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Imaging Biomarkers of Cardiovascular Disease

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Abstract

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality worldwide. Current clinical techniques that rely on stenosis measurement alone appear to be insufficient for risk prediction in atherosclerosis patients. Many novel imaging methods have been developed to study atherosclerosis progression and to identify new features that can predict future clinical risk. MRI of atherosclerotic vessel walls is one such method. It has the ability to non-invasively evaluate multiple biomarkers of the disease such as luminal stenosis, plaque burden, tissue composition and plaque activity. In addition, the accuracy of *in vivo* MRI has been validated against histology with high reproducibility, thus paving the way for application to epidemiological studies of disease pathogenesis and, by serial MRI, in monitoring the efficacy of therapeutic intervention. In this review, we describe the various MR techniques used to evaluate aspects of plaque progression, discuss imaging based measurements (imaging biomarkers), and also detail their validation. The application of plaque MRI in clinical trials as well as emerging imaging techniques used to evaluate plaque compositional features and biological activities are also discussed.

Keywords

Carotid Artery MRI; Clinical Trial; Atherosclerotic Plaque; Stroke

INTRODUCTION

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality worldwide. In the United States, it accounts for 34.2% of all deaths with 2,400 people dying of CVD every day (1). Imaging techniques, such as X-ray angiography or CT angiography, are used to predict potential myocardial infarction or strokes based on the luminal stenosis caused by atherosclerotic lesions. Utilizing stenosis severity as the criterion for CVD prevention, however, is not a strong predictor for future events because of the potential for outward remodeling of the atherosclerotic artery (2). During this process, only the external boundary of the artery expands while the luminal boundary remains the same (2), making lesions difficult to detect by angiography based imaging techniques. A recent imaging study of carotid atherosclerosis revealed the presence of significant plaque burden in arteries with zero percent stenosis by angiography, as shown in Fig.1 (3).

Recent studies have stressed the importance of identifying vulnerable plaques (VPs) with the increased risk of causing thromboembolic events (4). Histologically, features of VPs include: a thin fibrous cap overlaying a lipid rich necrotic core (LRNC); the presence of intraplaque hemorrhage (IPH); plaque inflammation and activity; and luminal stenosis (5-9).

These features are related to plaque burden, plaque tissue composition and plaque activity. The goal of non-invasive imaging should be to: identify the presence of these features; quantify their amount; use the newly acquired information to better understand the progression and regression of atherosclerosis; and ultimately, identify high risk lesions prior to clinical events. This review summarizes recent developments in magnetic resonance imaging of imaging biomarkers, and their use in prospective studies to monitor atherosclerosis progression, regression, and medical treatment.

Among all vascular beds, the carotid artery has been the most frequently studied by imaging. This is primarily due to the fact that carotid artery wall imaging techniques have been well validated by histology because of the availability of corresponding carotid endarterectomy specimens obtained from surgery. Additionally, the carotid artery's proximity to the skin's surface and its strong association with stroke also contribute to making it a frequent target of imaging. This review primarily focuses on imaging studies of the carotid artery.

In addition to magnetic resonance, various imaging approaches have been proposed to evaluate atherosclerotic plaques. B-mode ultrasound (US) can measure intima-media thickness (IMT) in the carotid arteries (10), with an increase in IMT shown to be associated with increased overall CVD risk (11). The IMT measurement has been successfully used in clinical trials for risk assessment and drug therapy evaluation (12-13). However, the IMT measurement has drawbacks in that: it only examines the thickness of a single layer of the vessel wall; is not a direct measurement of plaque; and the measurement accuracy is limited by the plane of acquisition and imaging angle (14). Computed tomography (CT) can detect and quantify plaque calcification (15), yet CT is limited by its radiation as well as its inability to detect other plaque components (16). Other imaging modalities, such as positron emission tomography (17), near-infrared spectroscopy (18) and optical coherence tomography (19) have also been used to study atherosclerotic plaque, but they are still in their early development phases and are primarily used as research tools.

PLAQUE BURDEN MEASUREMENTS

Vessel wall area is used as a measure of plaque burden to indirectly measure the size of the disease in the vessel wall.

Faithful characterization of the carotid artery plaque often requires the description of very small structural details. To maintain a clinically feasible scan time, an in-plane resolution of 0.5-0.6mm, together with a 2-3mm slice thickness has been widely used for carotid artery imaging (20-22). Images acquired at this resolution have been validated to provide accurate plaque burden measurements (23-24), as well as plaque component quantification (25).

Black-blood (BB) imaging, which refers to MR techniques that can suppress the MR signal from flowing blood (26), is critical for plaque burden assessment since the artery is better visualized when the bright lumen signal is absent (27). Accurate delineation of plaque, however, represents technical challenges in a number of ways. First, sufficient blood signal suppression around the carotid bifurcation is difficult because plaque mimicking artifacts tend to arise from the slow-flowing or stagnant blood around the bifurcation. Second, successful acquisition of high resolution MR images without sacrificing coverage, blood suppression, or time efficiency can also be difficult. Other challenges include the demand for high lumen/wall contrast as well as robust boundary segmentation.

