE	TSE	BLADE
TR (ms)	4.6	5.1	4.6	3.0	15	9.1	15	12	224	24	24	24	800	1500
TE (ms)	1.6	1.6	1.7	1.1	4.8	5.5	4.8	5.3	4.6	4.6	4.6	4.6	10	53
TI (ms)	NA	NA	NA	NA	500	304	NA	500	NA	NA	NA	NA	282, 61	659
Flip angle (°)	27	30	27	22	15	15	15	15	20	20	20	20	90	60
No. of slices	150	150	150	88	75	80	82	128	75	30	75	22	15	18
Slice thickness (mm)	1.0	0.8	1.0	0.9	0.6	0.6	0.6	0.6	2.0	2.0	2.0	1.0	2.0	2.0
FOV (mm)	320x320	360x360	320x320	297x340	160x160	160x160	160x160	160x160	180x180	160x160	180x180	160x145	160x160	160x160
Acquisition matrix	508x 508	348x346	508x508	210x320	228x228	268x266	268x2.68	256x256	300x300	268x266	300x300	256x243	260x256	320x320
Acquired voxel size	0.6x0.6	1.0x1.0	0.6x0.6	1.1x1.4	0.7x0.7	0.6x0.6	0.6x0.6	0.6x0.6	0.6x0.6	0.6x0.6	0.6x0.6	0.6x0.6	0.6x0.6	0.5x0.5
Reconstruction matrix	640x640	768x768	640x640	512x512	288x288	560x560	288x288	256x256	320x320	528x528	320x320	256x243	528x528	320x320
Reconstructed voxel size	0.5x0.5	0.5x0.5	0.5x0.5	0.6x0.7	0.5x0.5	0.3x0.3	0.5x0.5	0.6x0.6	0.6x0.6	0.3x0.3	0.6x0.6	0.6x0.6	0.3x0.3	0.5x0.5
Echo train length	NA	NA	NA	NA	26	30	27	64	NA	NA	NA	NA	10	9
Parallel imaging	Yes	Yes	Yes	Yes	2	No	2	No	2	No	2	Yes	No	Yes
No. of signal averages	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Fat suppression	No	No	No	No	Yes	Yes	Yes	Yes	No	No	No	No	Yes	Yes

1: UCL, 2: Maastricht, 3: Verona, 4: Basel. CE-MRA, contrast-enhanced MR angiography; MP-RAGE magnetization-prepared rapid acquisition gradient; TOF, time of flight; FFE, fast field echo; FLASH, fast low angle shot; IR-TFE, inversion recovery turbo field echo; TSE, turbo spin echo; TR, repetition time; TE, echo time; TI, inversion time; NA, not applicable; FOV, field of view.

Axial reconstructed images of the mask, MP-RAGE, and TOF images were anonymized and scored by the two trained observers (MK and SdK) independently of each other and blinded to their scores of the other sequences. A minimal one-month period between scoring IPH on each MRI weighting was set to minimize prior knowledge. Dedicated software (Vesselmass) was used for MR image analysis. The presence of IPH was defined as high signal intensity within the bulk of the plaque compared with the adjacent muscle tissue. The presence of IPH on mask images was scored two times independently with and without the help of black blood T1 weighted images with a minimal two-months period between these two sessions. In the first session the mask images of the CE-MRA and the TOF and MP-RAGE images were co-registered with pre-contrast/post-contrast 2D T1 weighted (T1w) double/quadruple inversion recovery turbo spin echo (TSE) images or any other black blood sequence such as blade. If the automated co-registration was not perfect, then the images were manually aligned. The luminal and outer vessel wall were defined on T1w images in this first session. In the second session IPH was scored without the help of T1w images. In this case, the lumen of the carotid artery was delineated on the post-contrast MRA. However, the outer vessel wall is difficult to observe only on CE-MRA. Therefore, any hyperintense signal surrounding the lumen at the side of the bifurcation compared with the adjacent sternocleidomastoid was considered to be IPH. A 4-poinst certainty score was used (4: very certain and 1: uncertain). In addition, a 5--point image quality score was used (5 high, 1 low) [14]. For an exploratory analysis, IPH volume was quantified on MP-RAGE images by delineating the region with hyperintense signal in the bulk of the plaque on each IPH-positive slice.

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STATISTICAL ANALYSIS

All analyses were performed with a dedicated statistical package (IBM SPSS statistics version 26). The Cohen κ coefficient was calculated to evaluate the inter-observer agreement on scoring IPH on MP-RAGE, mask and TOF images. $\kappa < 0.4$ is considered poor agreement, 0.4 to 0.75 is fair to good and $\kappa > 0.75$ is excellent [15]. The sensitivity and specificity of IPH detection on mask MRA and TOF were calculated with MP-RAGE as reference standard and were expressed in percentages with 95% confidence intervals. The Mann-Whitney test was used to compare the median volume of IPH between the true positive and false negative cases.

RESULTS

217 patients out of 455 patients underwent baseline carotid MRI within 2nd European Carotid Surgery Trial (ECST-2). CE-MRA with mask images available, MP-RAGE (IR-TFE) and TOF have been acquired in 36 patients (72 carotid arteries). Six arteries were excluded due to motion artifacts on MP-RAGE (two arteries) or location of the plaque

was located outside the field of view (four arteries). Finally, the data of 66 arteries were eligible for final analysis. The image quality of mask (1.42±0.66) and TOF (2.42±0.66) was significantly lower ($p<0.05$) than MP-RAGE (3.47±0.61).

SCORING IPH ON MASK IMAGES OF CE-MRA WITH THE AID OF HIGH RESOLUTION BLACK BLOOD T1W IMAGES

The inter-observer agreements for identifying IPH on MP-RAGE, mask and TOF with the aid of black blood high resolution T1w images were excellent (κ: 0.93, 0.96 and 0.85, respectively). There were two, one and three disagreement cases of IPH scores on MP-RAGE, mask and TOF, respectively (Table 2).

CE-MRA mask showed very high specificities (97.8% [95%CI: 88.2 to 99.9%] and 97.9% [85%CI 88.7 to 99.9%]) and high sensitivity (81.0% [95%CI: 58.1 to 94.5%] and 84.2% [95%CI: 60.4% to 96.6]) of identifying IPH using MP-RAGE as reference standard by reader 1 and reader 2, respectively. However, TOF demonstrated high specificity (95.6% [95%CI: 84.8% to 99.5] and 97.9% [95%CI: 88.7 to 99.9%]) and poor sensitivity (50.0% [95%CI: 27.2 to 72.8%] and 57.9% [95%CI: 33.5 to 79.7%]) by reader 1 and 2, respectively. False positive and negative results for both readers are presented in Table 3. Examples of true and false negative findings are shown in Figure 1 and 2.

The exploratory analysis demonstrated that the median IPH volume of the true positive cases (tended) to be significantly larger than the false negative cases (0.18 ml [interquartile range (IQR) 0.10-0.27] vs. 0.02 ml [0.003-0.05], p=0.001 for reader 1; 0.18 ml [0.09-0.27] vs. 0.05 ml [0.003-0.16], p=0.06 for reader 2).

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Tabel 2. Level of Agreement on IPH detection between two observers for the three different sequences

CE-MRA mask	**Reader 1**		**Total**
Reader 2	IPH -	IPH +	
IPH -	48	1	49
IPH +	0	17	17
Total	48	18	66
MP-RAGE	**Reader 1**		**Total**
Reader 2	IPH -	IPH +	
IPH -	45	2	47
IPH +	0	19	19
Total	45	21	66
TOF	**Reader 1**		**Total**
Reader 2	IPH -	IPH +	
IPH -	52	2	54
IPH +	1	11	12
Total	53	13	66

CE_MRA, contrast-enhanced MR angiography; IPH, intraplaque hemorrhage, MP-RAGE, magnetization-prepared rapid acquisition gradient; TOF, time-of-flight.

Table 3. Sensitivity and specificity of identification of IPH on the mask images of CE-MRA and on the TOF images using MP-RAGE as reference standard and using black blood T1w images to define the outer vessel wall

CE-MRA mask	**MP-RAGE**		
	Reader 1	IPH -	IPH +
	IPH -	44 (97.8%)	4
	IPH +	1	17 (81.0%)
TOF	**MP-RAGE**		
	Reader 1	IPH -	IPH +
	IPH -	43 (95.6%)	10
	IPH +	2	10 (50.0%)
CE-MRA mask	**MP-RAGE**		
	Reader 2	IPH -	IPH +
	IPH -	46 (97.9%)	3
	IPH +	1	16 (84.2%)
TOF	**MP-RAGE**		
	Reader 2	IPH -	IPH +
	IPH -	46 (97.9%)	8
	IPH +	1	11 (57.9%)

CE_MRA, contrast-enhanced MR angiography; IPH, intraplaque hemorrhage, MP-RAGE, magnetization-prepared rapid acquisition gradient; TOF, time-of-flight

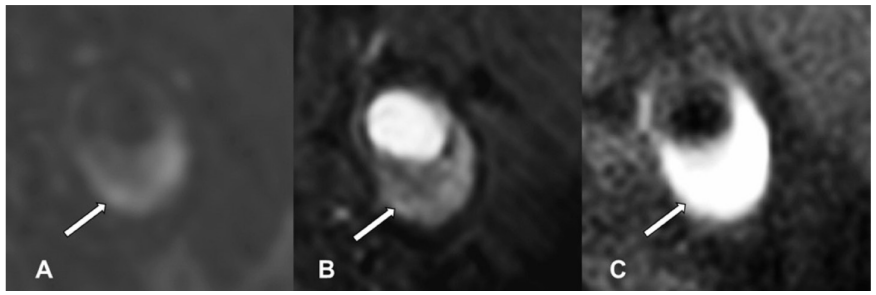

Figure 1. *An example of a true positive finding. IPH is shown as a region within the bulk of the plaque with high signal intensity (arrow) on all three MRI weightings: A) contrast-enhanced MR angiography (CE-MRA) mask image, and B) Time-Of-Flight (TOF) and C) Magnetization Prepared-Rapid Acquisition Gradient Echo (MP-RAGE) image.*

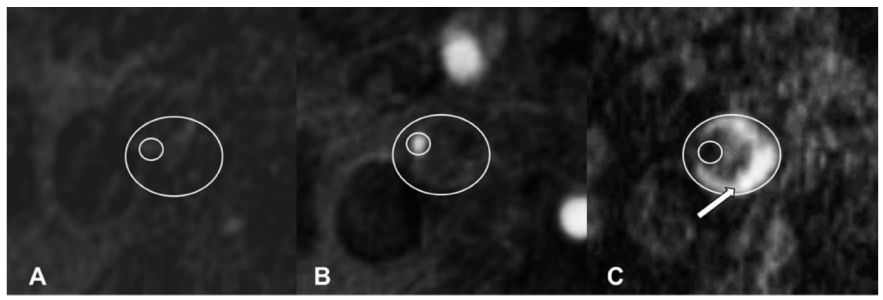

Figure 2. *An example of a false negative finding. IPH is not shown on the A) contrast-enhanced MR angiography (CE-MRA) mask image and B) time-of-flight (TOF) image, while IPH is clearly seen as region within the bulk of the plaque with high signal intensity (arrow) on the C) Magnetization-Prepared Rapid Acquisition Gradient Echo (MP-RAGE) image. White boundaries indicate inner (lumen) and outer carotid wall.*

SCORING IPH ON MASK IMAGES OF CE-MRA WITHOUT THE AID OF BLACK BLOOD T1W IMAGES

The intra-observer agreement of IPH detection on mask images when black blood T1w was not used was lower (κ =0.88 and κ =0.87) for reader 1 and 2, respectively. When we excluded cases with a low certainty score (≤2), the intra-observer agreement increased (κ =0.92 and κ =0.91) for reader 1 and 2, respectively. The inter-observer agreement between two readers of scoring IPH on mask images without using T1w TSE images was still excellent (κ =0.87). The specificities of scoring IPH on mask images without the black blood T1w images using MP-RAGE as reference standard were 100% [95%CI: 92.1 to 100%] and 97.8% [95% CI: 88.4 to 99.9%], while the sensitivities were 71.4% [47.8 to 88.7%] and 61.9% [95%CI: 38.4 to 81.9%] for reader 1 and reader 2, respectively (Table 4). When cases with certainty scores ≤2 were excluded, the specificity and sensitivity became 100 [95%CI: 92.3 to 100%] % and 72.2% [95%CI: 46.5 to 90%] for reader 2, while the results of reader 1 remained unchanged.

Table 4. Sensitivity and specificity of identification of IPH on the mask images of CE-MRA using MP-RAGE as reference standard without using black blood T1w images to define the outer wall

	MP-RAGE		
	Reader 1	IPH -	IPH +
	IPH -	45 (100%)	6
	IPH +	0	15 (71.4%)
CE-MRA mask without T1w	**MP-RAGE**		
	Reader 2	IPH -	IPH +
	IPH -	44 (97.8%)	8
	IPH +	1	13 (61.9%)

CE_MRA, contrast-enhanced MR angiography; IPH, intraplaque hemorrhage, MP-RAGE, magnetization-prepared rapid acquisition gradient.

DISCUSSION

We demonstrated that there is an excellent inter-observer agreement for identification of IPH on mask MRA and TOF images. Moreover, we showed very high specificities of IPH detection on mask and TOF images, while the sensitivity was high for mask but poor for TOF when observers were allowed to use black blood T1w TSE MR images to detect the outer vessel wall. The specificity and sensitivity of scoring IPH on mask images without the aid of these black blood T1w images was high and moderate, respectively.

Numerous studies have established that IPH on MRI is a strong predictor of stroke and is therefore of high relevance for the identification of a subgroup of patients with an increased risk for stroke[3, 7]. Therefore, during the past decades, there has been an

increasing interest in MRI sequences to visualize IPH [16]. In 2003, Moody discovered that MP-RAGE was capable of detecting IPH [17]. Compared to two-dimensional T_1-weighted fast spin echo and 3D TOF, Ota et al demonstrated that MP-RAGE is the optimal T1w sequence to detect the presence of IPH as it has the highest specificity and sensitivity (97% and 80%, respectively) [8]. In line, Cappendijk *et al* showed a high detection rate (81-93%) for IPH on MP-RAGE images and less (72-91%) on T1w TSE images using histology as reference standard [9]. Recently, expert consensus recommendations on vessel wall MR imaging protocol stated that MP-RAGE is well suited to detect IPH [6, 18].

Our results are in line with earlier findings by Qiao *et al* [11]. They showed in 15 patients a specificity of 99% and sensitivity of 87% when IPH was identified on mask images using histology as a reference standard. Also, the inter-observer agreement for IPH detection on mask images (κ=0.91) was comparable to the present study. On TOF images, these authors showed a high specificity and sensitivity of detecting IPH (87% and 79%) using histology as reference standard. However, in our study we confirmed the high specificity but found a lower sensitivity of scoring IPH on TOF when MP-RAGE was used as reference standard.

We showed that the sensitivity of detecting IPH on mask images became lower when the black blood sequence was not used to visualize the outer vessel wall, while the specificity remained very high. Distinction of the outer wall could be challenging on mask images since no fat suppression was applied. Therefore, small high signal intensity regions on mask images can be erroneously identified as perivascular tissue. We also observed a few false negative cases. Since the false negative cases showed a smaller median IPH volume than the true positive ones, this was probably due to the lower spatial resolution of mask images compared to the MP-RAGE images which can limit the ability to visualize small regions of IPH. Two false positive cases on TOF images were associated with ulceration that also appears as a high signal intensity region on TOF images.

More recently, Simultaneous Non-contrast Angiography and intraplaque hemorrhage (SNAP) MR imaging was proposed to detect both luminal stenosis and hemorrhage in patients with carotid plaques with a single sequence [19]. Strong agreement (κ=0.82, $p<0.001$) was identified between SNAP and MP-RAGE images for detecting IPH. Also, fast simultaneous non-contrast angiography and intraplaque hemorrhage (fSNAP) performs similar to SNAP but with 37.5% less scan time [20]. In addition, simultaneous T_1 and T_2 mapping of carotid plaque (SIMPLE) [21] and golden angle radial k-space sampling (GOAL-SNAP) were proposed to score IPH [22]. Moreover, MATCH was developed to image IPH and other vulnerable plaque components using a single multi-contrast sequence [23]. However, most of these sequences are not available in the standard MR system configuration. Software patches or work in progress (WIP) packages are required to use these sequences on clinical MR systems.

In general, high-resolution MRI is sensitive to patient motion. Therefore, we recommend to fixate the head of the patient by using cushions and soft belts. Swallowing artefacts can be reduced by positioning a regional saturation slab over the pharynx. In addition, the MP-RAGE sequence contains a non-selective inversion pre-pulse. However, this non-selective inversion pre-pulse is limited to the field-of-view of the body coil. Therefore, incomplete blood suppression can occur especially in tall patients due to inflow of incompletely suppressed blood. This can be prevented by positioning the patient slightly off-center towards the feet direction.

A limitation of our study is the lack of histological validation. Patients were randomized for optimized medical treatment or revascularization in ECST-2. Storage of the surgery specimen and histological analysis was not part of the study design. Alternatively, we have used MP-RAGE as a reference standard, which has been validated with histology in numerous previous studies [9, 17]. Another limitation is that we validated the use of the mask images in asymptomatic and symptomatic patients with moderate to severe stenosis with a 5-year risk of ipsilateral stroke < 20% estimated by the Carotid Artery Risk score. Future studies need to investigate whether intraplaque hemorrhage on mask images can also be detected in patients with mild carotid stenosis or a non-stenotic carotid plaque. The strength of the present study is the larger sample size compared to that of the previous study [11].