Blood Suppression Techniques

Currently, BB imaging is achieved by using one of three techniques: in-flow suppression (IS) (26), double-inversion recovery (DIR) (28) or motion-sensitized driven equilibrium (MSDE) (29-30).

The IS technique pre-saturates flowing blood using a slab-selective radio-frequency (RF) pulse before it enters the imaging plane (26). Compared to other BB techniques, the IS technique is more susceptible to flow artifacts. As a result, most reported plaque burden measurement techniques are obtained from other techniques. Nevertheless, the IS technique is still used in a wide range of clinical applications because it allows for direction-selective suppression and can be achieved in a relatively short time.

The DIR technique (28) has been shown to have more effective blood suppression than IS in plaque imaging, although its suppression efficiency is still challenged by a number of factors such as flow direction dependency, time efficiency, and blood T_1 dependency (31-32). To overcome these limitations, multislice DIR and quadruple inversion recovery (QIR) techniques have been proposed for improved acquisition efficiency (32-35) and T_1 insensitive blood suppression (31). The sequence design in these techniques, however, requires increased imaging volume and/or reduced TI time. Both changes will limit the blood replenishing efficiency at the time of imaging. As a result, blood suppression efficiency in these variations can be compromised.

The recently developed MSDE technique (29-30), unlike the IS and DIR techniques, can suppress blood signal with minimum dependence on the outflow volume (30,36), thus it was found to have the best blood suppression efficiency among all available techniques, as shown in Fig.2. MSDE has been combined with 3D acquisition techniques for effective blood suppression in a large volume (37-38) because of its low dependency on flow direction. It can also be utilized post-contrast due to its T_1 insensitivity. One limitation of MSDE, caused by its inherent T_2 and diffusion weighting, is signal drop, which is approximately 12% in a typical clinical scan setting (30).

Three-Dimensional (3D) Acquisition

Compared to traditional two-dimensional (2D) acquisition techniques, 3D alternatives can offer higher SNR in less time (39-41). Acquiring images in an isotropic 3D fashion allows for flexible image reconstruction in various viewing angles (41). Despite these advantages, however, previous implementations of 3D black blood imaging have been relatively limited due to insufficient blood suppression. MSDE provides an ideal solution for effective, large volume, flow-direction independent blood suppression in 3D acquisition. It has been combined with local excitation to achieve time efficient large coverage 3D acquisition in the longitudinal direction (37). It has also been used for coronal acquisition in time-efficient BB imaging (38). In addition, as long as proper blood suppression is selected, 3D acquisition can be achieved with a variety of pulse sequences, such as fast spin echo (42), gradient echo (38) or steady-state free precession (40).

A limitation of the current 3D acquisition approach is the lack of a histology confirmed contrast weighting. Most 3D sequences have been particularly optimized for fast acquisition so that the actual contrast can be a mixture of many. A recently developed 3D technique (3D-MERGE) has been able to accurately identify some plaque components when compared against traditional 2D images (38). Such studies are promising for the future development and validation of novel 3D techniques for time efficient, high resolution plaque imaging.

Motion Artifacts and its Reduction

In carotid atherosclerotic plaque MR imaging, the data acquisition can be compromised by various motion artifacts (43), which can significantly impact the accuracy of plaque burden measurement. Although artifacts caused by periodic motion, such as pulsation, can be reduced by synchronizing acquisition with physiological signal (44), artifacts generated from non-periodic motion, such as involuntary swallowing, may be difficult to remove. Such non-periodic motion is considered the major source of poor image quality in carotid scans.

A number of studies have investigated novel techniques to suppress motion artifacts from non-periodic motion. Motion-detection navigator used to detect the motion of the tongue and only accept data acquired when it is relatively still has been designed (45). It has been shown to improve carotid vessel wall image quality and to reduce artifacts caused by non-periodic motion. Other than navigator based techniques, external coils that can accurately detect neck motion have also been proposed for improved carotid artery image quality. In an *in vivo* study on five healthy volunteers, the adoption of the external coil was found to significantly improve the image quality and minimize swallowing artifacts (46) (Fig. 3).

Plaque Burden Quantification and Validation

Although BB MRI can clearly visualize diseased vessel wall boundaries, specialized image processing tools are needed to accurately quantify plaque burden. To assist in the segmentation of lumen and wall boundaries, several semi-automated image processing algorithms, based on active contours or snakes, have been proposed (47-49). The most direct plaque burden measurements are vessel lumen and wall areas obtained from cross sectional images. From these area measurements, a series of parameters can be derived, such as, vessel wall volume, percent wall volume (PWV) – which is defined as the ratio between the wall volume and the total vessel volume – and normalized wall index (NWI). Wall thickness measurement techniques based on lumen and outer wall boundaries are developed (50-51). A dedicated image processing software package, compatible with many of these techniques, has also been developed to facilitate the plaque burden measurements (52).

The accuracy of plaque burden measurement using carotid BB MRI has been validated in a number of studies. By comparing *ex vivo* and *in vivo* MR images, the measurement accuracy of plaque burden parameters obtained on *in vivo* BB MRI, such as wall area, lumen area and wall volume, have been well established (20,23,53). In a multi-contrast MR imaging protocol, different contrast weighted images tend to provide similar results in lumen and vessel wall area measurement (24), as long as the same BB technique is utilized. The measurement accuracy on 3D imaging has also been validated by comparison to the corresponding 2D images (41). Beyond measurement accuracy, the reproducibility of MR plaque measurement has also been proven (20,54). It is reported that the slice-based lumen and wall area measurement variability ranges between 6.2% - 12% (54), and the intra-class correlation (ICC) ranges between 0.90-0.98 (20). Volume based measurements provide more reproducible measurements compared to slice-based measurements: the variation for lumen and wall volume ranges between 4.3%-5.8% with ICC ranging between 0.97-0.99 (55). Of note, among all plaque burden measurement parameters, plaque wall volume is shown to be the most reproducible parameter in images acquired at different time points (55).

PLAQUE COMPOSITION MEASUREMENTS

Atherosclerotic plaque composition measurement refers to the detection and quantification of common plaque tissues such as lipid rich necrotic core (LRNC), fibrous cap, intraplaque hemorrhage, calcification and other components. Such measurements can provide insights into plaque vulnerability beyond simple plaque burden measurements. Histologically, a large LRNC with thin overlaying fibrous cap has long been considered the main feature of

vulnerable plaque (56-57). And indeed, the existence and size of LRNC and/or intraplaque hemorrhage has been found to be associated with plaque progression (58-59). Other studies found that the presence and the distribution of calcification may also play a role in determining the stability of atherosclerotic plaque (60-61).

Multi-Contrast Plaque Component Imaging and Validation

A common approach for conducting plaque component imaging is to use multi-contrast based MRI (27,62-64). MR plaque component identification relies on differences in the inherent magnetic properties (such as T_1 and T_2 relaxation times) among plaque components. A number of studies revealed that the signal intensities from different plaque components can present a significant amount of overlap on a single contrast weighting (65-66), thus multi-contrast MRI is a better approach for obtaining a comprehensive plaque component characterization. A typical multi-contrast carotid MR imaging protocol usually contains: bright-blood time-of-flight (TOF) images, proton-density (PD), T_1 and T_2 weighted spin echo images acquired in combination with proper black blood techniques (67). An example of histology validated multi-contrast MR imaging is shown in Fig.4.

LRNC is a mixture of lipid, cellular debris, blood and water in various concentrations. When compared to the signal intensities of the adjacent sternocleidomastoid muscle the LRNC (without hemorrhage) is iso-/hyper-intense on T_1 W images but hypointense on T_2 W images (27). By using these criteria for LRNC identification, the sensitivity and specificity were found to be 92% and 74% on *ex vivo* specimens (68) and 92% and 65% *in vivo* (25), when compared to the corresponding histology as a reference.

The majority of intraplaque hemorrhage (IPH) is caused by microvessel leakage into the necrotic core created by compromised endothelial integrity and/or biomechanical deformation. IPH is generally detected because of the presence of the oxidation product (methemoglobin), which presents a strongly shortened T_1 relaxation time (69). On a multi-contrast protocol, IPH can be effectively detected using a combination of T_1 weighted and TOF images. The sensitivity and specificity of *in vivo* IPH detection in atherosclerosis patients was found to be 82-90% and 74-77%, respectively (25,62).

Calcification is found in atherosclerotic plaques as a result of dystrophic calcium build-up in the vessel wall. Due to its low proton content, it gives a hypointense signal on all contrast weightings (25,64). A high sensitivity and specificity of 98% and 99% was reported for *ex vivo* calcification identification accuracy (68). High *in vivo* sensitivity and specificity of 84% and 91% were also reported when compared against histology (25).

Based on the plaque composition measurement provided by multi-contrast MRI, Cai et al took the analysis a significant step further by showing that *in vivo* MRI can accurately classify human carotid atherosclerotic plaques according to a modified version of the American Heart Association system for lesion classification (70). By carotid plaque MRI, intermediate to advanced atherosclerotic lesions were reliably identified (weighted kappa = 0.79) by comparison with histology based classifications and criteria (70).

Other Contrast Weightings for Component Imaging

Other than the standard multi-contrast MR imaging approach, a few other MR contrasts have also been found to be useful for plaque component quantification.

In an effort to improve the lipid rich necrotic core *in vivo* quantification, Yuan et al found that the LRNC presented a much lower signal enhancement when compared to other components after the injection of a contrast agent (71). A later study confirmed that the combination of both pre- and post- contrast T_1 weighted images provided an improved

LRNC delineation and size measurement when compared to the non-contrast approach (72). A separate study also found that contrast-enhanced MR images provided a better LRNC delineation than by using only T₂ weighted images (73).

Taking advantage of IPH's short T₁ relaxation time, Moody et al found that IPH can be potentially identified with a single contrast weighting (74). A heavily T₁ weighted 3D gradient echo sequence (refer to as direct thrombus imaging, or DTI) was developed for IPH imaging. When compared to histology, they found that *in vivo* IPH detection sensitivity and specificity were 84% and 84%, respectively (74).