CONCLUSION

In addition to measuring the degree of stenosis using only the contrast-enhanced images, our results showed that the mask images of CE-MRA can also be used to score the presence of IPH. Using an additional black blood sequence to visualize the outer vessel wall can further improve the accuracy. Scoring IPH during the routine assessment of CE-MRA can provide additional information about the risk of stroke and could contribute to treatment decisions.

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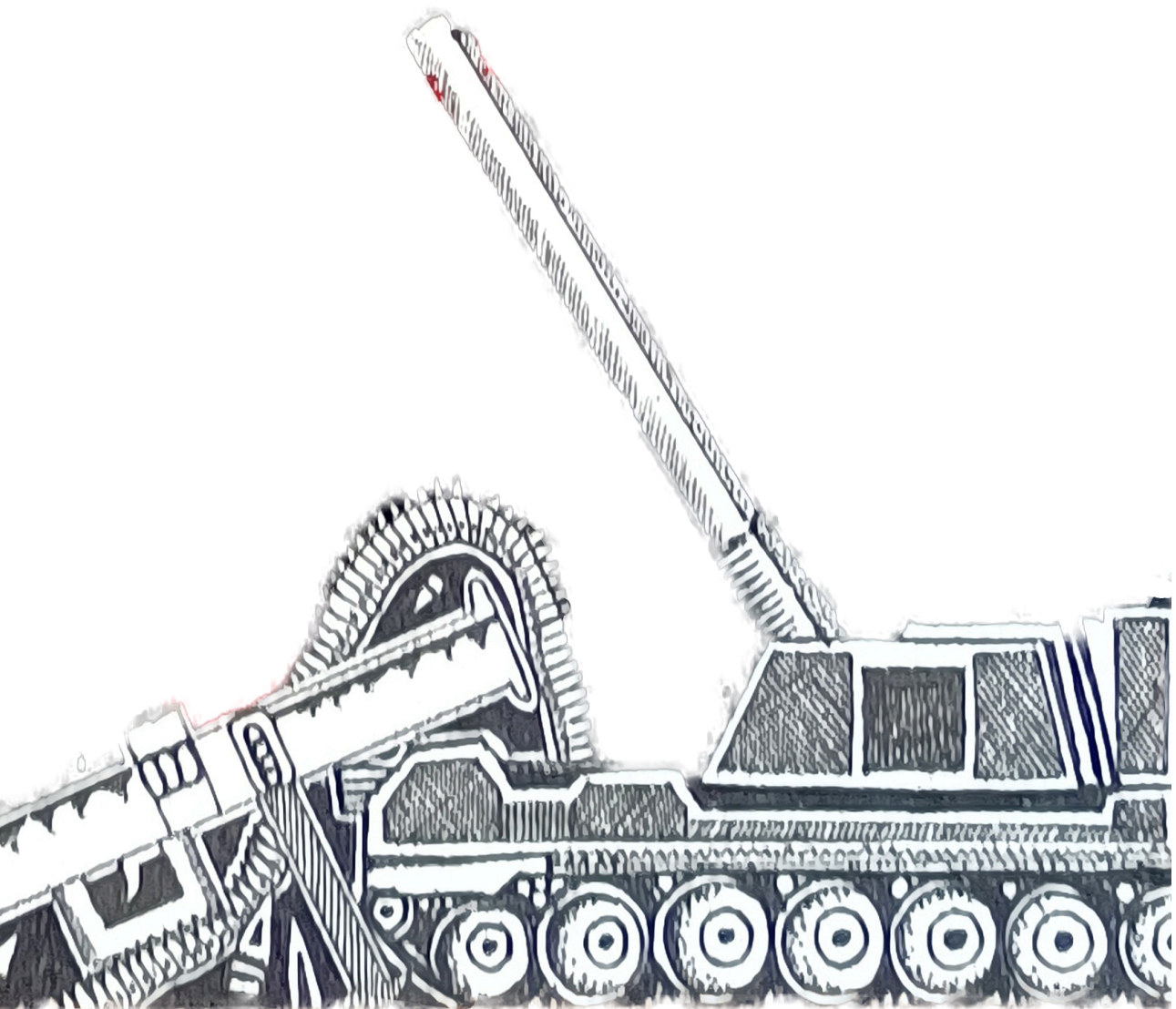
CHAPTER 6

Quantification of carotid plaque composition with a multi-contrast atherosclerosis characterization (MATCH) MRI sequence



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ABSTRACT

BACKGROUND AND PURPOSE

Carotid atherosclerotic plaques with a large lipid-rich necrotic core (LRNC), intraplaque hemorrhage (IPH), and a thin or ruptured fibrous cap are associated with an increased stroke risk. Multi-sequence MRI can be used to quantify carotid atherosclerotic plaque composition. Yet, its clinical implementation is hampered by long scan times and image mis-registration. Multi-contrast Atherosclerosis Characterization (MATCH) overcomes these limitations. The main objective was to compare quantification of plaque composition with MATCH and multi-sequence MRI.

METHODS

MATCH and multi-sequence MRI were used to image 54 carotid arteries of 27 symptomatic patients with ≥2mm carotid plaque on a 3.0 Tesla MRI scanner. The following sequence parameters for MATCH were used: repetition time/echo time (TR/TE): 10.1/4.35 msec, field of view (FOV): 160x160x2 mm, matrix size: 256x256, acquired in-plane resolution: 0.63x0.63 mm^2, number of slices: 18, and flip angles: 8°,5°,10°. Multi-sequence MRI (black blood pre- and post-contrast T1w, time of flight, and magnetization prepared rapid acquisition gradient echo; acquired in-plane resolution: 0.63x0.63 mm^2) was acquired according to consensus recommendations. Image quality was scored (5-point scale). The interobserver agreement in plaque composition quantification was assessed by intraclass correlation coefficient (ICC). The sensitivity and specificity of MATCH in identifying plaque composition were calculated using multi-sequence MRI as a reference standard.

RESULTS

A significantly lower image quality of MATCH compared to multi-sequence MRI was observed ($p<0.05$). The Scan time for MATCH was shorter (7 vs 40 minutes). Interobserver agreement in quantifying plaque composition on MATCH images was good-to-excellent (ICC≥0.77) except for total volume of calcifications and fibrous tissue that showed moderate agreement (ICC≥0.61). The sensitivity and specificity of detecting plaque components on MATCH were ≥89% and ≥91% for IPH, ≥81% and 85% for LRNC, and ≥71% and ≥32% for calcifications. Overall, good-to-excellent agreement (ICC≥0.76) of quantifying plaque components on MATCH with multi-sequence MRI as reference standard was observed except calcifications (ICC=0.37-0.38), and fibrous tissue (ICC=0.59-0.70).

DISCUSSION AND CONCLUSION

MATCH images can be used to quantify plaque components such as LRNC and IPH but not for calcifications. Although MATCH images showed a lower mean image quality score, short scan time and inherent co-registration are significant advantages.

KEYWORDS

Magnetic resonance imaging, atherosclerotic plaque, carotid arteries, stroke, MATCH.

INTRODUCTION

Stroke is the second global cause of both disability and mortality [1]. Approximately 85% of all strokes are classified as ischemic strokes. [2]. Around 20% of ischemic strokes are linked to carotid atherosclerosis [3]. At present, the degree of carotid stenosis is essential in determining the type of (secondary) stroke prevention treatment. Ischemic stroke related to carotid stenosis is mainly caused by embolization occurring after plaque rupture rather than by low perfusion resulting from the stenotic plaque [4-6]. A vulnerable plaque is a plaque that is more susceptible to rupture and distal embolization. The morphological features of a vulnerable plaque include the presence of a large lipid-rich necrotic core (LRNC), intraplaque hemorrhage (IPH), and a thin or ruptured fibrous cap [7-9]. It has been demonstrated that these features are strongly associated with an increased risk of (recurrent) stroke [10-12].

In the last two decades, magnetic resonance imaging (MRI) has emerged as the preferred imaging modality for visualizing and assessing plaque composition [9, 13, 14]. By employing a combination of different MRI pulse sequences, especially pre- and post-contrast T1 weighted (T1w) turbo spin echo (TSE), magnetization-prepared rapid acquisition gradient echo (MPRAGE), and time of flight (TOF) MRI, various plaque components can be distinguished. [15]. Recently, expert consensus recommendations on using the above-mentioned multi-sequence carotid vessel wall imaging protocol have been published [15, 16]. However, multi-sequence carotid MRI still has some limitations. Limited slice resolution associated with 2D sequences, long acquisition time, and image mis-registration due to patient motion between scans may hamper clinical implementation.

A few years ago, a new pulse sequence, i.e., Multi-contrast Atherosclerosis Characterization (MATCH), was introduced [17]. MATCH employs a 3D spoiled segmented spoiled gradient echo (GRE) readout to acquire data with three different contrast weightings, including hyper-T1w, grey-blood, and T2-weighted (T2-w), following a non-selective inversion pulse and various inversion-recovery times. On the hyper-T1w and T2w images, luminal blood signals are suppressed using flow-sensitive dephasing (FSD) magnetization preparation [18]. IPH and calcifications can be delineated on the

black-blood hyper T1w and grey-blood images, respectively. The T2w images provide information on overall plaque morphology and the presence of LRNC [17]. In addition to the unique tissue contrast weightings available in MATCH, all three image sets are simultaneously acquired in a 5-min scan and therefore are inherently co-registered and more immune to patient intolerance than conventional multi-sequence protocols. In a small group of six patients, good agreement between MATCH and multi-sequence MRI was demonstrated for detecting plaque components [17]. However, this study had a few limitations, including a small sample size and no quantitative analysis of the plaque components. Later, in a study conducted on a larger group of patients (46 patients), MATCH and multi-sequence MRI showed similar performance in detecting plaque components [19]. An essential limitation of both studies was the absence of a dedicated sequence for detecting IPH or LRNC in the multi-sequence protocol. We used a dedicated sequence for the identification of IPH (MPRAGE) and LRNC (pre- and post-contrast T1w TSE images) as part of the multi-sequence protocol in the current study, as recommended in the consensus paper [16].

Therefore, the current study aims to compare the diagnostic performance of MATCH with multi-sequence MRI, including a dedicated sequence for IPH and LRNC to quantify carotid plaque composition.

MATERIALS AND METHODS

STUDY POPULATION

Baseline carotid MRI data between 2018-2022 were derived from three different prospective studies in our institution. The studies were registered at ClinicalTrial.gov (NCT03291093, NCT02640313, NCT04569006). The common objective of these previous trials was evaluating novel PET tracers and novel MRI sequences. Our institutional review board has approved all three studies. A signed informed consent form was obtained from each patient. The inclusion criterium was the presence of a carotid plaque of at least 2 mm in the ipsilateral carotid artery on either duplex ultrasonography or computed tomography angiography (CTA). Both symptomatic patients that had experienced a transient ischemic attack (TIA), stroke or retinal ischemia or asymptomatic patients were eligible for inclusion. Only patients who underwent MATCH and multi-sequence MRI were eligible for inclusion in our analysis.

MRI PROTOCOL

All examinations were performed on a 3.0 Tesla hybrid integrated PET-MRI scanner (Biograph mMR, Siemens Healthineers). MATCH and multi-sequence MRI were combined in one exam. A 4-channel special purpose coil (Siemens Healthineers) was used to image the carotid bifurcation, allowing sub-millimeter resolution imaging. The scan parameters for MATCH were as follows; repetition time/echo time (TR/TE): 10.10/4.35 ms, field of view (FOV): 160x160 mm, acquired matrix size: 256x256, in-plane acquired resolution: 0.63x0.63 mm^2, slice thickness: 2 mm, flip angles: 8°,5°,10°, number of slices: 18, bandwidth: 130 Hz/pixel. The multi-sequence MRI protocol acquired 14 adjoining transverse 2 mm slices covering the entire plaque. The parameters of multi-sequence MRI and MTACH are listed in Table 1. A gadolinium-based contrast medium (Gadovist, Bayer AG) with a dose of 0.1 mmol/kg was used for post-contrast T1w imaging with a delay of 6 min post-injection.

IMAGE ANALYSIS

All image datasets were anonymized and processed using dedicated software (VesselMass, Department of Radiology, Leiden University Medical Centre). Two trained observers (MK, KN) with 1-3 years of experience reviewed the MR images independent of the clinical data and each other. The multi-sequence MRI and MATCH images were evaluated blindly from the delineations and the scores of the other MRI method with a time interval of at least one month.

The observers manually co-registered the images of the different contrast weightings of the multi-sequence protocol in-plane (x- an y-direction) and out-of-plane (z-direction) using the same dedicated software package (VesselMass). The time required for manual coregistration was included in the overall image analysis time. In the case of MATCH images, coregistration was inherent and did not require additional manual adjustments. Multi-sequence and MATCH images were assessed based on the previously validated criteria [17, 20]. 1) The inner and outer vessel wall of the plaque was delineated on pre -contrast T1w TSE images (multisequence protocol), or on the T2w images (MATCH protocol). 2) The LRNC was defined as a region within the plaque that exhibits no contrast enhancement on the post-contrast T1w images (multi-sequence protocol) or a hypo-intense region within the plaque on T2w images and isointense on hyper-T1w images (MATCH protocol). 3) IPH was characterized as a hyperintense signal in the bulk of the plaque compared to surrounding muscle tissue. For the multi-sequence protocol, this was done using MPRAGE images, while for the volume of the vessel wall [21]. The normalized wall index (NWI) was defined as wall area/ (lumen area + wall area), and the wall area was defined as the area between the lumen and outer wall [22]. The percent wall volume (PWV) is calculated by dividing the volume of the vessel wall by the total volume of the carotid artery segment and then multiplying this number by 100 to express it as a percentage [23]. The scan time (acquisition time and planning) and the image analysis time (coregistration time and time to delineate the vessel wall and plaque components) were recorded for MATCH and multi-sequence MRI. MATCH protocol, hyper-T1-weighted images were utilized.

Table 1. MATCH and Multi-sequence carotid MRI protocol

Pulse sequence	pre/post-contrast T1w TSE	TOF FFE	MPRAGE	MATCH
Acquisition plane	Transversal	Transversal	Transversal	Transversal
Acquisition time (min:sec)	5:14 x 2	2:47	3:25	4:44
Mean scan time (min:sec)	39:31			7:25
Image mode	2D	3D	3D	3D
TR (ms)	800	20	13.2	10.1
TE (ms)	10	3.6	6.5	4.35
TI (ms)	683	n/a	500	450, 1100, 3600
shot interval (ms)	n/a	n/a	800	4239
Flip angle (°)	90	20	15	8,5,10
No. of slices	14	14	14	18
Slice thickness (mm)	2	1	2	2
FOV (mm)	160x160	160x160	160x160	160x160
Acquisition matrix	256x256	256x256	256x256	256x256
Acquired voxel size (mm)	0.63x0.63x2.0	0.63x0.63x1.0	0.63x0.63x2.0	0.63 x 0.63 x 2.0
Reconstructed voxel size (mm)	0.31x0.31x2.0	0.31x0.31x1.0	0.31x0.31x1.0	0.31x0.31x2.0
Echo train length	10	n/a	44	53
GRAPPA acceleration factor	n/a	n/a	n/a	2
No. of signal averages	1	1	1	1
Fat suppression	SPAIR	no	water excitation	water excitation

T1w, T1-weighted; TSE, turbo spin echo; TOF, time of flight; FFE, fast field echo; MPRAGE, magnetization prepared rapid gradient echo; TR, repetition time; TE, echo time; TI, inversion time; n/a, not applicable; FOV, field of view; SPAIR, spectral attenuated inversion recovery.

4) For the multi-sequence protocol, calcifications were identified as areas with a hypo-intense signal relative to the sternocleidomastoid muscle on at least two different MRI weightings. Juxtaluminal calcifications can be obscured on the dark blood MRI weightings, thus, the TOF images are used to identify juxtaluminal calcifications. For the MATCH protocol, calcifications were identified as a hypo-intense signal on the grey-blood images. The quantification of the total fibrous tissue involved subtracting the combined volume of the LRNC (including IPH) and calcifications from the overall
A region of interest (ROI) was drawn in the sternocleidomastoid muscle at the level of the carotid bifurcation. The signal-to-noise ratio (SNR) was calculated as the ratio between the mean signal intensity and the standard deviation (SD) of this ROI. The contrast-to-noise (CNR) of IPH was calculated for MPRAGE and MATCH as the difference in mean signal intensity of IPH and muscle tissue divided by the noise of muscle tissue (SD) [24]. Image quality was scored on a 5-point scale on a slice-by-slice basis: 1 low image quality and 5 excellent [20].

HISTOLOGICAL ANALYSIS

In one patient that underwent carotid endarterectomy as part of routine clinical care one day after the MRI examination, the MRI findings were compared to histology. The specimen was collected directly after carotid endarterectomy. The specimen was cut

into ~3 mm slices, coded, and alternately frozen and stored at -80°C. The sample was fixated in 4% paraformaldehyde-phosphate buffered saline (PBS) solution for 18-48 h, decalcified using ethylenediaminetetraacetic acid (EDTA) for 4 h, and then embedded in paraffin. Cross-sectional sections of 4 mm were stained using hematoxylin and eosin (HE). An experienced vascular pathologist macroscopically assessed the carotid plaque composition. All procedures conducted during the research adhered to the guidelines outlined in the Dutch Code of Conduct for Observational Research with Personal Data (2004) and Tissue.

STATISTICAL ANALYSIS

Two-way mixed effects model of intraclass correlation coefficients (ICC) and Cohen's kappa test (κ) were used to assess the inter-observer agreement for quantification and identification of carotid plaque components on multi-sequence and MATCH images. The ICC is a numerical value ranging from 0 to 1. Values below 0.5 suggest poor agreement, values between 0.5 and 0.75 indicate moderate agreement, values between 0.75 and 0.9 represent good agreement, and any value above 0.9 indicates excellent agreement [25]. Kappa values also range from 0 to 1, where values from 0 to 0.2 indicate slight, 0.21 to 0.4 fair, 0.41 to 0.60 moderate, 0.61 to 0.8 substantial, and 0.81 upward excellent agreement [26]. The comparison between MATCH and muti-sequence protocol was as follows: hyper T1w versus MPRAGE, grey-blood versus TOF, and T2w MATCH versus post-contrast T1w TSE. Sensitivities and specificities of MATCH in identifying plaque components on artery basis were calculated using the multi-sequence protocol as a reference standard. A paired t-test or Wilcoxon signed ranked test was used to evaluate the differences in vessel wall volume, NWI, and volumes of the various plaque components between the MATCH and multi-sequence protocol, as appropriate. In addition, the differences between the IPH, LRNC, calcifications, and NWI measurements on MATCH and multi-sequence images were plotted against the mean difference as Bland-Altman plots. Limits of agreement were calculated as mean difference$\pm$1.96*standard deviation of difference. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corporation). Normally distributed continuous variables were presented as mean $\pm$ standard error. Otherwise, the median and interquartile range [IQR] was presented. P <0.05 indicated statistical significance.

RESULTS

Twenty-seven patients (54 carotids) underwent MATCH and multi-sequence carotid MRI. A flow chart of the patient inclusion is presented in Figure 1. One artery was excluded due to total occlusion. Two patients did not undergo contrast injection because of a low

glomerular filtration rate (GFR) (<30ml/min). 21/27 patients were male. The patients had a mean age of 70 ± 6.8 years. Patient characteristics are provided in Table 2. The mean MATCH and multi-sequence scan time was 7 min 25 sec and 39 min 31 sec, respectively. The mean time to delineate the vessel wall and the plaque components, including co-registration, for one artery on MATCH was shorter than for the multi-sequence images (7:42 min ± 2:30 min vs 13:24 ± 1:55 min; p<0.05, respectively.

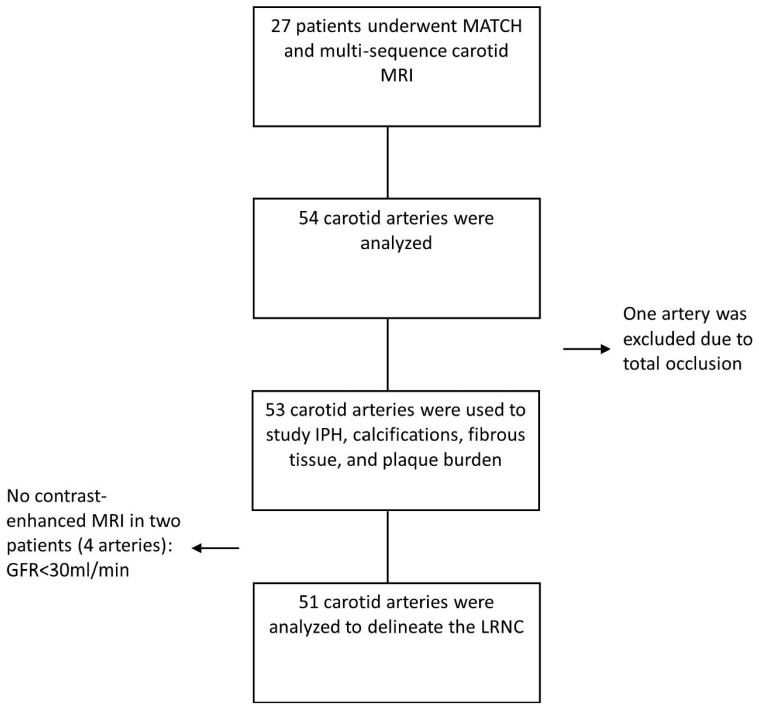

Figure 1. Flow chart of the study
MRI= magnetic resonance imaging, LRNC= lipid-rich necrotic core, IPH= intraplaque hemorrhage

IMAGE QUALITY

A significantly lower image quality of hyper-T1w, grey-blood, and T2w MATCH images was found compared with MPRAGE, TOF, and post-contrast T1w TSE (median score 1, IQR [1-1] vs median score 4, IQR [3-4]; 2, [2-3] vs 4, [3-4]; 3, [3-4] vs 4, [4-4]; p<0.05, respectively). A direct comparison between the MATCH and the equivalent multi-sequence images is shown in Table 3.

The SNR was significantly lower for the MATCH images and the equivalent multi-sequence images: hyper T1 images compared with MPRAGE (3.8±0.3 vs 12.8±0.6; p<0.001), grey-blood compared with TOF (9.2±0.6 vs 11.9±0.7; p=0.007) and T2w MATCH images compared with post-contrast T1w TSE (8.9±0.6 vs 12.5±0.7; p=0.001). The results are summarized in Table 3. In addition, IPH on MATCH showed a significantly lower mean CNR than on MPRAGE (10.4±1.1 vs 13.4±1.4; p=0.02).

Table 2. Subject characteristics (n:27, 53 arteries)

Characteristic	Value
Age, years; mean ± SD	70 ± 6.8
Sex, Male; number (%)	21 (80%)
Stenosis degree (NASCET, %):	
<50%	37/54 (69%)
50-69%	11/54 (20%)
70-99%	5/54 (9%)
Time event to MRI (days), median (IQR)	9 (6-17)
Type of event, number (%)	
Stroke	13 (48%)
TIA	10 (37%)
Retinal ischemia	4 (15%)
Smoking status, number (%)	
Current	10 (37%)
Former smoker	8 (30%)
Never	9 (33%)
Hypertension; number (%)	15 (56%)
Hypercholesterolemia; number (%)	17 (63%)
Diabetes Mellitus; number (%)	3 (11%)
Previous CVD and PAD; number (%)	6 (22%)
BMI (kg/m2); mean ± SD	25.9 ± 3.2

The data are presented as mean ± standard deviation (SD), median, interquartile range (IQR), or absolute numbers of patients (%). NASCET= North American Symptomatic Carotid Endarterectomy Trial, CVD= Cardiovascular disease, PAD= Peripheral arterial disease, BMI= Body mass index

Table 3. Image quality comparison between MATCH and multi-sequence protocol

Comparison	Median image quality	IQR	P-value	Mean SNR±SE	P-value
Hyper T1w vs MPRAGE	1 vs 4	[1-1] vs [3-4]	P<0.05	3.8±0.3 vs 12.8±0.6	P<0.001
Grey-blood vs TOF	2 vs 4	[2-3] vs [3-4]	P<0.05	9.2±0.6 vs 11.9±0.7	P=0.007
T2w MATCH vs post-contrast T1w	3 vs 4	[3-4] vs [4-4]	P<0.05	8.9±0.6 vs 12.5±0.7	P=0.001

IQR: interquartile range, T1w: T1-weighted, MPRAGE: magnetization prepared rapid gradient echo, TOF: time of flight, T2w: T2-weighted, SNR: signal-to-noise ratio, and SE: standard error.

INTEROBSERVER AGREEMENT

The interobserver agreement is summarized in Table 4. The agreement between two readers on multi-sequence images was excellent for IPH (κ=0.82) and calcifications (κ=0.84) and substantial for LRNC (κ=0.73) detection. On MATCH images, excellent

interobserver agreement for detection of IPH (κ=0.84), fair for calcifications (κ=0.21), and substantial for LRNC (κ=0.71) was observed.

Table 4. Interobserver agreement for multi-sequence and MATCH images

Plaque component	Multi-sequence kappa (κ)	MATCH kappa (κ)	Multi-sequence ICC (95% CI)	MATCH ICC (95% CI)
Total vessel wall volume	-	-	0.81 (0.67-0.89)	0.77 (0.64-0.86)
The presence and total volume of LRNC	0.73	0.71	0.96 (0.93-0.98)	0.94 (0.90-0.97)
The presence and total volume of IPH	0.82	0.84	0.97 (0.94-0.98)	0.78 (0.62-0.87)
The presence and total volume of CA	0.84	0.21	0.63 (0.36-0.79)	0.61 (0.41-0.76)
Total volume of fibrous tissue	-	-	0.68 (0.44-0.82)	0.70 (0.53-0.81)
NWI	-	-	0.86 (0.75-0.92)	0.85 (0.75-0.91)

ICC: intercorrelation coefficient, LRNC: lipid-rich necrotic core, IPH: intraplaque hemorrhage, CA: calcifications and NWI: normalized wall index.

The interobserver reproducibility (ICC; 95% confidence interval (CI)) of the quantification was as follows for multi-sequence MRI: excellent for total volume of LRNC (0.96; 0.93-0.98) and IPH (0.97; 0.94-0.98), good for total vessel wall (0.81; 0.67-0.89) and NWI (0.86; 0.75-0.92), moderate for fibrous tissue (0.68; 0.44-0.82), and calcifications (0.63; 0.36-0.79). When using MATCH images, interobserver reproducibility for the total volume was excellent for LRNC (0.94; 0.90-0.97), good for IPH (0.78; 0.62-0.87), total vessel wall (0.77; 0.64-0.86), fibrous tissue (0.70; 0.53-0.81), NWI (0.85; 0.75-0.91), and moderate for calcifications (0.61; 0.41-0.76). In addition, the results of the quantitative analysis of the vessel wall and the plaque components on multi-sequence versus MATCH images for both readers are listed in Supplementary Table 6 and Figure 5.

THE PERFORMANCE OF MATCH FOR IDENTIFYING AND QUANTIFYING CAROTID PLAQUE FEATURES

The results of true positive/negative and false positive/negative identification of plaque components on MATCH images compared with multi-sequence protocol as reference standard for both readers are listed in Table 5. For the reader 1 and 2, the sensitivity and specificity of detecting IPH on hyper T1w images of the MATCH protocol were ≥88.9% and 90.9%, and for the detection of LRNC on T2w images of the MATCH, they were ≥81.8% and ≥85.4%, respectively. However, lower sensitivity and specificity were observed for scoring calcifications on the MATCH protocol grey-blood images of 71.4% and 32.0%, respectively. Figure 2 shows an example of good agreement between MATCH and multi-sequence images for identifying plaque composition.

Table 5. Concordance between multi-sequence and MATCH images.

	Multi-sequence (MPRAGE)			
			IPH -	**IPH +**
MATCH	**IPH -**	Reader 1	41 (95.3%)	1
		Reader 2	40 (90.9%)	1
	IPH +	Reader 1	2	9 (90.0%)
		Reader 2	4	8 (88.9%)
	Multi-sequence (pre- and post-contract T1w)*			
			LRNC -	**LRNC +**
MATCH	**LRNC -**	Reader 1	35 (94.6%)	2
		Reader 2	35 (85.4%)	2
	LRNC +	Reader 1	2	10 (83.3%)
		Reader 2	6	9 (81.8%)
	Multi-sequence			
			CA -	**CA +**
MATCH	**CA -**	Reader 1	10 (47.6%)	8
		Reader 2	8 (32.0%)	8
	CA +	Reader 1	11	24 (75.0%)
		Reader 2	17	20 (71.4%)

4 carotids were excluded (contraindication of contrast injection). Intraplaque hemorrhage (IPH), lipid-rich necrotic core (LRNC), calcifications (CA), T1-weighted (T1w), magnetization prepared rapid gradient echo (MPRAGE), time of flight (TOF), T2-weighted (T2w)

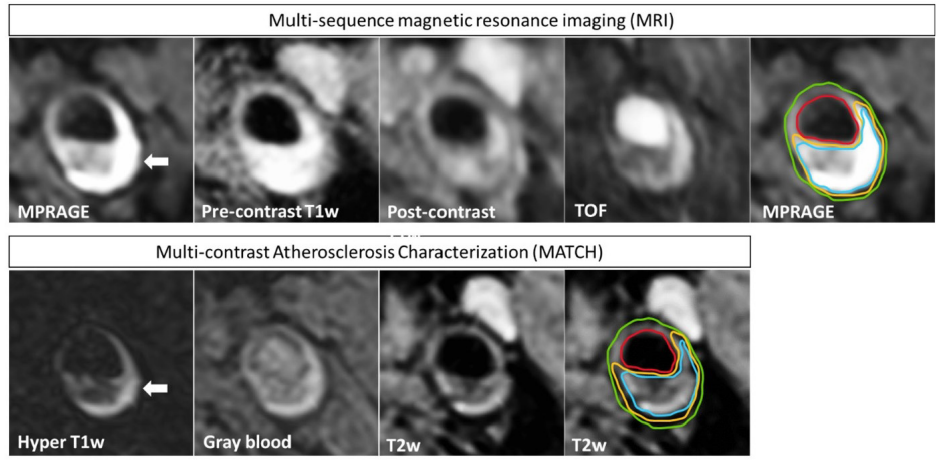

Figure 2.
An example of a patient that exhibits an atherosclerotic plaque in the left carotid artery. The four multi-sequence MRI images have been manually co-registered and are displayed in the upper row. Additionally, the MATCH images with inherent co-registration are shown in the bottom row.. IPH appears hyper-intense (white arrow) on hyper T1w MATCH and MPRAGE images. Contours are shown on T2w MATCH and MPRAGE: green for the outer vessel wall, red for the lumen, yellow for LRNC, and blue for IPH. A good agreement is shown between the contours that are delineated on the MATCH versus the multi-sequence MR images, although slight deviations can be observed.
Intraplaque hemorrhage (IPH), lipid-rich necrotic core (LRNC), T1-weighted (T1w), magnetization prepared rapid gradient echo (MPRAGE), time of flight (TOF), T2-weighted (T2w)

Figure 3 also shows the presence of IPH, LRNC, and calcifications on MATCH and multi-sequence images with histology as a reference in a CEA specimen in a patient that underwent CEA one day after the MRI examination as part of routine clinical care. The results of quantitative analysis of plaque components on multi-sequence and MATCH images for both readers are listed in Supplementary Table 6 and shown in figure 4 as Bland-Altman plots. For reader1, there was overall significant good correlation (ICC>0.75, p<0.01) between both protocols for the quantitative plaque assessment, except for poor correlation for total volume of calcifications (ICC=0.38, p=0.4) and moderate t for total volume of fibrous tissue (ICC=0.59, p<0.001). For reader 2, overall good correlation for the quantification of total vessel wall volume, total volume of LRNC, PWV, and NWI (ICC>0.75, p<0.01), poor for total volume of calcifications (ICC=0.37, p=0.06) and moderate for total volume of fibrous tissue (ICC=0.70, p<0.01) was observed.

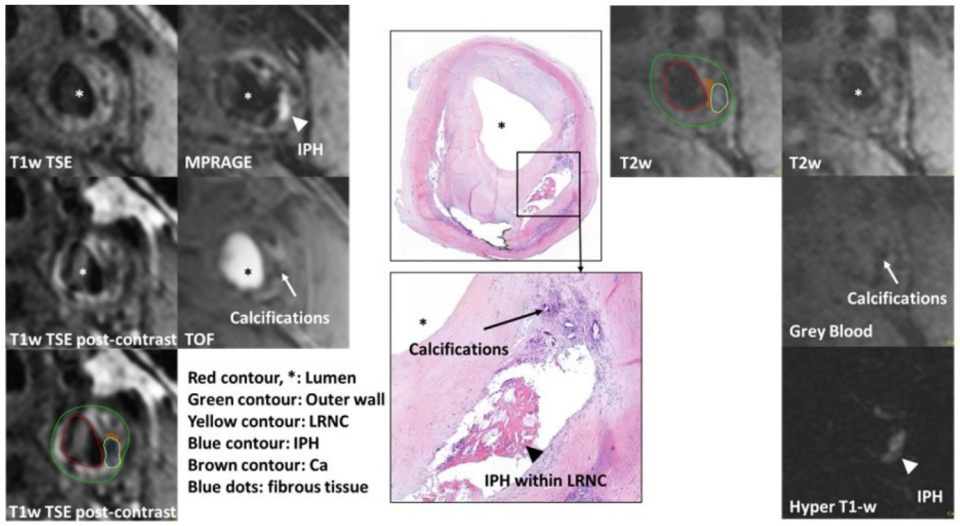

Figure 3.
Example of histological comparison of MATCH and multi-sequence protocol at left common carotid artery. IPH appears hyper-intense (white arrowhead) on hyper T1w MATCH and MPRAGE images. A dark calcified nodule on grey-blood image and TOF (white arrows). Histological specimen with hematoxylin-eosin staining confirms the presence of IPH, LRNC (black arrowhead), and calcifications (black arrows). Contours are shown on T2w MATCH and T1w post-contrast: green for outer vessel wall, red for lumen, yellow for lipid-rich necrotic core, brown for calcifications and blue for IPH. Intraplaque hemorrhage (IPH), lipid-rich necrotic core (LRNC), T1 weighted (T1w) turbo spin echo (TSE), magnetization prepared rapid gradient echo (MPRAGE), time of flight (TOF), and T2-weighted (T2w).