Multiple plaque components have been associated with the plaque progression or subsequent neurological events (59). It is therefore important to obtain comprehensive plaque component information for plaque vulnerability evaluation. Since no single contrast weighting has been demonstrated to provide such information thus far, multi-contrast MRI is still considered the method of choice for plaque component imaging. The identification criteria and validation for the major plaque components using multi-contrast MRI are summarized in Table 1.

Reproducibility and Applicability in Serial MRI

A major rationale for identifying atherosclerotic plaque components using non-invasive imaging is to track disease progression and regression and the response to therapies at multiple time points in clinical trials making plaque component measurement reproducibility obtained from MRI (Table 1) important. In a study aimed at understanding the intra- and inter- reader reproducibility of plaque MRI measurements of 31 atherosclerosis patients, very good intra- (LRNC: 0.89, Calcification: 0.9, hemorrhage: 0.74) and inter- (LRNC: 0.92, Calcification: 0.95, hemorrhage 0.73) reader reproducibility were obtained for all plaque components (25).

Saam et al (55) investigated the needed sample size requirements for observing plaque changes when using carotid MRI. By studying the measurement coefficient of variation (CV) of the LRNC using MR, they found that only 14 subjects per treatment arm would be required to identify a 5% treatment effect if the plaque wall volume was used as an endpoint. This study illustrated the considerable savings in time and expense that can be achieved by using carotid MRI in a well designed clinical study. Recently Li et al (75) studied the corresponding coefficient of variations at 3T and found that similar values and sample sizes can be expected at 3T as were previously used for 1.5T (55).

Automated Component Identification

Due to the complexities in signals for individual plaque components, a full image review of a carotid MR exam can take a relatively long time to accomplish for even well-trained radiologists. Computer assisted automated plaque component identification and quantification can address this challenge and provide a quicker review process.

Ex vivo based segmentation techniques have been proposed and found to correlate well with histology data (76). In addition, a number of *in vivo* automated plaque identification techniques were developed (77-78) and validated against manual MR drawings but did not have histology confirmation. In an advanced effort, Liu et al developed Morphology Enhanced Probabilistic Plaque Segmentation (MEPPS) based on the maximum a posteriori probability Bayesian theory (66). The fully automated method was applied to *in vivo* images from 26 patients and validated by histology. High correlations were found for all major plaque components, but more importantly, the method was demonstrated to provide higher correlation with histology than manually identified regions from experienced radiologists

(66). Although the technique was developed and optimized for 1.5T MR images, its performance on 3T images has also been validated (79).

PLAQUE ACTIVITY MEASUREMENTS

In addition to anatomical information about plaque morphology and composition, MRI can also provide functional information about plaque activity. Plaque activity generally refers to the plaque neovasculature, inflammation and other processes at the cellular level. A class of iron oxide based contrast agents, known as ultra small super paramagnetic particles of iron oxides (USPIOs), has been approved for clinical use in some countries. USPIO enhanced MRI has the ability to provide information about plaque activity measures, such as inflammation or angiogenesis, which has been linked to plaque vulnerability.

Due to USPIO's T_2 or T_2^* shortening effects, gradient echo sequences with long TEs are particularly useful for their detection. Macrophages, which are considered markers of inflammation, accumulate USPIO. The accumulation of the iron particles in macrophages was validated by scanning patients before and after USPIO injection and then comparing their surgically removed carotid specimens to MRI (80). The USPIO/macrophage-positive histology sections were visualized in 24 of 27 patients, with an average reduction in signal intensity of the images induced by the USPIO particles of 24% (80) showing that USPIO accumulation can be a biomarker of inflammation. Tang et al also showed that USPIOs can be used to detect inflammation in otherwise asymptomatic plaques (81). In examining carotid plaques from patients with coronary artery disease (CAD), plaques from patients had a signal intensity decrease of 16.4% after USPIO infusion compared to 8.4% signal intensity increase in controls. Further studies have shown that asymptomatic arteries can also exhibit signal loss after USPIO infusion (82), which is suggestive of occult inflammation. Trivedi et al showed that the optimum time for USPIOs to aggregate within macrophages is between 24-48 hours with a peak effect at 36 hours (83). Beyond 48 hours, USPIO signal decreases but is still visible up to 96 hours (84). Newer imaging techniques such as IRON or GRASP (85) may potentially improve the detection of USPIOs in atherosclerotic plaques by providing a positive contrast instead of a reduced signal.

PLAQUE IMAGING BIOMARKERS AND THEIR ROLE IN VARIOUS STUDIES

Using the techniques specifically developed and validated for plaque burden, plaque components, and plaque activity characterization, MRI has been applied to risk-assessment or evaluation of therapeutic effects in atherosclerosis patients. Since atherosclerosis usually progresses and regresses slowly over time, many pertinent studies require large sample sizes and multiple scan centers. Such studies often require imaging at multiple time-points. MR imaging in such studies is challenging because of the need to 1) achieve comparable measurements at multiple imaging centers; and 2) establish accurate disease characterization over a long time course.