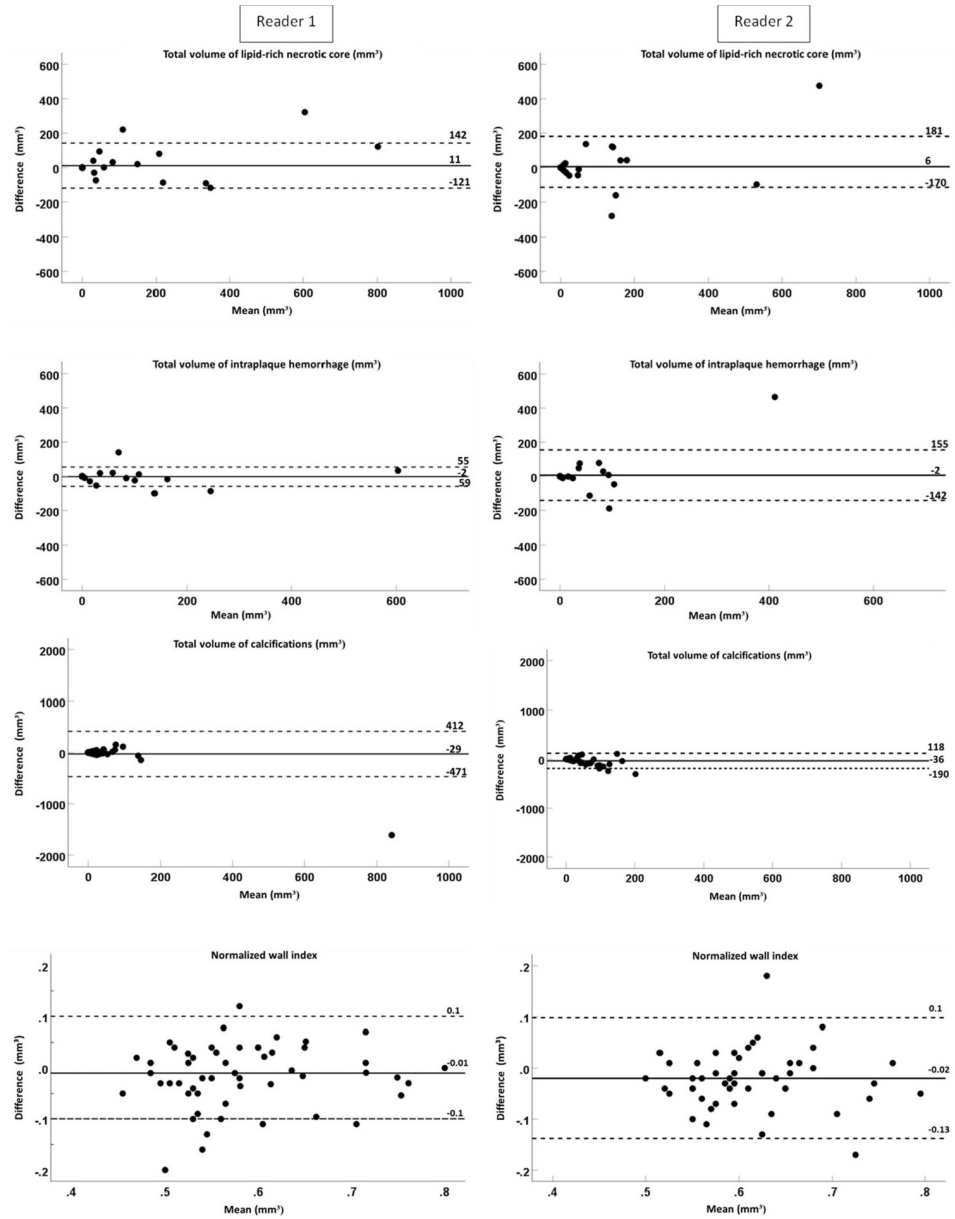

Figure 4.
Bland-Altman plots of difference (mm^3) between two protocols (multi-sequence – MATCH) versus the mean value for the total volume of lipid-rich necrotic core intraplaque hemorrhage, calcifications, and normalized wall index with a mean bias (continuous lines) and two times the standard deviation (SD) (dashed lines).

No significant bias between multi-sequence MRI and MATCH was shown for the quantification of LRNC, IPH, calcifications, and NWI (p>0.05) for reader 1. However, the total volume of the vessel wall (1335.1±55.8 vs 1421.3±63.2 mm^3), the total volume of fibrous tissue (1227.8±50.6 vs 1441.5±68.5 mm^3), and PWV (57.6±1.3 vs 59.5±1.3 %) were significantly different (p<0.05) between multi-sequence and MATCH. For reader 2, no significant bias was found for quantifying LRNC, IPH, and fibrous tissue (p>0.05). However, the total volume of the vessel wall (1453.7±42.8 vs 1584.2±65.2 mm^3), the volume of calcifications (23.5±5.7 vs 59.6±10.9), PWV (60.5±1.1 vs 62.5±1.0 %) and NWI (0.60±0.01 vs 0.62±0.01) were significantly different (p<0.05) between multi-sequence and MATCH.

DISCUSSION

In the present study, we validated MATCH with a multi-sequence carotid MRI protocol that was recommended in a white paper [16]. Our results showed a substantial-to-excellent interobserver agreement of detecting and quantifying all plaque features with MATCH except for calcification which showed fair to a moderate agreement. In addition, the sensitivity and specificity of identifying IPH and LRNC on the MATCH images using multi-sequence MRI as a reference standard were high. Moreover, the quantification of vulnerable carotid plaque components such as IPH and LRNC using MATCH were in agreement with the quantification using the multi-sequence protocol, while a moderate and poor agreement was seen for total volume of fibrous tissue and calcifications, respectively. These findings are important since the scan time and the time needed for image analysis were substantially less for MATCH than for the conventional multi-sequence protocol.

Dai et al. demonstrated that MATCH showed a comparable, if not superior, performance compared to a conventional protocol in identifying and quantifying major carotid plaque components. [19]. This previous study used T1w TSE, T2w TSE, and TOF images as a reference. Therefore, LRNC was determined using T2w TSE, which is less accurate than contrast-enhanced T1w TSE that has been used in the present study [27]. In addition, in the current work, we also measured additional practical outcome variables, such as the total scan time, which includes acquisition time, planning, etc. Similarly, we compared how long it takes to identify and quantify carotid plaque features and composition of one carotid plaque. Comparing MATCH to MPRAGE, they both have similar scan times and can be used to identify IPH, an important risk factor for predicting stroke [11,12]. The advantage of MATCH is that without a time penalty, it also provides multiple co-registered images, which can be used to identify additional plaque features, such as LRNC volume, that are not possible by using only

MPRAGE. In addition, the MPRAGE sequence contains a nonselective inversion pre-pulse. This nonselective inversion pre-pulse is limited to the field-of-view of the body coil. Therefore, in case we observe that the luminal blood is not entirely suppressed, the patient's position can be slightly off-centered towards the feet-direction. In the MATCH protocol, flow-sensitive dephasing (FSD) prepulses are applied to suppress the signal originating from the luminal blood. [17, 18]. The FSD-prepared is flow dependent but does not rely on the inflow of blood [18].

We observed poor agreement on the quantification of calcifications. The visualization of small calcifications on MR images can be challenging due to their dark appearance. Therefore partial volume effects can obscure small regions of calcifications on MRI. In line, in a previous study on quantitative assessement of carotid atherosclerosis also a higher measurement error for calcifications was reported compared to the LRNC and wall volume [28]. In the multisequence MRI protocol, regions that were not identified as LRNC and that appeared dark on at least two different weightings were identified as calcifications. In the MATCH protocol, calcifications are identified on the grey-blood image. This may explain the poor agreement on the quantification of calcification. Also, this will lead to a lower agreement in the quantification of fibrous tissue since the fibrous tissue is identified as the remaining tissue after delineating the LRNC, IPH and calcifications. Prrevious studies have demonstrated that IPH and LRNC are important parameters for risk prediction of stroke [11, 29]. Importantly, these parameters can be quantified with good-to-excellent agreement. The association with stroke risk risk has not been reported for calcifications and fibrous tissue as identified on MRI.

Despite the lower image quality observed in the hyper T1w and T2w MATCH images, good agreement was still observed for the quantification of IPH and the LRNC, respectively. Also, although the CNR between IPH and muscle tissue was lower for the hyper T1w MATCH images compared to the MPRAGE images, the CNR remained relatively high, allowing for a clear appearance of IPH. These findings suggest that despite the lower image quality of the MATCH images, the the vulnerable plaque components (IPH and LRNC) remained discernible.

MRI-based tissue quantification is accurate and reproducible [30, 31]. The requirement of acquiring multiple weightings to obtain accurate measurements can lead to longer scan times. Moreover, the acquisition of different weightings increases the risk of image mis-registration, resulting in misalignment of anatomical structures and potential inaccuracies in tissue quantification. Despite these limitations, ongoing research and technological advancements aim to optimize MRI protocols to enhance the efficiency, accuracy, and reproducibility of MRI-based tissue quantification. Apart from MTACH, other novel multicontrast MR sequences, such as Simultaneous Non-

contrast Angiography and IPH (SNAP) [32] and Bright-blood and black-blOOd phase SensiTive inversion recovery sequence (BOOST) [33], have recently been developed. These sequences also offer the advantage of acquiring multi-contrast images using a single sequence, thereby greatly reducing scan time and providing inherent image co-registration. However, it is important to note that these novel sequences still require additional validation to ensure their reliability and accuracy in clinical practice. In addition to the advancements in MR sequences, the integration of Artificial Intelligence (AI) holds tremendous potential in further enhancing MR image quality and shortening analysis times. AI techniques can aid in automating image analysis tasks, enabling more efficient and streamlined processing of MR images

A limitation of the MATCH sequence is that no criteria are available for determining the fibrous cap status on MATCH images. A thin or ruptured fibrous cap on MRI is a well-known risk factor for recurrent stroke [29, 34], and it can be easily scored on post-contrast T1w as part of the multi-sequence protocol [27, 35]. Thus, if time allows it, it is still beneficial to add a post-contrast dark blood T1w MRI sequence to score the fibrous cap status. This sequence, which takes 3-5 minutes, will require contrast injection and needs to be acquired approximately 6 minutes after contrast injection. These 6 minutes could be used to acquire a contrast-enhance MRA to quantify the degree of stenosis. In the present study, the MATCH images were only acquired before contrast injection. Future studies, could investigate whether post-contrast MATCH images could be of added value.

A limitation of our study is that most patients had only an intermediate or mild degree of stenosis (<70%). Symptomatic patients with high-grade carotid stenosis (70-99%) benefit from carotid endarterectomy [36]. Thus, comparing MATCH with histology as gold standard can be considered in future. Symptomatic patients with mild to moderate stenosis (<70%) are still at increased risk of recurrent ischemic events, and the usefulness of carotid endarterectomy has not yet been determined [37]. Therefore, studying the risk factors of plaque components such as IPH and LRNC using a short carotid MRI protocol and inherently co-registered images is beneficial. In addition, while we made efforts to minimize bias by conducting the image evaluation with a time interval of at least one month, it is important to acknowledge that blinding readers to whether they delineated the plaque components on MATCH or multi-sequence MR images is inherently impossible since the observers can recognize the weightings. Last, although the sensitivity and specificity for the identification of the plaque components were similar between the two readers, reader 2 had a larger number of false positive findings. These results suggest that although both readers were well-trained, there is still a difference in their performance.

In conclusion, MATCH can be used for identifying and quantifying carotid plaque composition except for calcifications and fibrous cap status. Inherently co-registered images and short scan- and analysis times are major advantages of MATCH.

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SUPPLEMENTARY MATERIAL

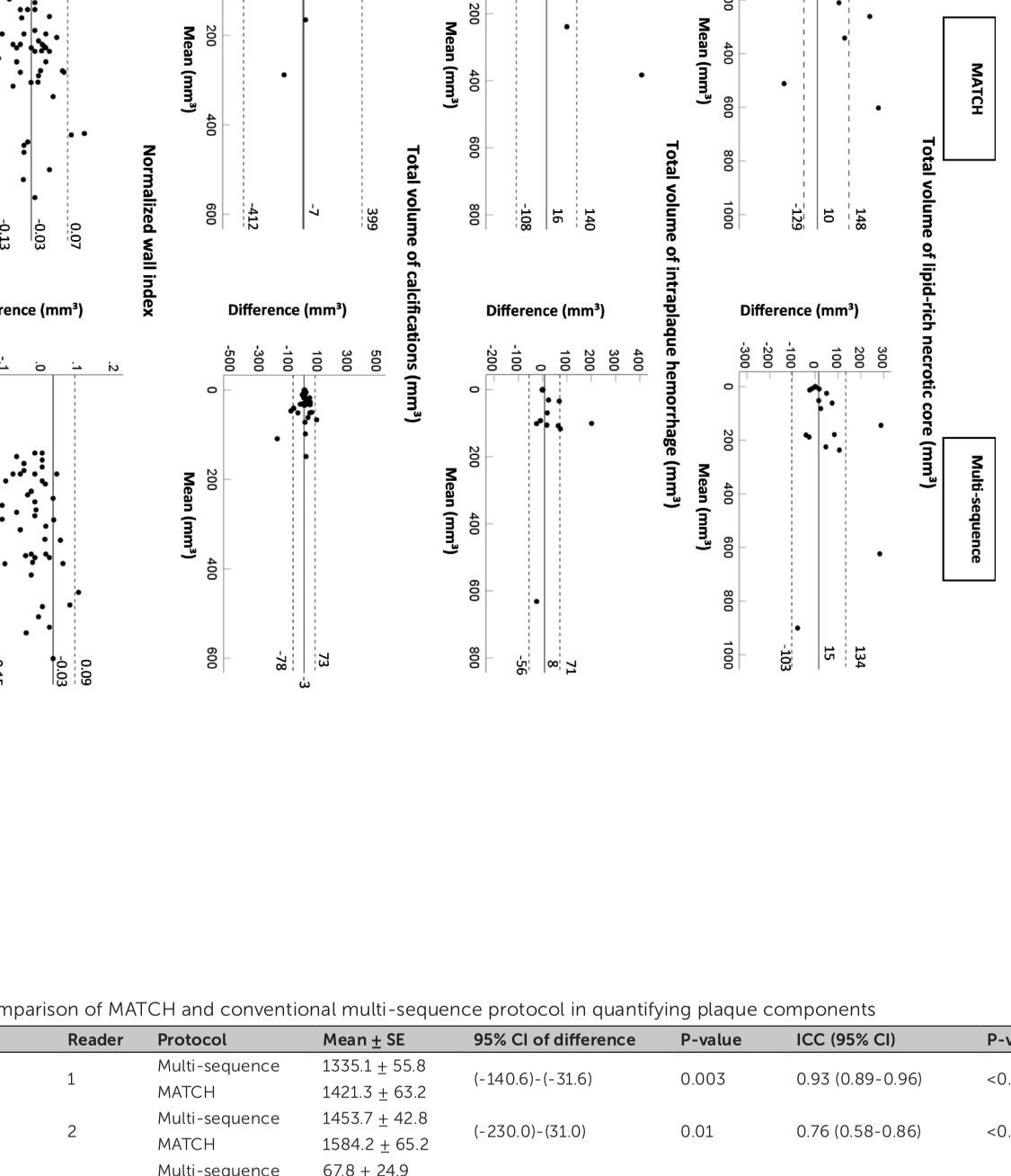


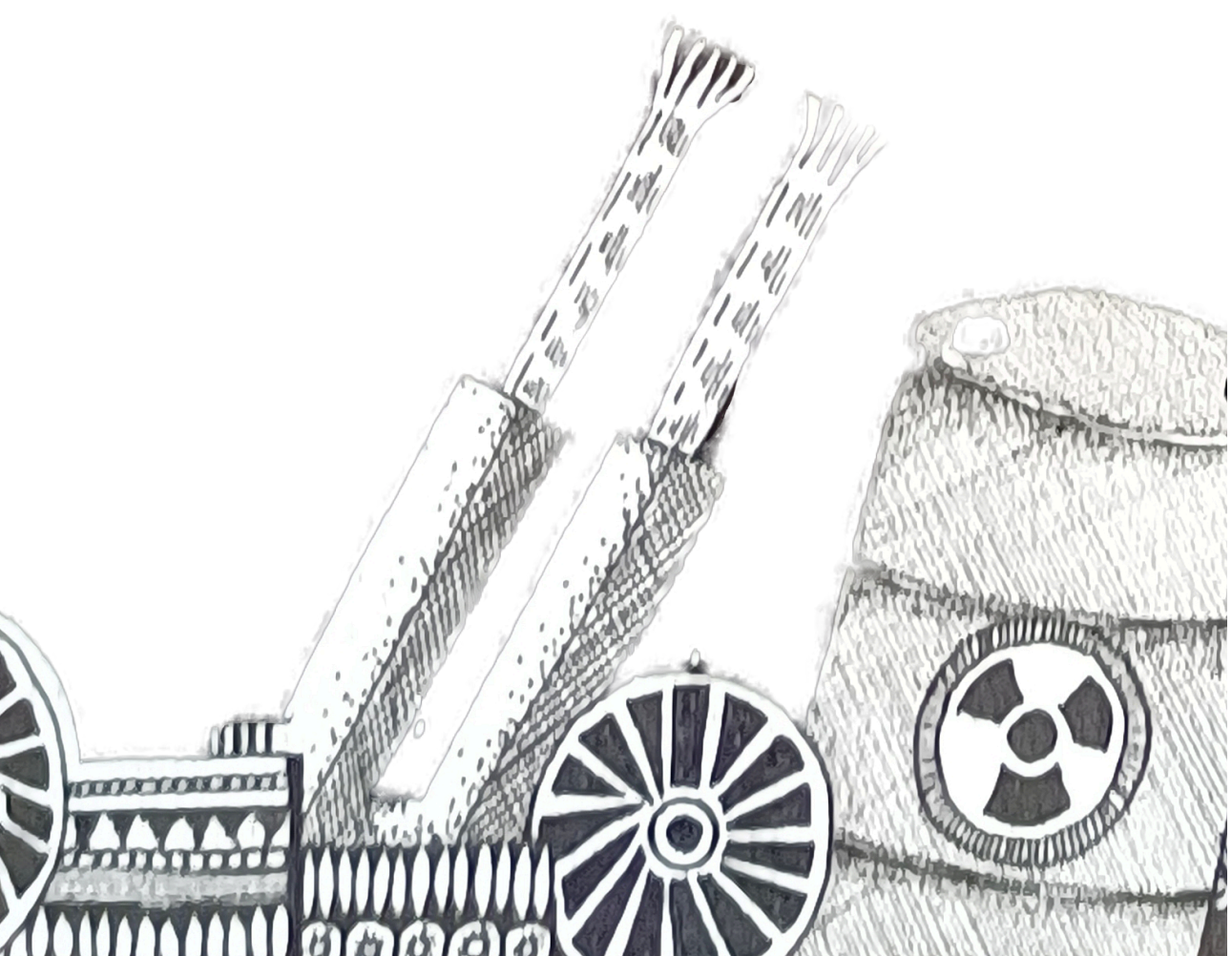
Figure 5.
The Bland-Altman plots of the difference (mm^3) between two readers and the mean value for the total volume of the lipid-rich necrotic core, intraplaque hemorrhage, calcifications, and the normalized wall index for MATCH and the multi-sequence protocol. The continuous lines display the mean bias and the dashed lines two times the standard deviation (SD).

Supplementary Table 6: Comparison of MATCH and conventional multi-sequence protocol in quantifying plaque components

Parameter	Reader	Protocol	Mean ± SE	95% CI of difference	P-value	ICC (95% CI)	P-value
Total vessel wall volume (mm^3)	1	Multi-sequence	1335.1 ± 55.8	(-140.6)-(-31.6)	0.003	0.93 (0.89-0.96)	<0.01
		MATCH	1421.3 ± 63.2				
	2	Multi-sequence	1453.7 ± 42.8	(-230.0)-(31.0)	0.01	0.76 (0.58-0.86)	<0.01
		MATCH	1584.2 ± 65.2				
Total LRNC volume (mm^3)	1	Multi-sequence	67.8 ± 24.9	(-8.5)-(29.9)	0.27	0.95 (0.92-0.97)	<0.01
		MATCH	57.1 ± 20.9				
	2	Multi-sequence	50.5 ± 21.4	(-19.8)-(31.2)	0.7	0.88 (0.79-0.93)	<0.01
		MATCH	44.8 ± 15.7				
Total IPH volume (mm^3)	1	Multi-sequence	30.1 ± 13.9	(-10.1)-(5.8)	0.60	0.97 (0.96-0.99)	<0.01
		MATCH	32.2 ± 14.1				
	2	Multi-sequence	24.0 ± 13.5	(-15.3)-(27.0)	0.6	0.84 (0.77-0.90)	<0.01
		MATCH	17.5 ± 6.1				
Total calcifications volume (mm^3)	1	Multi-sequence	24.1 ± 5.5	(-91.2)-(32.9)	0.35	0.38 (0.23-0.46)	0.4
		MATCH	53.2 ± 33.6				
	2	Multi-sequence	23.5 ± 5.7	(-58.5)-(-13.7)	<0.01	0.37 (-0.1-0.64)	0.06
		MATCH	59.6 ± 10.9				
Total fibrous tissue volume (mm^3)	1	Multi-sequence	1227.8 ± 50.6	(-336.6)-(-90.6)	0.001	0.59 (0.29-0.76)	<0.01
		MATCH	1441.5 ± 68.5				
	2	Multi-sequence	1369.6±39.7	(-161.8)-(33.8)	0.2	0.70 (0.48-0.83)	<0.01
		MATCH	1433.6±52.5				
Percent wall volume (PWV) %	1	Multi-sequence	57.6 ± 1.3	(-3.7)-(-0.2)	0.03	0.85 (0.74-0.91)	<0.01
		MATCH	59.5 ± 1.3				
	2	Multi-sequence	60.5 ± 1.1	(-3.4)-(-0.6)	<0.01	0.87 (0.78-0.93)	<0.01
		MATCH	62.5 ± 1.0				
Normalized wall index (NWI)	1	Multi-sequence	0.58 ± 0.01	(-0.5)-(0.0)	0.06	0.85 (0.74-0.91)	<0.01
		MATCH	0.60 ± 0.01				
	2	Multi-sequence	0.60 ± 0.01	(-0.04)-(-0.0)	0.01	0.82 (0.68-0.90)	<0.01
		MATCH	0.62 ± 0.01				

CHAPTER 7

General discussion



INTRODUCTION

Stroke is the second-leading cause of death globally, with an annual mortality rate of approximately 5.5 million [1]. However, the impact of stroke goes beyond mortality, as up to 50% of the survivors experience significant morbidity and become chronically disabled [1]. Approximately 80% of stroke cases are ischemic strokes, whereas hemorrhagic strokes account for the remaining 20% [2]. 15-20% of ischemic strokes are related to carotid stenosis due to the presence of an atherosclerotic carotid plaque [3, 4]. Carotid endarterectomy (CEA) is a surgical procedure to remove plaque buildup in the carotid artery to prevent (recurrent) stroke. CEA is typically performed to prevent recurrent stroke in symptomatic patients with significant carotid artery stenosis. The degree of carotid stenosis and symptomatology are mainly used to stratify patients for CEA in combination with medical treatment versus medical treatment only. The results of a pooled analysis of the first European Carotid Surgery Trial (ECST), the North American Carotid Endarterectomy Trial (NASCET), and the Veterans Affairs trial showed a clear benefit of CEA over medical treatment alone in patients with ≥70% carotid stenosis (n=1095, absolute risk reduction: 16.0%, p<0.001) for the 5-year risk of ipsilateral stroke [5]. For patients with 50-69% stenosis, marginal benefits were observed (n=1549, absolute risk reduction: 4.6%, p=0.04) [5]. No proven benefit was found for patients with 30-49% stenosis (n=1429, absolute risk reduction: 3.2%, p=0.6) [5]. Notably, surgery was associated with an increased 5-year risk of ipsilateral stroke in patients with less than 30% stenosis (n=1746, absolute risk reduction: -2.2%, p=0.05) [5]. In addition, it was found that the number needed to treat (NNT) was six in patients with 70-99% stenosis, whereas in male patients with stenosis greater than 50%, the NNT was nine, and in female patients, it was thirty-six [5]. Therefore, relying solely on the degree of carotid stenosis and symptomatology as a marker of identifying patients who will benefit from CEA to prevent recurrent stroke is suboptimal.

The most important underlying cause of stroke in patients with carotid stenosis is the rupture of a vulnerable plaque. A vulnerable plaque is a plaque that is prone to rupture and consists of a thin fibrous cap overlying a large lipid-rich necrotic core. When this cap ruptures, the luminal blood is exposed to the thrombogenic lipid-rich necrotic core of the plaque, leading to thrombus formation that can embolize and obstruct a distal cerebral artery, potentially resulting in a stroke [6].

Research in the last decade suggests the need for a paradigm shift in the assessment of stroke risk based on the atherosclerotic plaque composition, thereby moving away from relying solely on the degree of stenosis as an imaging marker and towards identifying high-risk patients based on the presence of vulnerable plaque characteristics that can cause plaque rupture. Extensive evidence shows that intraplaque hemorrhage (IPH) is a key factor in the destabilization and rupture of plaques, thereby contributing to cardiovascular events such as stroke [7, 8]. The presence of IPH on carotid MRI increases the risk of future ipsilateral stroke independently in patients with symptomatic and asymptomatic carotid stenosis with HRs of 10.2; 95% confidence interval (CI): 4.6 to 22.5 and 7.9; 95% CI: 1.3 to 47.6 respectively, as shown in a large meta-analysis using data from 7 cohort studies of patients with a carotid plaque [9]. In light of this matter, the 2017 guidelines by the European Society for Vascular Surgery (ESVS) advocate the utilization of imaging techniques in order to detect plaques with vulnerable characteristics (echolucency, IPH, surface irregularity) [10].

However, the mechanism that results in the accumulation of erythrocytes within plaques (intraplaque hemorrhage) has been the subject of debate. Some researchers believe that repeated plaque fissuring and subsequent formation of a non-occlusive thrombus within the lumen, which is then incorporated into the plaque, is responsible [11]. Also, it has been hypothesized that without the intact endothelial cell layer due to the plaque fissures, the entrance of erythrocytes into the plaque can be expected [12]. Others argue that IPH results from leakage from intra-plaque neo-capillaries [13]. IPH correlates with increased age, sex, hypertension, smoking, and the use of platelet antiaggregant [12-14], while statin use is associated with a lower prevalence of IPH [15]. A previous cross-sectional analysis from the PARISK study showed that using antiplatelet agents prior to the index event was associated with IPH in symptomatic patients with carotid stenosis below 70% [14]. From the population-based Rotterdam Study, a higher frequency of IPH in carotid plaques was related to antithrombotic treatment [16]. Most of these previous studies had a cross-sectional design and, therefore, could not conclude on causality. The longitudinal relationship between antiplatelet agents use and IPH is warranted [19].

Magnetic resonance imaging (MRI) enables longitudinal studies and facilitates the examination of both symptomatic and asymptomatic patients, irrespective of the severity of carotid stenosis. Previous studies have demonstrated that IPH can be identified as a hyperintense signal within the plaque bulk on T1-weighted inversion recovery turbo field echo (IR-TFE) MR images, also known as magnetization-prepared rapid acquisition with gradient echo (MP-RAGE) images [17, 18]. High-resolution MRI also has high sensitivity and specificity (>90%) in the noninvasive detection of carotid IPH [19, 20]. IPH and lipid-rich necrotic regions can appear echolucent on ultrasound, making it challenging to distinguish between them. Similarly, Computed tomography angiography (CTA) has limited utility in accurately identifying IPH due to the overlap of Houndsfield values between IPH and the lipid-rich necrotic core. These strengths render MRI the ideal imaging modality for investigating temporal changes in IPH.

CTA enables the evaluation of plaque surface morphology, distinguishing between smooth vs. fissured/ulcerated surface [21]. Chapters 3 and 4 of this thesis investigated the relationship between the fibrous cap status on MRI, plaque surface status on CTA, and the use of antiplatelet agents on IPH and its volume changes over time.

A few years ago, the Vessel Wall Imaging Study Group of the American Society of Neuroradiology published a white paper recommending a multisequence MRI protocol for visualizing carotid plaque components. The recommended protocol includes dark blood pre-contrast T1-weighted imaging (T1w), post-contrast T1w imaging (or alternatively, T2w MRI), 3D time-of-flight (TOF) imaging, and 3D MPRAGE imaging [22]. However, limitations of the recommended protocol are the long scan time and difficulties with image misregistration of the different MRI weightings. Also, in most centers, additional MR sequences beyond the contrast-enhanced MR angiography (CE-MRA) or Time-of-flight (TOF) MRA to measure the degree of stenosis are not acquired during a routine carotid MRA examination. Due to the magnetic properties of blood products, IPH was identified as an area of high signal intensity within the bulk of the plaque on CE-MRA and TOF in a single-center study of 15 patients [23]. Chapter 5 focused on investigating the validation of routine imaging protocols, i.e., CE-MRA and TOF, for the identification of IPH. The aim was to assess the accuracy and reliability of these imaging techniques in detecting IPH.

Moreover, a few years ago, a new pulse sequence, i.e., Multi-contrast Atherosclerosis Characterization (MATCH), was introduced to overcome the long scan time and mis-registration of the multisequence protocols [24]. MATCH simultaneously provides three contrast weightings, hyper-T1w, grey-blood, and T2w, in a 5-min scan. The validation of using MATCH as a novel MR sequence to identify and quantify carotid plaque composition is still lacking compared to the recommended multisequence MRI protocol.

The main aim of this thesis was to get more insight into factors that contribute to the changes in carotid IPH volume on MRI over time. Furthermore, the accuracy of detecting intraplaque hemorrhage (IPH) in carotid arteries using routine MRA and quantifying the components of atherosclerotic carotid plaque through a fast novel MRI sequence was validated. The ultimate objective behind the latter efforts is to effectively apply carotid plaque MRI in clinical practice. We have answered the following research questions:

1- What is the relation between carotid fibrous cap status or plaque surface morphology and the progression of carotid IPH volume on carotid MR imaging?

2- How does the initiation of antiplatelet therapy after an ischemic cerebrovascular event impact the progression of carotid IPH on MRI over a two-year follow-up period?
3- What is the sensitivity and specificity of detecting carotid IPH on the mask images derived from routine contrast-enhanced magnetic resonance angiography (CE-MRA) compared to the reference standard MPRAGE?
4- Can the fast novel MRI sequence known as MATCH be utilized to accurately study carotid plaque composition?

In the following paragraphs, the main findings of this thesis are discussed. In addition, the etiology of intraplaque hemorrhage and the potential applications of carotid plaque MRI in clinical practice are described.

WHAT IS THE RELATION BETWEEN CAROTID FIBROUS CAP STATUS OR PLAQUE SURFACE MORPHOLOGY AND THE PROGRESSION OF CAROTID IPH VOLUME?

In Chapter 3, we reported that symptomatic patients with a carotid plaque of at least 2-3 mm thick and less than 70% stenosis with a thin or ruptured fibrous cap (TRFC) on magnetic resonance imaging (MRI) or a disrupted plaque surface on computed tomography angiography (CTA) had a significantly larger IPH volume at baseline than those with a thick cap/smooth plaque surface. During the two years of follow-up, the volume of IPH tended to decrease in the patients with TRFC or disrupted plaque surface, indicating plaque healing in some patients two years after the ischemic cerebrovascular symptoms. However, the patients with TRFC or disrupted plaque surface were still at increased risk of IPH progression. In contrast, patients with a thick FC or smooth plaque surface showed hardly any IPH at baseline and follow-up. Furthermore, an additional explorative analysis showed that patients who initially had TRFC but developed a thick fibrous cap during the follow-up period experienced a (non-significant) decrease in the volume of IPH, indicating regression in IPH volume and a path toward plaque stability. On the other hand, patients who maintained a TRFC at both baseline and follow-up showed a (non-significant) increase in IPH volume, indicating progression in IPH volume and a continuation of plaque vulnerability. This was not the case in patients with thick fibrous cap/smooth plaque surface where the small volume of IPH or the absence of IPH did not change over time. The results of our additional exploratory analysis with a small sample size were not statistically significant. Larger studies are warranted to further investigate this.

There is an ongoing debate regarding the mechanisms underlying the occurrence of intraplaque hemorrhage (IPH). One point of view is that IPH is primarily caused by repeated plaque fissuring or rupture. According to this viewpoint, the integrity of the plaque's fibrous cap is compromised multiple times, leading to the release

of blood into the plaque. However, an alternative point of view suggests that IPH arises from the leakage of blood from neo-capillaries within the plaque itself [25]. Proponents of this argument argue that these newly formed vessels within the plaque can be fragile and prone to leakage, resulting in the development of IPH. This debate highlights the differing opinions among researchers regarding the primary cause of IPH and underscores the complexity of understanding its underlying mechanisms. Several cross-sectional studies have shown an association between TRFC/disrupted plaque surface and IPH [26-30]. Lovett et al. showed that ulcerated plaque surface on angiography is strongly associated with IPH (OR=17.0, 95% CI=2.0-147, P=0.02) [25]. In addition, the presence of IPH, as assessed on MRI, is significantly associated with plaque ulceration on CTA [30]. The PARISK study also confirmed the association between IPH on MRI and disrupted plaque surface on MDCTA (OR: 3.13; 95% CI: 1.25-7.85) [31]. A study by Cui et al. showed an independent positive association between the volume of IPH and fibrous cap disruption in carotid arteries as determined on TOF images in 37 symptomatic patients (OR: 1.8, 95% CI:1.1-3.1) [32]. The previous studies were cross-sectional and did not examine the longitudinal changes in IPH volume over time. As a result, it is not possible to establish a causal relationship based on these previous studies. Chapter three explored the changes in IPH volume over time and its correlation with the fibrous cap/plaque surface status at baseline and at follow-up. A cross-sectional association has also been suggested between the volume of IPH and cerebrovascular events [33, 34]. Saba et al. showed that patients with cerebrovascular symptoms (n=46) had significantly larger IPH volume on CT images than asymptomatic patients (n=77) (115 vs. 2 mm^3; p=0.001) [33]. Moreover, they found that a threshold of 50 mm^3 showed the best association with cerebrovascular symptoms. Also, Liu et al. demonstrated in 687 patients with carotid atherosclerotic plaques that the cross-sectional size of IPH is independently associated with an ipsilateral acute cerebral infarction (OR=2.5, p=0.003) [34]. In chapter three, we showed in an explanatory analysis that patients who showed IPH progression after two years of follow-up were at higher risk for future stroke. However, this novel explorative analysis had a small sample size since only nine cerebrovascular events occurred after the two-year carotid MRI examination, while typically, at least ten events are required for each degree of freedom.

Note that after a TIA or stroke, patients in the Netherlands receive optimized medical treatment (statins, antihypertensive medication, and antiplatelet agents) for long-term secondary prevention after stroke. Although the current study showed no significant difference in medication usage between the groups (thick vs. TRFC, smooth surface vs. disrupted, and progression vs. regression), it is important to acknowledge the inability to eliminate the influence of medication during the follow-up period due to the absence of a control group receiving no medical treatment. However, it would be

unethical to perform such a study. Another constraint of our study is that future studies should also investigate the relationship between changes in IPH volume over time and changes in vessel wall K^{trans}, an indicator of reduced microvascular flow, density, and leakiness. This can be achieved using dynamic contrast-enhanced MRI. Conducting such research would provide valuable insights into the dynamic nature of intraplaque hemorrhage and its association with microvascular alterations within the plaque.

In conclusion, the findings of the current study indicate that TIA/stroke patients with a thin ruptured fibrous cap (TRFC) or a disrupted carotid plaque surface exhibit a notably larger initial volume of intraplaque hemorrhage (IPH) compared to patients with a thick fibrous cap and a smooth plaque surface. Interestingly, over the following two years, some of these patients demonstrated a healing process characterized by reduced IPH volume. This suggests that the plaque experienced a recovery or stabilization during that period. However, patients with a TRFC or disrupted plaque surface at baseline remain at an increased probability of IPH progression compared to those with a thick fibrous cap or smooth plaque surface. Consequently, individuals with a TRFC and disrupted plaque surface may benefit from more frequent monitoring. The present longitudinal study gave us more insight into the relationship between TRFC or surface disruption and IPH volume.