Plaque Imaging Biomarkers vs. Stenosis

Although luminal stenosis is an indicator of late stage atherosclerotic disease, early lesions may remain undetected by luminography. Babiarz et al showed that carotid atherosclerosis was prevalent in arteries with minimal luminal stenosis by angiography (86). Stenosis measurements by contrast enhanced MRA correlated with stenosis measurements by black-blood vessel wall imaging ($r = 0.77$). However, lumen area measurements on black-blood MRI exceeded corresponding MRA measurements in 20 of 24 cases, indicating that MRA underestimates lumen area measurement compared to black-blood MRI. Further, Dong et al showed that atherosclerotic lesions were present in carotids with zero percent stenosis on MR angiography (3). Advanced atherosclerotic lesions with LRNC, calcification, IPH and

fibrous cap rupture were present in 67.4%, 65.2%, 8.7% and 4.3% of the arteries graded as having zero stenosis by MRA. Other studies have also noted the presence of high-risk plaque features such as IPH and fibrous cap rupture in 8.1% of subjects with <16% stenosis by ultrasound (87). These studies underscore the importance of vessel wall imaging in identifying the true extent of carotid disease, especially in early disease, when there is a higher chance of reversing the disease process with adequate therapeutic intervention.

Plaque components such as large necrotic cores with thin fibrous caps, plaque neovasculture, and increased inflammation have been implicated as features associated with vulnerable plaques in histological studies of carotid and coronary arteries (88-89). High resolution vessel wall imaging allows direct visualization of the diseased vessel wall with excellent soft-tissue contrast and can visualize such high risk plaque components. Yuan et al showed that fibrous cap status detected on MRI can distinguish between symptomatic and asymptomatic subjects. Subjects with a ruptured fibrous cap on MRI were 23 times more likely to have had a recent TIA or stroke compared to subjects with thick fibrous caps (90). This was followed by a series of studies that identified other plaque features associated with neurological symptoms. For example, the presence of IPH detected by MRI has been shown to be predictive of neurological symptoms (22), and plaque inflammation as detected by USPIO imaging was different between symptomatic and asymptomatic patients (91). These studies strongly suggest that vessel wall imaging by multi-contrast MRI currently offers the best prospect for a non-invasive tool to identify lesions that are most likely to cause symptomatic events.

Multicenter Studies and Their Findings

While early clinical trials were restricted to single MRI centers, the feasibility of multicenter studies for carotid plaque imaging has recently been established (92). Five imaging sites with 1.5T GE scanners participated in a double-blind, randomized trial (drug vs. placebo) and scanned subjects at four time points using a standardized multi-contrast carotid MRI protocol. All sites achieved consistently good image quality (3.5 to 4.2 on a 5-point scale with image quality 3 being acceptable for plaque compositional analysis) with matched coverage (1.6–1.8 cm) across the four time points. Measurement variability assessed in a subset of patients was found to be comparable to single-site measurement variability. Thus clinical trials may recruit subjects at multiple sites, thereby greatly reducing the overall study time. Further studies have shown that such studies may be performed at MRI centers using different scanner platforms (93). Saam et al scanned 32 subjects with >15% stenosis, by ultrasound, on 1.5T GE, Philips and Siemens scanners. Although there were small differences in wall measurements for different scanner platforms, the intra-platform CVs were comparable. Additionally, AHA lesion types could be consistently identified across the same ($\kappa = 0.75$) and different scan platforms ($\kappa = 0.75$). Thus a multicenter study can involve multiple scanner platforms, with the caveat that the subjects should be imaged on the same scanner platform throughout the study.

Risk Assessment

Carotid plaque imaging by MRI has proven to be an effective technique in longitudinal studies designed to evaluate the natural history of disease progression. A prospective study (PRIMARI) aimed at assessing the pathophysiology of plaque progression recruited subjects with 50–79% carotid stenosis by duplex ultrasound examination and no symptoms of carotid artery disease 6 months prior to enrollment. Patients were followed by carotid MRI every 18 months. Patients with IPH at baseline were more likely to have new IPH than controls (43% vs 0%). Between the baseline scan and 18 month scan, patients with intra-plaque hemorrhage (IPH) had a higher percent change in wall volume (6.8% vs -0.15%) and LRNC volume (28.4% vs -5.2%) compared to controls (21). Over a mean follow-up period

of 38.2 months, 12 carotid cerebrovascular events occurred ipsilateral to the index carotid artery. The presence of IPH, thin or ruptured fibrous cap, larger mean IPH area, and maximum %LRNC were significant predictors of future cerebrovascular events.

The predictive value of IPH in symptomatic stroke or TIA has also been established in other prospective studies. Singh et al reported similar results using an inversion-recovery prepared gradient echo sequence (22). Over a 1-year follow-up period, six cerebrovascular events occurred in patients with IPH compared to no events in patients without IPH. The hazard ratio for IPH on MR and increased risk of cerebrovascular events was 3.59. Another prospective study of symptomatic patients with mild to moderate (30%-69%) carotid stenosis by T₁w gradient echo imaging noted 5 ipsilateral strokes and 14 ipsilateral ischemic events in a 2 year period (94). All strokes and eight ischemic events occurred in patients with ipsilateral carotid IPH (HR = 9.8).