Moving forward, future studies with a larger sample size should investigate the predictive value of carotid IPH progression for future events and the impact of changes in fibrous cap status or plaque surface morphology on IPH progression. These areas warrant further exploration to enhance our understanding of IPH development and management of this patient population.

DOES THE INITIATION OF ANTIPLATELET THERAPY AFTER AN ISCHEMIC CEREBROVASCULAR EVENT IMPACT THE PROGRESSION OF CAROTID IPH ON MRI OVER A TWO-YEAR FOLLOW-UP PERIOD?

As a standard therapeutic procedure, medical professionals commonly prescribe platelet aggregation inhibitors in TIA or stroke patients to mitigate the risk of reoccurrence of ischemic events. However, the risk of bleeding complications will increase [35]. In chapter four, we hypothesized that individuals who initiate antiplatelet therapy following an ischemic stroke (referred to as “new antiplatelet users”) are at higher risk of experiencing new carotid IPH and IPH progression on MRI after two years of follow-up compared to patients who were already utilizing antiplatelet agents prior to the initial event.

The results of our study confirmed the previously established association between the presence of IPH at the baseline and prior use of antiplatelet therapy in symptomatic

patients with ipsilateral nonsignificant carotid artery stenosis (<70%). However, over the course of the two-year follow-up period, neither the frequency nor the volume of IPH demonstrated a significant increase in either the new users or the continued users of antiplatelet agents. Furthermore, although a slightly higher prevalence of new IPH was observed among the new antiplatelet users, we did not find a significant association between the start of using antiplatelet agents and the development of new IPH or progression in IPH volume during the two-year follow-up.

In line with our baseline analysis, a higher occurrence of IPH was observed in coronary segments obtained during autopsy from patients using antiplatelet therapy [36]. In a histopathological analysis of 154 endarterectomy specimens, another study demonstrated a significantly larger percentage of multiple carotid IPH in patients who were using antiplatelet therapy (80.1% vs. 19.7%; p<0.001) [37]. However, another histopathological study conducted on a large group of carotid endarterectomy patients did not find any association between using antiplatelet agents and the prevalence of IPH [13].Within a subpopulation of the Rotterdam Study, comprising individuals with a carotid plaque larger than 2.5 mm in at least one of the carotid arteries, there was a (nonsignificant) association observed between current and past use of antiplatelet agents and a higher prevalence of carotid IPH [16]. A prior investigation based on baseline data from the PARISK study of the first 100 patients also revealed a link between the presence of IPH and the use of antiplatelet agents before the index event [14]. Moreover, in another study, the utilization of dual antiplatelet agents by patients with asymptomatic carotid artery stenosis was found to be associated with the progression from moderate stenosis (50-69%) to severe stenosis (>70%) after 2.6 years of follow-up [38]. However, this study did not examine plaque composition or IPH. Furthermore, a recent investigation involving 34 symptomatic patients with carotid IPH and a follow-up period of at least six months revealed that atherosclerotic plaques were significantly more likely to be in the progressed IPH group if the patients were using antiplatelet therapy at baseline (86.7% vs. 53.3%, p=0.046) [39]. However, the study did not report whether the patients were still using antiplatelet therapy during the follow-up period. In our study, all patients were under antiplatelet therapy throughout the entire follow-up period.

Our study cohort was restricted to individuals presenting with ipsilateral carotid artery stenosis below the threshold of 70% without clinical indication for revascularization surgery or stenting. Patients with symptomatic severe stenosis exceeding the 70% threshold are promptly referred for surgical intervention in the Netherlands, thereby precluding the possibility of conducting follow-up imaging for IPH. The current study is limited by the absence of a control group comprising patients who did not receive antiplatelet therapy at baseline and during the follow-up period. Antiplatelet agents

are recommended after a stroke; therefore, such a study would be unethical [40]. Future investigations could be conducted involving asymptomatic individuals (not mandatory to use antiplatelet agents) with and without IPH in the carotid plaque. By including both individuals who are using antiplatelet agents and those who are not, the relationship between the use (or non-use) of antiplatelet therapy and the temporal changes in the presence or volume of IPH can be explored.

Summarizing, among stroke patients with mild to moderate carotid artery stenosis, those who had a history of antiplatelet therapy exhibited a notably higher prevalence of IPH at the baseline assessment. However, our study did not identify any significant association between the initiation of antiplatelet agents and the occurrence of newly developed IPH or the progression of IPH volume over a two-year follow-up period. Further investigations are warranted to delve deeper into the relationship between the use of antiplatelet agents and the progression of IPH in asymptomatic individuals with carotid stenosis.

WHAT IS THE SENSITIVITY AND SPECIFICITY OF DETECTING CAROTID IPH ON THE MASK IMAGES DERIVED FROM ROUTINE CONTRAST-ENHANCED MAGNETIC RESONANCE ANGIOGRAPHY (CE-MRA) COMPARED TO THE REFERENCE STANDARD MPRAGE?

Our findings in Chapter 5 indicate a strong consensus among observers in identifying intraplaque hemorrhage (IPH) using mask MRA and TOF images. Additionally, we observed a notably high specificity in detecting IPH on both mask and TOF images, particularly when observers utilized black blood T1w MR images to identify the outer vessel wall. However, the sensitivity of IPH detection differed between mask and TOF images. On mask images, the sensitivity was high, whereas, on TOF images, it was found to be poor. Furthermore, the specificity and sensitivity of scoring IPH on mask images without the aid of these black blood T1w images were high and moderate, respectively. When it came to TOF images, the specificity was moderate, while the sensitivity was poor.

Numerous research studies have confirmed the value of IPH on MRI as a strong predictor of stroke [43, 44]. Consequently, there has been a growing interest in utilizing MRI sequences to visualize IPH over the past few decades [19]. In 2003, Moody discovered that MP-RAGE (Magnetization Prepared-Rapid Gradient Echo) was capable of detecting IPH [19]. Compared to two-dimensional T1-weighted fast spin echo and 3D TOF sequences, Ota et al. conducted a comparative analysis and demonstrated that MP-RAGE is the most accurate T1-weighted sequence for detecting IPH in terms of specificity (97%) and sensitivity (80%) [45]. Similarly, Cappendijk et al. observed a high detection rate (81-93%) for IPH on MP-RAGE images, whereas the detection rate

was lower (72-91%) on T1-weighted Turbo Spin Echo (TSE) images when compared to histology as the reference standard [20]. Recent expert consensus recommendations on vessel wall MR imaging protocols have also advised that MP-RAGE is particularly well-suited for IPH detection [25, 46]. Despite the acknowledged diagnostic value of MP-RAGE and similar sequences in detecting IPH, they have not yet become a routine part of clinical investigations for carotid artery disease.

Our findings in chapter five align with earlier research conducted by Qiao et al. [26]. In their study involving 15 patients, they reported a specificity of 99% and sensitivity of 87% in detecting IPH on mask images, using histology as a reference standard. Furthermore, the inter-observer agreement for IPH detection on mask images (κ=0.91) was comparable to our present study. On TOF images, Qiao et al. demonstrated a high specificity and sensitivity of IPH detection (87% and 79%) using histology as the reference standard. However, while we confirmed the high specificity in our study, we observed a lower sensitivity in scoring IPH on TOF images in a larger group of patients when MP-RAGE was used as the reference standard.

Detecting the outer vessel wall can be challenging when examining mask images, especially in the absence of fat suppression. As a result, small regions with high signal intensity in the plaque on mask images can be mistakenly identified as perivascular tissue instead of intraplaque hemorrhage. Our study revealed that the sensitivity of IPH detection on mask images decreased when the high-resolution black blood sequence, which aids in visualizing the outer vessel wall, was not utilized. However, the specificity remained consistently high. Our study encountered a few false negative cases where IPH was not detected. These cases typically exhibited a small IPH volume. This discrepancy may be attributed to the lower spatial resolution of mask images in comparison to MP-RAGE images, which could limit the visualization of small IPH regions.

Additionally, two false positive cases were identified on TOF images. These cases were associated with ulceration, which can also appear as a high signal-intensity region on TOF images. The similarity in signal intensity between ulceration and IPH on TOF images may lead to false positive results. Therefore, relying solely on TOF images for IPH detection may lead to difficulties in distinguishing flowing blood within an ulcer from true IPH regions. However, mask images exhibited fewer false-positive cases since flowing blood does not appear bright in these images.

In the near future, the inclusion of IPH identification in the clinical assessment of carotid atherosclerotic disease is expected to improve patient stratification for the appropriate treatment strategy. Current imaging modalities, such as ultrasound, are

less suited to differentiate IPH from the lipid-rich necrotic core, while CT angiography also has limited utility due to the overlapping distribution of Houndsfield values between IPH and lipid core. In contrast, MR imaging provides superior soft-tissue contrast compared to other modalities, making it suitable for in vivo detection of IPH. Recently, a novel approach called Simultaneous Non-contrast Angiography and Intraplaque Hemorrhage (SNAP) MR imaging has been introduced, aiming to detect both luminal stenosis and hemorrhage in patients with carotid plaques using a single sequence [47]. Strong agreement (κ=0.82, p<0.001) was found between SNAP and MP-RAGE images in detecting intraplaque hemorrhage (IPH). Additionally, a faster variant known as fast SNAP (fSNAP) demonstrated comparable performance to SNAP but with a 37.5% reduction in scan time [48]. Another development, Multi-Contrast Atherosclerosis Characterization (MATCH), enables imaging of IPH and other vulnerable plaque components using a single multi-contrast sequence [27]. However, it is important to note that most of these sequences are not readily available on standard clinical MRI scanners. CE-MRA, or non-contrast-enhanced methods like TOF, are routinely acquired in many medical centers worldwide. Hence, the ability to detect carotid IPH on mask images holds clinical relevance for both symptomatic and asymptomatic patients, as it serves as a robust predictor for future ischemic stroke.

Our study has a few limitations that should be acknowledged. First, there was a lack of histological validation. Our study was a substudy of the second European Carotid Surgery Trial (ECST-2), and the storage of surgical specimens and subsequent histological analysis in the surgery arm were not included in the study design. Instead, we utilized MP-RAGE as a reference standard, which has been validated with histology in numerous previous studies [20, 49]. Another limitation is that, based on the ECST-2 inclusion criteria, our validation of mask images was conducted in asymptomatic and symptomatic patients with moderate to severe stenosis, characterized by a 5-year risk of ipsilateral stroke estimated to be less than 20% according to the Carotid Artery Risk score. It remains uncertain whether intraplaque hemorrhage detection on mask images can be extended to patients with mild carotid stenosis or non-stenotic carotid plaques.

Apart from the conventional practice of measuring the degree of stenosis of the carotid artery using CE-MRA, our study revealed that the mask images CE-MRA can serve as a valuable tool for scoring the presence of IPH. Furthermore, incorporating an additional black blood sequence to visualize the outer vessel wall can significantly enhance the accuracy of IPH assessment. By integrating IPH scoring into the routine assessment of CE-MRA, clinicians can gain supplementary information about the risk of stroke and make informed decisions regarding treatment strategies.

CAN THE FAST NOVEL MULTI-CONTRAST ATHEROSCLEROSIS CHARACTERIZATION (MATCH) MRI SEQUENCE ACCURATELY QUANTIFY CAROTID PLAQUE COMPOSITION?

In Chapter 6 of this thesis, we conducted a validation study of the MATCH sequence using a multi-sequence carotid MRI protocol that had been recommended in a white consensus paper as a reference standard. Our findings revealed a substantial-to-excellent level of agreement among observers when it came to detecting and quantifying the carotid atherosclerotic plaque components using MATCH, with the exception of calcifications, which exhibited a fair-to-moderate agreement.

Furthermore, we observed high sensitivity and specificity in identifying intraplaque hemorrhage (IPH) and lipid-rich necrotic core (LRNC) on the MATCH images, using the multi-sequence MRI as a reference standard. Additionally, the quantification of vulnerable carotid plaque components, i.e., IPH and LRNC, on the MATCH images demonstrated good to excellent agreement with the quantification obtained using the multi-sequence protocol. However, we observed moderate and poor agreement for the total volume of fibrous tissue and calcifications, respectively. Interestingly, The MATCH demonstrated significantly shorter scan and image analysis times. These findings highlight the advantages of MATCH over the conventional multi-sequence protocol in terms of shorter scan and image analysis time. MATCH substantially reduces scan and image analysis time while maintaining reliable performance in identifying and quantifying the most important carotid plaque components.

Dai et al. [49] conducted a study demonstrating that MATCH performs on par with, if not surpasses, a conventional MRI protocol for the identification and quantification of the major carotid plaque components. This previous study utilized T1 weighted (T1w) turbo spin echo (TSE), T2w TSE, and time of flight (TOF) images as a reference standard. However, it is worth noting that the quantification of the LRNC using T2w TSE, as employed in this previous study, is inferior compared to contrast-enhanced T1w TSE, which was used in this thesis [50]. Also, this study did not use a dedicated sequence for the detection of intraplaque hemorrhage, as recommended in a white paper. When comparing MATCH to magnetization-prepared rapid acquisition gradient echo (MPRAGE), both techniques exhibit similar scan times and can effectively identify IPH, an important risk factor in stroke prediction [9, 51]. However, MATCH has the advantage of providing multiple co-registered images without any additional time penalty. These images provide the opportunity to also identify additional plaque features, including the LRNC, which cannot be accomplished solely with MPRAGE. Moreover, the MPRAGE sequence incorporates a nonselective inversion pre-pulse for dark blood images, which is limited to the body coil's field of view. In MPRAGE images, inadequate blood suppression can occur due to blood flowing from outside the field

of view of the body coil and entering the imaging slices.

Consequently, in cases where the suppression of luminal blood signals is not entirely achieved, slight off-centering of the patient's position towards the feet direction may be necessary. In contrast, the hyper T1w sequence used in MATCH employs a flow-sensitive dephasing (FSD) preparation prior to the acquisition, effectively suppressing luminal blood signals [27, 52]. Unlike the MPRAGE technique, the FSD-prepared sequence in MATCH is flow-dependent and not reliant on the inflow of blood [52].

Building upon this previous work, our current study goes further by incorporating additional practical outcome variables for assessment. We specifically measured the total scan time, encompassing the acquisition and planning time. Furthermore, we compared the time required to accurately identify and quantify carotid plaque features and composition for an individual carotid plaque. By considering these aspects and conducting these measurements, our study offers a comprehensive evaluation of MATCH, highlighting its potential advantages over the conventional protocol in terms of accuracy, efficiency, and practicality.

One limitation of the MATCH sequence compared to the multisequence MRI protocol is that, as far as we know, MATCH does not allow us to assess the fibrous cap status. A thin or ruptured fibrous cap on MRI is a well-established risk factor for recurrent stroke [53, 54]. This assessment can be easily conducted using post-contrast T1w images as part of a multi-sequence protocol [50, 55]. However, this MRI weighting is not available since MATCH is a non-contrast technique. Although there was a limitation in terms of lower image quality, particularly in hyper T1 images, it is worth noting that hyper T1 images are specifically employed to identify IPH. The contrast-to-noise ratio of IPH on hyper T1 images is typically high. Consequently, the lower quality of hyper T1 images did not have an impact on the accurate identification and quantification of IPH. Additionally, the lower image quality observed in T2-weighted and gray-blood MATCH images may account for the decreased performance of MTACH in quantifying the overall volume of fibrous tissue and calcifications.

A limitation of our study is that the majority of patients exhibited only intermediate or mild degrees of stenosis (<70%). Carotid endarterectomy is considered beneficial for patients with symptomatic carotid stenosis in the high-grade range (70-99%) [56]. Therefore, future investigations may compare MATCH results with histological findings as the gold standard in this patient group. It is worth noting that symptomatic patients with mild to moderate stenosis (<70%) still face an increased risk of recurrent ischemic events, and the appropriateness of carotid endarterectomy in this context remains uncertain [57]. Thus, studying the risk factors associated with plaque components

such as IPH and LRNC using a concise carotid MRI protocol that includes inherently co-registered images can provide valuable insights for improved patient management. In summary, MATCH offers an attractive time-efficient approach to identifying and quantifying carotid plaque composition, with the exception of assessing calcifications and fibrous cap status. The inherent co-registration of the images and the efficient time required for scanning and image analysis are important advantages of the MATCH technique.

DECIPHERING THE ETIOLOGY OF INTRAPLAQUE HEMORRHAGE

Intraplaque hemorrhage (IPH) is a crucial phenomenon observed within atherosclerotic plaques, contributing to plaque vulnerability and increasing the risk of neurovascular events. Investigating the etiology of IPH provides crucial insights into the underlying mechanisms contributing to its formation and progression, enabling the development of targeted interventions to prevent or mitigate its adverse consequences. Advanced imaging techniques, particularly magnetic resonance imaging (MRI), have revolutionized our ability to detect, quantify, and characterize IPH, offering valuable diagnostic and prognostic information.

The various chapters of this thesis have contributed significantly to our understanding of factors influencing the development and progression of intraplaque hemorrhage (IPH). In Chapter 3, our findings revealed that patients with thin or ruptured fibrous cap (TRFC) or disrupted plaque surface exhibited larger IPH volumes at the time of the index event compared to those with a thick fibrous cap/ smooth plaque surface. Additionally, we demonstrated that patients with TRFC or disrupted plaque surface face a higher risk of IPH progression on MRI during a two-year follow-up period.

In Chapter 4, our study examined the impact of antiplatelet usage on IPH progression. We found that patients who initiated antiplatelet therapy at the time of the index event did not exhibit a higher risk of IPH progression compared to patients who continued using antiplatelet medications. However, it is important to note that we were unable to directly compare the group starting antiplatelet therapy with those not using antiplatelet medication due to current clinical guidelines mandating antiplatelet use for all patients following an ischemic event.

The findings of chapters three and four provide valuable insights into the factors influencing IPH development and progression, highlighting the importance of plaque biomechanics and the potential role of antiplatelet therapy in mitigating IPH progression. Further research with larger cohorts could shed more light on these associations.

CAROTID MRI IN CLINICAL PRACTICE: OPPORTUNITIES AND CHALLENGES FOR IMPROVED PATIENT CARE

Over the past two decades, magnetic resonance imaging (MRI) has emerged as the preferred modality for studying carotid plaque features. Its high resolution and multi-contrast capabilities enable the identification and quantification of the key components of atherosclerotic plaque, including intraplaque hemorrhage. The validity of these techniques has been extensively established by comparing MRI findings to histopathology. However, the integration of the current MRI protocol into clinical practice is tough and faces various challenges.

These challenges include worldwide variations in MRI protocols due to MRI systems from different vendors, the need for dedicated carotid coils for high-resolution imaging, and the need for dedicated software for quantitative image analysis. But probably most of all, clinicians need to be convinced that plaque imaging by MRI is beneficial for patient selection and clinical decision-making in CEA. Lengthy scan times, the need for contrast medium administration for improved detection of the fibrous cap status, and the requirement for multiple MR weightings to comprehensively study carotid plaque composition further contribute to the obstacles towards clinical translation. These factors result in issues such as image mis-registration and prolonged analysis times, impeding the widespread adoption of these protocols.

In Chapter 5, we demonstrated the high specificity and sensitivity of detecting IPH on the source images (mask) of routine contrast-enhanced magnetic resonance angiography (MRA). These findings pave the way for incorporating IPH identification into the clinical assessment of carotid atherosclerotic disease.

In Chapter 6, we also showed high sensitivity and specificity in the identification of IPH and lipid-rich necrotic core (LRNC) using MATCH, a novel fast MR sequence. This indicates that MATCH has the potential to be integrated into daily clinical carotid MRI work.

Furthermore, novel MR sequences such as Simultaneous Non-contrast Angiography and IPH (SNAP) and Bright-blood and black-blOOd phase SensiTive inversion recovery sequence (BOOST) have been developed. These sequences also allow the generation of multi-contrast imaging with a single sequence, significantly reducing scan time and providing inherent image co-registration. However, these novel sequences need further validation.

Artificial intelligence (AI) may further enhance MR image quality, shorten analysis times, and offer tools for automated image analysis. Developing and validating clinical

risk prediction models incorporating carotid plaque MRI findings as well as clinical risk factors in randomized clinical trials can further improve risk stratification.

Overall, the advancements in carotid MRI techniques and the exploration of new sequences and AI integration hold promise for overcoming current challenges and translating plaque imaging with MRI toward clinical practice.

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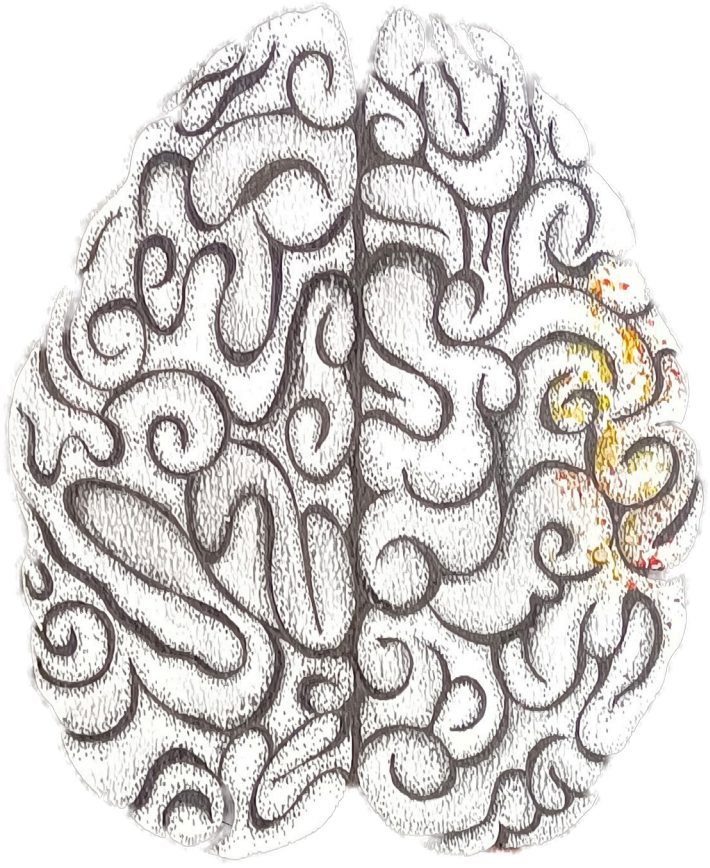

CHAPTER 8

SUMMARY

Estimations show that approximately 15% of transient ischemic attacks (TIAs) and ischemic strokes are associated with carotid atherosclerotic plaques. Currently, clinical decision-making in the treatment of stroke patients with carotid artery plaques primarily relies on the degree of stenosis of the internal carotid artery. However, recent findings have highlighted the importance of plaque composition, particularly the presence of intraplaque hemorrhage (IPH) detected on Magnetic Resonance Imaging (MRI), as a strong predictor of stroke risk in patients with carotid plaque.

Despite strong evidence linking IPH to increased stroke risk, the factors contributing to IPH development remain incompletely understood. IPH may originate from the entry of blood from the lumen due to the fissuring or rupture of the fibrous cap. Previous cross-sectional studies have demonstrated an association between the presence of IPH and thin or ruptured fibrous cap (TRFC) detected on MR images, as well as fissured or ulcerated plaque surface observed on computed tomography angiography (CTA) images.

Furthermore, the use of antiplatelet agents, while beneficial in reducing thrombus formation, has been associated with an elevated risk of bleeding complications and IPH. However, the longitudinal changes in IPH volume in relation to these factors have not been investigated before.

Additionally, the identification of IPH relies on advanced MR sequences beyond routine contrast-enhanced MR angiography (CE-MRA) or non-contrast MRA, such as time-of-flight (TOF), for stenosis assessment. However, these additional advanced sequences are often omitted in clinical practice due to time constraints and since they are not yet part of the clinical guidelines. Recently, a novel and fast MR sequence called Multi-contrast Atherosclerosis Characterization (MATCH) has been introduced for imaging carotid atherosclerotic plaque composition.

This thesis aims to explore various factors contributing to changes in IPH volume in symptomatic carotid plaques of stroke patients over a two-year follow-up period. Moreover, it seeks to determine the diagnostic accuracy of identifying IPH using routine carotid MRI (i.e., mask images of CE-MRA and TOF images) and quantifying carotid plaque composition using a novel MR sequence (i.e., MATCH).

WHAT IS THE RELATION BETWEEN CAROTID FIBROUS CAP STATUS OR PLAQUE SURFACE MORPHOLOGY AND THE PROGRESSION OF CAROTID IPH VOLUME ON CAROTID MR IMAGING?

In Chapter Three of this thesis, we reported that patients with a TRFC or a disrupted plaque surface had a significantly larger IPH volume at baseline than those with a thick cap/smooth plaque surface. During the two years of follow-up, the overall volume of IPH tended to decrease in the patients with TRFC or disrupted plaque surface, indicating plaque healing, while patients with a thick FC or smooth plaque surface showed hardly any IPH at baseline and follow-up. However, patients with TRFC or disrupted plaque surface were still at increased risk of IPH progression. Mainly patients who maintained a TRFC at both baseline and follow-up showed IPH progression. These observations indicate that a TRFC or disrupted plaque surface contributes to the development of IPH.

HOW DOES THE INITIATION OF ANTIPLATELET THERAPY AFTER AN ISCHEMIC CEREBROVASCULAR EVENT IMPACT THE PROGRESSION OF CAROTID IPH ON MRI OVER A TWO-YEAR FOLLOW-UP PERIOD?

In clinical practice, platelet aggregation inhibitors are frequently prescribed as a standard therapeutic approach to reduce recurrent ischemic events in patients diagnosed with TIA or stroke. However, it is important to acknowledge that antiplatelet therapy is also associated with an increased risk of bleeding complications, including IPH. In Chapter Four, we demonstrated that individuals who initiate antiplatelet therapy following an ischemic stroke are not at a higher risk of developing new carotid IPH or experiencing IPH progression on MRI during a two-year follow-up period, in comparison to patients who were already receiving antiplatelet treatment prior to the initial event. No significant association was found between new antiplatelet agent use and the formation of new IPH or IPH volume progression during the two-year follow-up.

WHAT IS THE SENSITIVITY AND SPECIFICITY OF DETECTING CAROTID IPH ON THE MASK ON TOF IMAGES DERIVED FROM ROUTINE CE-MRA COMPARED TO THE REFERENCE STANDARD MPRAGE?

The Magnetization-Prepared Rapid Acquisition Gradient (MP-RAGE) sequence is commonly employed for the detection of IPH. CE-MRA is routinely utilized for the assessment of stenosis and has the potential to be used for IPH identification. In Chapter Five of this thesis, we demonstrated a strong consensus among the observers in detecting IPH on both mask images of CE-MRA and TOF images, particularly when utilizing black blood T1-weighted (T1w) MR images to assess the outer vessel wall. Notably, we observed a high specificity in identifying IPH on both mask and TOF

images. Evaluation of the mask images also exhibited a high sensitivity, while TOF images displayed poor sensitivity for IPH identification. Moreover, the specificity of IPH detection on mask images remained consistent even without the assistance of black blood T1w images while the sensitivity reduced. However, for TOF images, the specificity was moderate, and the sensitivity was found to be poor.

IS THE FAST NOVEL MULTI-CONTRAST ATHEROSCLEROSIS CHARACTERIZATION (MATCH) MRI SEQUENCE ABLE TO ACCURATELY QUANTIFY CAROTID PLAQUE COMPOSITION?

In Chapter Six of this thesis, we conducted a validation study to assess the efficacy of the MATCH sequence as a fast imaging method that inherently aligns the images. We used a multi-sequence carotid MRI protocol as a reference standard. Our study findings demonstrated an excellent level of agreement among observers in the detection and quantification of various carotid atherosclerotic plaque components on MATCH images. However, there was fair to moderate agreement for calcifications. Moreover, MATCH images exhibited high sensitivity and specificity in identifying IPH and the lipid-rich necrotic core (LRNC). The quantification of vulnerable carotid plaque components, i.e., IPH and LRNC, on MATCH images showed good to excellent agreement with the quantification obtained using the multi-sequence protocol. However, moderate and poor agreement was observed for the total volume of fibrous tissue and calcifications, respectively. The acquisition and image analysis time of MATCH were significantly less compared to the conventional multi-sequence protocol. These findings underscore the advantages of utilizing MATCH over conventional multi-sequence protocols, as it significantly reduces scanning and image analysis time while maintaining reliable performance in the identification and quantification of the most important carotid plaque components.

DISCUSSION AND CONCLUSION

The findings presented in this thesis are discussed in Chapter Seven. This thesis contributes to the understanding of the development of intraplaque hemorrhage over time. Furthermore, our findings aim to facilitate the application of carotid plaque magnetic resonance imaging in clinical practice, providing valuable insights for medical professionals in assessing and managing patients with a carotid plaque. Unlike previous research, which predominantly focused on the association between intraplaque hemorrhage and other risk factors using cross-sectional study designs, in this thesis, longitudinal magnetic resonance imaging studies were performed, indicating the potential involvement of a thin/ruptured fibrous cap, fissures/ulcers on the development and progression of intraplaque hemorrhage. Also, we explored the relationship between the new onset of antiplatelet treatment in the development and progression of intraplaque hemorrhage. Additionally, we demonstrate that

intraplaque hemorrhage, as an independent risk factor for recurrent ischemic stroke, can be accurately identified on the mask images of routine contrast-enhanced MR angiography but not on time-of-flight images. Furthermore, we establish that the novel and fast MATCH MRI sequence can accurately quantify IPH and the LRNC. Large randomized trials to investigate the impact of carotid plaque MRI on patient outcome are warranted.

DUTCH SUMMARY - SAMMENVATTING

Ongeveer 15% van de “Transient Ischaemic Attacks” (TIA’s) en herseninfarcten zijn gerelateerd aan de aanwezigheid van een zogenaamde atherosclerotische plaque in de halsslagader (aderverkalking). De aanwezigheid van een dergelijke plaque kan leiden tot een vernauwing van het bloedvat. De plaque bestaat uit een vetrijke kern die afgeschermd wordt van het lumen (de centrale holte van het bloedvat waar het bloed doorheen stroomt) door een kapsel van bindweefsel. Als dit kapsel scheurt, dan komt het bloed in contact met de vetrijke kern van de plaque. Hierdoor kan er plotseling een bloedstolsel ontstaan op de plaque. Vervolgens kunnen er bloedpropjes losschieten uit dit stolsel, waardoor een bloedvat verderop in de hersenen verstopt kan raken, waardoor een herseninfarct ontstaat.

De behandeling van patiënten met een TIA of herseninfarct met een plaque in de halsslagader is momenteel vooral gebaseerd op de mate van vernauwing en niet op de kenmerken van de plaque zelf. Er is echter steeds meer bewijs dat de samenstelling van de plaque, met name de aanwezigheid van een zogenaamde intraplaque bloeding op Magnetische Resonantie Imaging (MRI), een sterke voorspeller is van het risico op een (terugkerend) herseninfarct bij patiënten met een plaque in de halsslagader.

Ondanks sterk bewijs dat een intraplaque bloeding het risico op een herseninfarct aanzienlijk verhoogd, zijn de mechanismes die bijdragen aan een dergelijke bloeding nog niet volledig opgehelderd. Intraplaque bloeding kan veroorzaakt worden door het binnendringen van rode bloedcellen uit het lumen ten gevolge van het scheuren van het kapsel van de plaque of door de aanwezigheid van kleine scheurtjes in het kapsel. Eerdere cross-sectionele studies, waarbij gegevens worden verzameld op één tijdstip, hebben een verband aangetoond tussen de aanwezigheid van een intraplaque bloeding en een dun of gescheurd kapsel op MRI, evenals oneffenheden in het oppervlakte van de plaque op “Computed Tomography” angiografie (CTA)-beelden.

Na een doorgemaakte TIA of herseninfarct gebruiken mensen veelal een bloedplaatjes-remmer om nieuwe stolselvorming te voorkomen. Echter, deze medicatie leidt ook tot een verhoogd risico op bloedingen waaronder ook mogelijk intraplaque bloedingen. Eerder is aangetoond dat patiënten die plaatjesaggregatatieremmers gebruikten, vaker een intraplaque bloeding hadden. De veranderingen in het volume van de intraplaque bloeding over de tijd in relatie tot deze factoren is echter niet eerder onderzocht.

Bovendien is de identificatie van een intraplaque bloeding afhankelijk van geavanceerde MRI-methoden en niet onderdeel van de in de klinische setting routinematig gebruikte

contrastversterkte MR-angiografie (CE-MRA) of time-of-flight (TOF) MRI, waarmee de mate van vernauwing in de halsvaten bepaald wordt. De geavanceerde MRI-methoden worden in de klinische praktijk echter vaak weggelaten vanwege tijdgebrek en omdat ze nog geen deel uitmaken van de klinische richtlijnen. Onlangs is een nieuwe en snelle MRI-methode genaamd Multi-contrast Atherosclerosis Characterization (MATCH) geïntroduceerd voor het afbeelden van de samenstelling van atherosclerotische plaques in de halsslagader.

Dit proefschrift heeft tot doel verschillende factoren te onderzoeken die bijdragen aan intraplaque bloeding in patiënten met een beroerte of TIA met een plaque in de halsslagader gedurende een periode van twee jaar. Een tweede doelstelling is het bepalen van de nauwkeurigheid van het identificeren van een intraplaque bloeding met behulp van een routinematige halsslagader-MRI protocol (d.w.z. maskerafbeeldingen van CE-MRA- en TOF-afbeeldingen) en het kwantificeren van de samenstelling van een halsslagaderplaque met een nieuwe snelle MRI-methode (MATCH).

WAT IS DE RELATIE TUSSEN DE STATUS VAN HET KAPSEL VAN DE HALSSLAGADER-PLAQUE OF ONEFFENHEDEN IN HET PLAQUEOPPERVLAK EN DE TOENAME VAN HET VOLUME VAN DE INTRAPLAQUE BLOEDING OP MRI AFBEELDINGEN VAN DE HALSSLAGADER?

In hoofdstuk drie van dit proefschrift rapporteerden we dat patiënten met een dun of gescheurd kapsel of oneffenheden in het plaque oppervlak direct na de TIA of het herseninfarct een significant grotere intraplaque bloeding hadden dan patiënten met een dik kapsel/glad plaque-oppervlak. Na twee jaar was er een tendens van een afname van de intraplaque bloeding bij de patiënten met dun/gescheurd kapsel of een oneffen plaque-oppervlak, wat duidt op genezing van plaque, terwijl patiënten met een dik kapsel of een glad plaque-oppervlak nauwelijks intraplaque bloeding vertoonden direct na de TIA of het herseninfarct en ook niet 2 jaar later. Patiënten met dun of gescheurd kapsel of een oneffen plaque-oppervlak hadden echter nog steeds een verhoogd risico op een toename van de intraplaque bloeding. Met name patiënten die direct na de TIA of het herseninfarct en 2 jaar later een dun/gescheurd kapsel hadden, lieten een toename zien van de intraplaque bloeding. De waarnemingen die beschreven staan in hoofdstuk 3 geven aan dat een dun/gescheurd kapsel en een oneffen plaque-oppervlak bijdragen aan de ontwikkeling van een intraplaque bloeding.