Therapeutic Effects Evaluation

MRI can precisely monitor changes in plaque burden and plaque composition over time in the same subject. Therefore it has emerged as an ideal tool to monitor the efficacy of therapeutic studies where it offers advantages in reduced sample size and time to the completion of study. Corti et al reported that simvastatin therapy can induce significant regression of atherosclerotic plaques (95). Subjects were followed by serial MRI studies at baseline, 6 months, 12 months, 18 months and 24 months. Vessel wall area (VWA) reduced by 14% and 10% in the carotids and aorta, respectively, at 12 months, but there was no change observed at 6 months. Similarly, vessel wall thickness (VWT) reduced by 10% and 9% in the carotid and aorta, respectively. With further follow-up there was an 18% and 15% reduction of VWA in the carotids and aorta, respectively, at 24 months. Corresponding VWT decrease was 17% and 15%, respectively. Interestingly, the lumen areas for carotid and aorta increased by 5% and 6% respectively at 24 months, showing that the arteries underwent a remodeling process. Yonemura et al showed that high dose atorvastatin (20mg/day) treatment reduced vessel wall thickness and vessel wall area of thoracic aorta plaques compared to low dose atorvastatin (5 mg/day) (96) (Fig.5). However there was no effect from either dose on abdominal aortic plaque.

In a case control study of eight patients with coronary artery disease on prolonged intensive lipid-lowering therapy for 10 years and matched controls, Zhao et al reported a difference in lipid composition in response to statin therapy (97). Treated patients had a smaller core lipid area (0.7 vs. 10.2 mm²) and percent lipid composition (1% vs. 17%). Calcium was higher in treated patients (6.4 vs. 1.8mm²) with a higher percentage of fibrous tissue (84% vs. 77%).

The ORION (Outcome of Rosuvastatin treatment on carotid artery atheroma: a magnetic resonance Imaging Observation) trial, a randomized, double-blind, parallel-group trial, compared low and high (5mg and 40 mg) doses of rosuvastatin on progression of atherosclerosis, using carotid MRI. Percent LRNC decreased by 41.4% in 24 months in all patients with LRNC at baseline even without any significant change in plaque burden (98).

In the prospective CPC (Carotid Plaque Composition) study (99), subjects with coronary artery disease or carotid disease were treated with atorvastatin and followed by serial MRI at 1 year intervals for 3 years. Percent LRNC area reduced from 8.4% to 5.2% over 3 years. When normal walls without LRNC were excluded, percent LRNC reduced from 14.2% to 7.4%. This measure decreased by 3.2%, 3.0% and 0.91% in the first, second and third years, respectively (100). The PWV also decreased by 3.8% over the 3-year course of the study. The reductions in the first, second and third years were 0.3%, 3.6% and 0.1%, respectively (101). These results indicate that the effects of lipid-lowering therapy on carotid atherosclerosis may be evaluated in a population with pre-existing carotid atherosclerotic

disease as early as the first year using the LRNC as the endpoint and 2 years using plaque burden as the clinical endpoint. Further, these results suggest that the maximal effect of statins may be seen at 2 years.

USPIO agents have also been used to assess plaque inflammation by therapy. The ATHEROMA (Atorvastatin THERapy: Effects on Reduction Of Macrophage Activity) study (102) compared low (10mg) and high dose (80mg) atorvastatin therapy on 40 subjects using MRI at baseline, 6 and 12 weeks. A significant reduction of USPIO-defined inflammation was observed in the 80-mg group at both 6 weeks and at 12 weeks (Fig.6). There was no difference with the low-dose regimen.

Recently several large scale carotid MR imaging studies have adopted the multi-site clinical trial approach. The MESA (Multi-Ethnic Study of Atherosclerosis) was a large scale study to investigate the prevalence, correlates, and progression of subclinical cardiovascular disease in a population-based sample of 6,500 men and women aged 45–84 years from six centers. The study included carotid and coronary vessel wall imaging among other imaging modalities. Wasserman et al showed that 71% of carotids with wall thickness >1.5mm had a LRNC (103). Among the established coronary heart disease risk factors only plasma total cholesterol was found to be strongly associated with the LRNC. Macedo et al showed that the maximum coronary wall thickness was greater for subjects with two or more cardiovascular risk factors compared to subjects with one or no risk factors (2.59 ± 0.33 mm vs. 2.36 ± 0.30 mm) (104). Positive remodeling of the coronaries in response to atherosclerosis was seen, with coronary outer wall area increasing 25.9 mm^2 per millimeter increase in coronary wall thickness, while corresponding lumen area increased only slightly at 3.1 mm^2 (105). These studies demonstrate the benefit of large scale imaging studies with standardized MRI techniques across multiple centers.

Several other multi-center clinical studies of atherosclerosis are currently underway. The dal-PLAQUE study evaluates the effect of dalcetrapib compared to placebo on atherosclerotic plaque in patients with coronary heart disease, and randomized 130 patients to be followed by MRI and PET/CT over 12 months across 10 centers. The primary endpoint by MRI is reduction in plaque burden and plaque size. The AIM-HIGH (Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides and Impact on Global Health Outcomes) study is a multicenter, randomized, double-blind clinical trial designed to test whether extended release niacin plus simvastatin is superior to simvastatin alone for delaying the time to a first major cardiovascular event. The carotid MRI sub study of AIM-HIGH is a multicenter (10 centers) carotid MRI study using GE and Philips 3T scanners. The purpose of this sub-study is to examine the effect of intensive LDL-lowering plus HDL-raising therapy, compared with LDL-lowering alone, on carotid atherosclerotic plaque lipid composition and burden and to determine if MRI based plaque characterization is predictive of future clinical cardiovascular events. The primary endpoint is the mean change in plaque lipid composition over 2 years. These ongoing studies are expected to not only demonstrate treatment efficacy monitoring by MRI but also to advance our knowledge of atherosclerotic progression/regression.

FUTURE IMAGING BIOMARKERS

Beyond the biomarkers that are currently being utilized in atherosclerosis clinical trials, novel imaging techniques are continuously being developed. Many of these techniques reach beyond the traditional morphology based analyses and provide new avenues to evaluate atherosclerotic disease, such as functional or local hemodynamic properties. Some techniques have already been extensively studied, validated and are expected to be applied in future clinical studies of therapeutic response in atherosclerotic arteries.

Inflammation Imaging Using Dynamic Contrast Enhanced (DCE) MRI

Inflammation is a key process in atherosclerosis progression and has been associated with increased frequency of plaque rupture (106). Although the current USPIO based contrast agent has been shown to be an effective indicator of plaque inflammation, the fact that it requires comparison between two separate scans (usually 24-48 hours apart) is a logistic challenge for many patients. In an effort to characterize the plaque inflammation process within a single scan, dynamic contrast enhanced (DCE) MRI in plaque imaging has been explored.

For dynamic contrast enhanced MRI, images are repetitively acquired at the same location after the administration of a gadolinium based contrast agent. Different plaque tissues can then be characterized based on their enhancement properties (107). With the proper kinetic models applied, Kerwin et al found two important quantitative parameters: the transfer constant K^{trans} and fraction plasma volume v_p (108). Sixteen atherosclerotic subjects were imaged with DCE MRI and the scans correlated with corresponding histology. The results showed v_p to be strongly associated with the fractional area of microvasculature ($r=0.8$). In a subsequent study on 30 patients (109), K^{trans} was also found to be strongly correlated with macrophage content ($r=0.75$) and numbers of plaque microvessels ($r=0.71$). Macrophages are indicators of inflammation and Kerwin et al further explored the possibility of evaluating the severity of inflammation in carotid plaque. In one of the group's recent findings (110), adventitial K^{trans} value was found to be highly associated with the serum biomarkers of inflammation ($r=0.57$).

Targeted Contrast Agents

Gadolinium contrast agents such as gadopentate and gadobenate dimeglumine are non-specific extracellular MRI contrast agents. However Gadolinium can be combined with other molecular structures for targeted imaging of atherosclerotic lesions. Thrombus can be imaged with a fibrin-targeted peptide (111). Inflammation specific enzymes such as matrix metalloproteinases have also been targeted with specific contrast agents (112-113). Gadofluorine (114-115) and polyethylene glycol (PEG)-micelles incorporated with Gadolinium DTPA (116) have been shown to detect the LRNC in animal studies due to their lipophilic nature. Targeted imaging of angiogenesis has also been demonstrated by $\alpha(v)\beta_3$ -integrin-targeted, paramagnetic nanoparticles (117). The use of targeted contrast agents may help improve the sensitivity and specificity of plaque component detection or enable identification of specific plaque components that are currently not detectable by routine multi-contrast MRI.

Hemodynamic Analysis

Hemodynamic analysis examines the etiology and evolution of atherosclerosis as a result of the interaction between arterial tissue and its mechanical environment. MRI has been used to analyze the mechanical behaviors of both the flowing blood and the vessel wall, by either building computational arterial models based on the geometric information provided by MRI, or by using flow and compliance measurements derived from MRI.

The focal widening of the internal carotid artery distal to the bifurcation is responsible for helical secondary flows that develop in this region. This complex flow leads to low and oscillating shear stress patterns that have been associated with the initiation of atherosclerotic disease (118). Computational fluid dynamics (CFD) has been extensively used to investigate the local hemodynamics of this atherosclerotic site, and MRI is the imaging technique most commonly used to render the needed luminal geometry (119-121). MRI-based CFD calculations have been reproducible with the exception of the bifurcation apex area, where secondary complex flow patterns develop (122).

Phase-contrast MRI (PC-MRI) is able to render three velocity components in a time-resolved manner. Although PC-MRI data can be used directly as a boundary condition of the CFD model by interpolating the image grid onto the inlet CFD nodes, the velocity data is mostly used to obtain flow rate waveforms (123). The velocity information provided by PC-MRI has been used to compute wall shear stress maps along different arterial segments, using interpolation techniques to overcome the relatively low spatial resolution (107,111).