WELKE INVLOED HEEFT DE START VAN PLAATJESAGGREGATIEREMMERS NA EEN TIA OF HERSENINFARCT OP DE PROGRESSIE VAN EEN INTRAPLAQUE BLOEDING IN DE HALSSLAGADER OP MRI GEDURENDE EEN PERIODE VAN TWEE JAAR?

In de klinische praktijk worden bloedplaatjesaggregatieremmers vaak voorgeschreven om de kans op een recidief te verminderen bij patiënten met een eerder doorgemaakte

TIA of herseninfarct. Plaatjesaggregatieremmers gaan ook gepaard gaat met een verhoogd risico op bloedingscomplicaties en dus mogelijk ook een intraplaque bloeding. In hoofdstuk vier hebben we aangetoond dat personen die na een TIA of herseninfarct starten met plaatjesaggregatieremmers geen hoger risico lopen op het ontwikkelen van een nieuwe intraplaque bloeding of progressie van de intraplaque bloeding op MRI gedurende een periode van twee jaar, in vergelijking met patiënten die voorafgaand aan de TIA of het herseninfarct al plaatsjesaggregatieremmers gebruikten. Er werd geen significant verband gevonden tussen nieuw gebruik van plaatjesaggregatieremmers en het ontstaan van een nieuwe intraplaque bloeding of progressie van de intraplaque bloeding in een periode van twee jaar.

WAT IS DE SENSITIVITEIT EN SPECIFICITEIT VOOR HET DETECTEREN VAN EEN INTRAPLAQUE BLOEDING IN DE HALSSLAGADERPLAQUE OP DE MASKERAFBEELDINGEN VAN ROUTINEMATIG VERKREGEN CE-MRA EN TOF AFBEELDINGEN IN VERGELIJKING MET DE REFERENTIESTANDAARD MPRAGE?

De Magnetization-Prepared Rapid Acquisition Gradient (MP-RAGE) MRI-methode wordt vaak gebruikt voor de detectie van een intraplaque bloeding. CE-MRA en TOF worden routinematig gebruikt voor de beoordeling van de mate van vernauwing van een bloedvat en kunnen mogelijk worden gebruikt voor het identificeren van een intraplaque bloeding. In hoofdstuk vijf van dit proefschrift hebben we een sterke overeenstemming aangetoond tussen 2 waarnemers in het detecteren van een intraplaque bloeding op zowel de maskerbeelden van CE-MRA- als TOF-afbeeldingen, met name wanneer gedetailleerde MRI beelden worden gebruikt om de buitenste vaatwand goed zichtbaar te maken. Intraplaque bloeding kon worden waargenomen met een hoge specificiteit op zowel de masker-CE-MRA als de TOF-afbeeldingen. De sensitiviteit was ook hoog voor de beoordeling van de masker-CE-MRA afbeeldingen, terwijl TOF-afbeeldingen een slechte sensitiviteit lieten zien voor de identificatie van een intraplaque bloeding. Bovendien bleef de specificiteit van een intraplaque bloeding-detectie op masker CE-MRA afbeeldingen goed, zelfs zonder de hulp van gedetailleerde MRI afbeeldingen, terwijl de sensitiviteit afnam. Voor TOF-afbeeldingen was de specificiteit zonder deze afbeeldingen echter matig en bleek de sensitiviteit slecht te zijn.

IS DE SNELLE MULTI-CONTRAST ATHEROSCLEROSIS CHARACTERIZATION (MATCH) MRI-METHODE IN STAAT OM DE SAMENSTELLING VAN HALSSLAGADERPLAQUE NAUWKEURIG TE KWANTIFICEREN?

In hoofdstuk zes van dit proefschrift hebben we een validatiestudie uitgevoerd om de effectiviteit van de MATCH-sequentie te beoordelen als een snelle beeldvormingsmethode die de verschillende MRI contrast-beelden intrinsiek co-registreert. We gebruikten een zogenaamd multi-sequentie MRI-protocol als referentiestandaard. Onze bevindingen toonden een uitstekende mate van overeenstemming aan tussen waarnemers voor de

detectie en kwantificering van verschillende atherosclerotische plaquecomponenten in de halsslagader op MATCH-beelden. Er was echter slechts een redelijke tot matige overeenstemming over de detectie van verkalkingen. Bovendien vertoonden MATCH-afbeeldingen een hoge sensitiviteit en specificiteit bij het identificeren van een intraplaque bloeding en de vet-rijke kern. De kwantificering van kwetsbare halsslagaderplaquecomponenten, d.w.z. een intraplaque bloeding en een vet-rijke kern op MATCH-beelden vertoonde een goede tot uitstekende overeenstemming met de kwantificering d.m.v. het multi-sequentie-protocol. Er werd echter matige tot slechte overeenstemming waargenomen voor kwantificatie van het totale volume aan bindweefsel en verkalkingen. De scantijd en beeldanalysetijd van MATCH was aanzienlijk korter in vergelijking met het conventionele multi-sequentie-protocol. Deze bevindingen onderstrepen de voordelen van het gebruik van MATCH ten opzichte van conventionele multi-sequentie MRI-protocollen, aangezien het de scan- en beeldanalysetijd aanzienlijk verkort, terwijl betrouwbare identificatie en kwantificering van de belangrijkste halsslagaderplaquecomponenten behouden blijven.

DISCUSSIE EN CONCLUSIE

De bevindingen in dit proefschrift worden bediscussieerd in hoofdstuk zeven. Dit proefschrift draagt bij aan een beter begrip van factoren die bijdragen aan intraplaque bloedingen. Bovendien hebben we simpelere en snellere MRI-methoden gevalideerd, om de toepassing van MRI van plaques in de halsslagader in de klinische praktijk te vereenvoudigen, Implementatie hiervan zou medisch specialisten informatie over plaque instabiliteit geven hetgeen zij mee kunnen wegen in de besluitvorming rondom de behandeling van patiënten met een plaque in de halsslagader. In tegenstelling tot eerder onderzoek, dat zich voornamelijk richtte op de associatie tussen intraplaque bloeding en andere risicofactoren in studies met een cross-sectionele opzet (op één tijdpunt), werden in dit proefschrift longitudinale MRI onderzoeken uitgevoerd naar de relatie tussen een dun/gescheurd kapsel en een oneffenheid van het plaque-oppervlak en het ontstaan en de progressie van een intraplaque bloeding. Ook hebben we de relatie onderzocht tussen het starten met plaatjesaggregatieremmers en de ontwikkeling en progressie van een intraplaque bloeding. Bovendien toonden we aan dat een intraplaque bloeding, een sterke onafhankelijke risicofactor voor een herseninfarct, nauwkeurig kan worden geïdentificeerd op de maskerbeelden van routinematig verkregen contrast-versterkte MR-angiografie, maar niet op time-of-flight MRI-beelden. Verder stelden we vast dat de nieuwe en snelle MATCH MRI-sequentie een intraplaque bloeding en een vet-rijke kern nauwkeurig kan kwantificeren. In de komende jaren moeten er grote gerandomiseerde studies worden uitgevoerd om te onderzoeken of het meewegen van gegevens over plaque instabiliteit op MRI bij de behandeling van patiënten met een plaque in de halsslagader leidt tot een vermindering van het aantal herseninfarcten.

SCIENTIFIC AND SOCIAL IMPACT

RELEVANCE

Stroke ranks as the second most prevalent cause of death in the European Union (EU) and stands as a primary contributor to adult disability [1]. Each year, approximately 1.1 million individuals in Europe experience the impact of stroke, with a staggering 440,000 lives lost as a result [2]. This concerning trend is further exacerbated by the aging population, which is expected to contribute to a rise in stroke cases.

The economic impact of stroke on healthcare systems and societies is substantial. In Europe, the provision of informal care alone reached an estimated €1.3 billion, with healthcare costs totaling €27 billion and an additional €12 billion attributed to lost productivity resulting from stroke in 2017 [3].

Approximately 85% of the strokes are ischemic, and approximately 20% of the ischemic strokes are related to carotid artery disease [4]. The rupture of a vulnerable carotid plaque is an important underlying cause of stroke. Therefore, symptomatic patients with severe carotid stenosis are currently treated by carotid endarterectomy (surgical removal of the plaque) or carotid artery stenting to prevent (recurrent stroke). However, relying solely on clinical symptoms and the degree of luminal stenosis to determine the need for surgical intervention or stenting has limitations. The number needed to treat (NNT) was six in patients with 70-99% stenosis, whereas in male patients with stenosis greater than 50%, the NNT was nine, and in female patients, it was thirty-six. This underscores the need to explore alternative plaque characteristics that can identify vulnerable, rupture-prone plaques.

One such characteristic is the presence of intraplaque hemorrhage (IPH), which has been shown to contribute to plaque destabilization and serve as a reliable predictor for recurrent events. Magnetic resonance imaging (MRI) has emerged as a promising tool for noninvasive identification of IPH. However, a comprehensive understanding of the mechanisms underlying the development of IPH is still lacking. The detection and quantification of IPH and other plaque compositions require dedicated carotid plaque MR sequences, which are currently not included in standard imaging protocols for carotid artery disease.

This thesis aims to investigate various factors that may influence the development of IPH. By delving into this area of research, we strive to enhance our understanding of plaque destabilization. Additionally, we seek to validate the efficacy of routine CE-MRA in identifying IPH, as well as introduce a novel carotid MR sequence for the identification and quantification of carotid plaque composition.

Addressing the burden of ischemic stroke and mitigating its impact on individuals and society is paramount. By uncovering novel insights into the pathophysiology of IPH and advancing novel carotid MRI techniques to facilitate its clinical use, this thesis aims to contribute to the development of more effective strategies for the management and treatment of patients with carotid atherosclerosis, ultimately leading to improved patient outcomes and reduced societal costs associated with stroke.

TARGET GROUPS

These findings have significant implications for clinicians who treat patients with recent ischemic cerebrovascular events. Currently, patients with mild to moderate carotid artery stenosis are not typically referred to vascular surgeons due to previous clinical trials that demonstrated no significant or marginal benefit in this group. However, IPH is a strong independent risk factor for recurrent stroke and can improve risk stratification of patients with carotid artery disease. Also, identifying factors that contribute to IPH occurrence can help clinicians predict the future risk of IPH development and subsequent stroke recurrence. Additionally, the results of this thesis contribute to ongoing research that aims to better stratify patients with carotid artery stenosis, leading to a more personalized treatment approach. Exploring factors that contribute to IPH can also guide the development of medications aimed at modifying these factors, reducing future IPH occurrences, and decreasing ischemic events.

Therefore, the results of this thesis are of interest to various medical professionals, including neurologists, radiologists, and vascular surgeons. The validation of routine CE-MRA as a tool to detect IPH, which is a strong risk factor for future events beyond the degree of carotid stenosis, is particularly significant. These results can be applied in screening procedures. Clinicians and research groups focused on atherosclerosis and carotid artery disease can utilize these findings to further understand the intricate process of plaque destabilization. By addressing these objectives, this research aims to make substantial contributions to the field of carotid artery disease, ultimately improving patient care and outcomes.

PRODUCTS

At present, the analysis of plaque components is conducted using dedicated software packages. This software is currently utilized by experienced observers; however, for broader adoption, a greater number of observers will be required. Moreover, the process of delineating plaque composition on MR images is time-consuming and susceptible to errors introduced by the observers. This indicates the necessity for the development of (semi)-automated software to streamline and enhance this process. Implementing such software would also enable more accurate detection

of changes in plaque composition volume over time. Moreover, it is recommended that MRI vendors prioritize the creation of multi-contrast MRI protocols for assessing plaque composition as a product since these sequences are currently only available for research and not for clinical care.

INNOVATION AND IMPLEMENTATION

In previous research, the presence of leaky plaque microvasculature was suggested as a key contributor to IPH development. However, in this thesis, we conducted a longitudinal study that indicates that erythrocytes can also enter the plaque from the luminal side. Additionally, while the use of standard antiplatelet therapy in the past was associated with IPH in cross-sectional studies, the onset of antiplatelet therapy had no impact on the occurrence of new IPH or the increase in IPH volume over a two-year period.

The findings of this thesis represent a significant advancement towards the integration of carotid atherosclerotic plaque MRI into daily clinical practice by easily and rapidly identifying patients with high-risk plaques as opposed to those with more stable plaques, personalized treatment options can be employed, resulting in improved stratification for surgical interventions compared to the current standard clinical routine.

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LIST OF ABBREVIATIONS

2D	2-dimensional
3D	3-dimensional
AI	Artificial Intelligence
BMI	Body mass index
BOOST	Bright-blood and black-blood phase Sensitive inversion recovery sequence
CA	Calcifications
CAR	Carotid artery risk score
CARIM	Cardiovascular Research Institute Maastricht
CAS	Carotid artery stenting
CEA	Carotid endarterectomy
CE-MRA	Contrast-enhanced MR angiography
CI	Confidence intervals
CNR	contrast-to-noise ratio
CTA	Computed tomography angiography
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
DANTE	Delay alternating with nutation for tailored excitation
DCE	Dynamic contrast-enhanced
DIR	Double inversion recovery
DM	diabetes mellitus
DWI	Diffusion-weighted imaging
ECST-2	2nd European Carotid Surgery Trial
EDTA	Ethylenediaminetetraacetic acid
ESOC	European Stroke Organization Conference
ESVS	European society for vascular surgery
FC	Fibrous cap
FFE	Fast field echo
FOV	Field of view
FSD	Flow-sensitive dephasing
fSNAP	fast simultaneous non-contrast angiography and intraplaque hemorrhage
FSPGR	Fast spoiled gradient echo
GFR	Glomerular filtration rate
GOAL-SNAP	Golden angle radial k-space sampling
GRE	Gradient echo
HE	Hematoxylin and Eosin
HR	Hazard ratio
ICC	Intraclass correlation coefficient
IPH	Intraplaque hemorrhage
IQR	Interquartile range
IR-TFE	Inversion recovery turbo field echo
LRNC	Lipid rich necrotic core
MATCH	Multi-contrast atherosclerosis characterization
MPRAGE	Magnetization-prepared rapid acquisition gradient echo
MRI	Magnetic resonance imaging
NASCET	The North American Symptomatic Carotid Endarterectomy Trial
NNT	Number needed to treat
NWI	Normalized wall index
NWO	Dutch research council
OMT	Optimized
OR	Odds ratio
PAD	Peripheral arterial disease
PARISK	Plaque at Risk
PET	Positron emission tomography
PWV	Flow-sensitive dephasing
QIR	Quadruple inversion recovery
ROI	interest
RR	Risk ratio
SD	Standard deviation
SE	Standard error
FSPGR	Fast spoiled gradient echo
SNAP	Simultaneous Non-contrast Angiography and IPH
SNR	Signal-to-noise ratio
SPAIR	Spectral attenuated inversion recovery
T1w	T1-weighted
T2w	T2-weighted
TE	TE echo time
TE	Echo time
TI	Inversion time
TIA	Transient ischemic attack
TOF	Time of flight
TR	Repetition time
TRFC	Thin/ruptured fibrous cap
TR-TFE	Inversion recovery turbo-field echo
TSE	Turbo spin echo

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Suzanne Hamoui, I was contemplating the cover of my thesis, and though I had an idea in mind, I struggled to find someone to bring it to life. Then, I recalled your artistic skills and the beautiful drawings you share regularly. Suzan, beyond being a Gynecologist, you are undeniably a talented artist. I'm overjoyed with what you created for me, especially considering the limited time you had. I highly encourage everyone to explore your Instagram and Facebook accounts; they are sure to discover some truly beautiful art. Thank you so much, Suzanne, for the outstanding work.

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Yasmin: As I sit down to write these few words, my heart is overflowing with gratitude for the incredible journey we've shared. You are not just my wife; you are my love, my friend, and the wonderful mother of our children.

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With all my love,

BIOGRAPHY

Mohamed Kassem, born on March 29, 1978, in Aleppo, Syria, began his academic journey at Aleppo University, where he obtained a Medical Diploma in June 2003.

He underwent rigorous training for six years in various Syrian academic hospitals specializing in neurosurgery. In January 2010, he achieved the Syrian Board in Neurosurgery through the Ministry of Health.

During the Syrian civil war, Mohamed was compelled to seek safety at the Syrian-Turkish border. He engaged in humanitarian relief work, providing aid to those affected by the war. In 2015, he sought asylum in the Netherlands, undertaking a perilous journey across the Mediterranean Sea. In the Netherlands, Mohamed enrolled in the Master of Biomedical Sciences program at Maastricht University, specializing in Imaging. He completed his Master's degree in June 2019, receiving recognition for his dedication and talent through a personal Hestia research grant from The Dutch Research Council (NWO).

Under the guidance of Prof. dr. M.E. Kooi and Prof. dr. R.J. van Oostenbrugge, Mohamed pursued his Ph.D. trajectory in stroke, atherosclerosis, and carotid MR imaging at Maastricht University's Cardiovascular Research Institute Maastricht (CARIM). He actively contributed as a tutor and supervisor in Biomedical Sciences Courses, leveraging his medical background. He has also received international recognition, including the Best Abstract award at the conference THE FIL ROUGE OF ATHEROSCLEROSIS: FROM BRAIN TO HEART IMAGING and Best E-Poster award at the 7th European Stroke Organization Conference 2021 (ESOC).

Mohamed's research within CARIM garnered him the CARIM-HS BAFTA 2022 award, recognizing his potential as a postdoctoral fellow. He currently serves as a Postdoctoral Researcher at the Forschungszentrum Jülich in Germany.

Mohamed's story and work have attracted media coverage from various media, including Maastricht University News, NWO News, The Limburger, Observant, Limburg 1 TV, and 2019/C2W (Chemisch2Weekblad).

Outside of his scientific research, Mohamed cherishes his role as a loving father to three children: Julie (15 years old), Akkad (5 years old), and his precious newborn baby girl (Inanna), bringing joy and balance to his life.