Use of hemodynamic analysis can potentially help predict future vulnerable plaques before they can be readily visualized by morphology or component based plaque MRI.

CONCLUSION

Vessel wall MRI has been utilized in characterizing atherosclerotic plaques from many different perspectives. A variety of techniques have been developed and validated to measure plaque burden, quantify plaque components and visualize plaque and arterial functions. Through the advancement of these techniques, MRI has been used in clinical trials to assess the risk factors of atherosclerosis disease and track therapeutic effects. Furthermore, novel imaging techniques are also being developed to improve the disease characterization from new perspectives.

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Fig.1.

Maximum intensity projection and multi-contrast carotid MRI images from a 62 year old male. Transverse images are consecutive slices corresponding to the horizontal lines on the MRA image. A substantial plaque with evidence of a lipid rich necrotic core (arrows) and calcification (arrowheads) is present on the cross sectional images, although there is no evidence on MRA. *: lumen of the carotid artery. Reprinted with permission from: Dong L et al. Geometric and compositional appearance of atheroma in an angiographically normal carotid artery in patients with atherosclerosis. *Am J Neuroradiol*, 31, 2, 311-6, 2010, © by American Society of Neuroradiology.

Fig.2.

Sample images illustrating the flow suppression capability of different BB imaging techniques: (a) IS, (b) SDIR, (c) MDIR, and (d) MSDE. The residual flow signal is eliminated by the MSDE sequence but visible for other techniques (arrows). All images are presented using the identical window settings. *, internal carotid lumen; **, external carotid lumen. Wang J et al. Improved suppression of plaque-mimicking artifacts in black-blood carotid atherosclerosis imaging using a multislice motion-sensitized driven-equilibrium (MSDE) turbo spin-echo (TSE) sequence, Vol. 58, No. 5, 2007, 973-981. Copyright 2007, Magn Reson Med; Reprinted with permission from John Wiley & Sons, Inc.

Fig.3.

Sample images comparison of image quality in the common carotid artery. Left column: the reject algorithm on; right column: the reject algorithm off. Notice the improved artery boundary delineation and blood suppression efficiency. Chan CF et al. Novel technique used to detect swallowing in volume-selective turbo spin-echo (TSE) for carotid artery wall imaging, Vol. 29, No. 1, 2009, 211-216. Copyright 2009, J Magn Reson Imaging; Reprinted with permission from John Wiley & Sons, Inc.

Fig.4.

Example of histological validation of MRI at 4 consecutive locations spanning the bifurcation. Contours have been drawn for lumen (red), outer wall (cyan), LR/NC (yellow), calcification (black), loose matrix (pink/white), and hemorrhage (orange). H&E indicates hematoxylin-eosin. Reprinted with permission from (25).

Fig.5.

Sample thoracic aorta images at baseline and after 12 months of atorvastatin treatment. After 12 months, three contiguous slices (no interslice gap) for each plaque were obtained, and the slice most closely matching the one at baseline was selected. Upper row: thoracic aortic plaque that showed regression (28% vessel wall area reduction) with 38% low-density lipoprotein-cholesterol (LDL-C) reduction by 20-mg (high dose) atorvastatin. Lower row: thoracic plaque that showed progression (+15%) with 20% LDL-C reduction by 5 mg (low dose). Reprinted with permission from (96).

Fig.6.

Example and Quadrant Analysis Approach T2*-weighted imaging of a right common carotid artery before and after ultrasmall superparamagnetic iron oxide (USPIO) infusion at the 2 time points of 0 (b) and 12 (d) weeks. USPIO uptake can clearly be seen in the plaque at baseline (yellow arrowheads). The plaque enhances at 12 weeks (blue arrowheads), indicating that the high-dose statin treatment has dampened the USPIO-defined inflammation. Reprinted with permission from (102).

Table 1

Carotid atherosclerotic plaque component imaging and validation

	Contrast behavior	Validation ^a	Reproducibility ^a
Lipid-Rich Necrotic Core (LRNC) imaging	Without Gd ^b : Iso/hyper- intense on T ₁ w, hypo-intense on T ₂ w (27)	Without Gd ^b : 92% sensitivity and 65% specificity (25)	Intra-: 0.89 Inter-: 0.92 (25)
	With Gd ^b : Low enhancement on post-Gd T ₁ w (72)	With Gd ^b : improved reproducibility (72)	
Intraplaque Hemorrhage (IPH) imaging	Multi-contrast: Hyperintense on T ₁ , Hyperintense on TOF (62)	Multi-contrast: 82-90% sensitivity and 74-77% specificity (25,62)	Intra-: 0.74 Inter-: 0.73 (25)
	Single-contrast: Hyperintense on DTI (74)	Single-contrast: 84% sensitivity and 84% specificity (74)	
Calcification imaging	Hypointense on all contrast weightings (64)	84% sensitivity and 91% specificity (25)	Intra-: 0.9 Inter-: 0.95 (25)

^a all measurements were obtained by comparison with histology^b Gd stands for (the administration of) gadolinium based contrast agent