Multi-sequence *In vivo* MRI can Quantify Fibrous Cap and Lipid Core Components in Human Carotid Atherosclerotic Plaques

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Objectives. Risk of thrombo-embolic stroke is thought to be better reflected by carotid plaque composition than by luminal stenosis. We set out to determine whether high resolution MRI was a valid method of quantifying plaque components *in vivo*.


Materials. Twenty-five recently symptomatic patients with severe internal carotid artery stenosis underwent pre-operative *in vivo* multi-sequence MRI of the carotid artery using a 1.5 T system.

Methods. Individual plaque constituents were characterized on axial MR images according to net signal intensities. Analysis of fibrous cap and lipid core content was quantified proportional to overall plaque area. Bland–Altman plots were generated, and intra-class coefficients computed to determine the level of agreement between the two methods and inter-observer variability.

Results. The intra-class correlation coefficients between two MR readers were 0.94 and 0.88 for quantifying fibrous cap and lipid core components, respectively. There was good agreement between MR and histology derived quantification of both fibrous cap and lipid core content; the mean % difference for fibrous cap was 0.75% (±2.86%) and for lipid core was 0.86% (±1.76%).

Conclusion. High resolution carotid MRI can be used to quantify plaque components and may prove useful in risk stratification.

Key Words: Atherosclerosis; MRI; Plaque burden; Vulnerable plaque; Carotid.

Introduction

Thrombo-embolism from extra-cranial atherosclerosis accounts for the neurological deficit in approximately half of people with ischaemic stroke.1,2 Selection for disease eradicating interventions is conventionally determined by measuring luminal stenosis that results from *in situ* atheromatous plaques.3,4 These traditional stratification parameters are no longer thought to be the most accurate predictors of thrombo-embolism, as the process of vessel wall remodeling can maintain luminal patency,5 and consequently, very large friable plaques may remain unidentified. Furthermore, natural history studies suggest that a large number of patients with severe ICA stenosis are asymptomatic and that the risk of subsequent thrombo-embolism in this population is low.6,7 Histological studies of coronary atherosclerosis suggest that plaques at risk of rupture, so called vulnerable plaques, can be identified by thin fibrous caps that overly large, often necrotic lipid cores.8,9 The prospective identification of vulnerable carotid plaques *in vivo*, may therefore improve risk stratification and selection for intervention. Recently, high resolution MRI has emerged as an accurate non-invasive tool that is able to characterize carotid plaque components *in vivo*.10,11 There are limited reports of flow-suppressed MRI techniques to measure overall lumen size and total plaque area as surrogates for plaque burden.12-14 These *in vivo* studies have, however, lacked quantification of the fibrous cap and lipid core components, the relative amounts of which are thought to contribute directly to plaque vulnerability.15 In the present study we set out...
to determine the potential of high resolution multi-sequence MR imaging to quantify these plaque components in human carotid arteries in vivo.

Materials and Methods

Patients

Approval for the study was obtained from the Institutional ethics committee. Twenty-five consecutive symptomatic patients with severe ICA stenosis (18 males; mean age 64, range 45–88 years; mean % SD stenosis = 80 ± 7.2; mean ± SD temporal interval from symptoms to surgery of 16.9 weeks ± 7.1) scheduled for carotid endarterectomy were recruited from a specialist neurovascular clinic after giving informed consent to participation. All patients underwent high resolution multi-sequence MR imaging of the carotid bifurcation pre-operatively.

MRI

Imaging studies were conducted on a 1.5 T whole body system (CV/i, GE Medical Systems, Milwaukee, WI, USA) using a custom designed four-channel phased array surface coil (Flick Engineering Solutions BV, Winterswijk, The Netherlands) wrapped around the neck, and secured by a soft cervical collar. Additionally, patients were rested in foam head rest to minimize motion artifacts. After an initial coronal localizer scan, an axial 2D time of flight (ToF) MR angiogram study was performed to identify the location of the carotid bifurcation and region of stenosis. Axial images were acquired through the common carotid artery (CCA), 6 mm (two slices) below the carotid bifurcation to a point 6 mm (two slices) distal to the extent of the stenosis identified on the ToF sequence. This method ensured that the entire plaque was imaged and also facilitated image co-registration (see below). The following 2D, ECG-gated, blood-suppressed, fast spin echo pulse sequences were used: intermediate T2W (TR/TE: 2*RR/46) with fat saturation, T2W (TR/TE: 2*RR/100), short T1 inversion-recovery (STIR) (TR/TE/TI: 2*RR/46/150), and T1W (TR/TE: 1*RR/ 7.8). The pixel size was 0.39 × 0.39 × 3 mm³ in all cases. The field of view was 10 cm and matrix size 256 × 256.

Histological analysis

Atheromatous plaques were excised intact, to facilitate the image co-registration, and immediately rinsed in 0.9% sodium chloride solution before fixation in 10% formalin solution. Plaques were then cut into 3 mm sections with reference to the start of the bifurcation (often three sections below and up to six sections above) following decalcification in ethylenediaminetetraacetate (EDTA) solution, and embedded in paraffin blocks. From these blocks 4 μm sections were obtained from the caudal end. Sections were dehydrated and underwent haematoxylin and eosin (H&E) and Elastic van Gieson (EVG) stains to define the fibrous cap, lipid core, calcium and haemorrhage components. The fibrous cap stained positively on the EVG section. The lipid core was identified by the area of necrotic material within which residual cholesterol crystals were seen on both H&E and EVG stains. Areas of calcification did not stain on EVG but had a blue hue from the haematoxylin in H&E sections, following the decalcifying process. Plaque haemorrhage was defined easily by the presence of fibrin and extravasated blood on the H&E section.

Image co-registration

The location of axial MR images (3 mm thickness) in relation to the carotid bifurcation was known from the planning ToF images. These were matched to corresponding histological sections from blocks located by reference to the carotid bifurcation of the ex vivo specimen, by two operators in consensus. Histological sections were then visually co-registered for orientation with the axial MR images. The process of image co-registration with reference to anatomical markers on MR and the ex vivo plaque resulted in little difficulty in matching corresponding pairs.

Image analysis

Axial MR images and histological sections were reviewed independently by experienced readers. Only MR images on which the entire vessel border was clearly visible and the lumen was free of flow artifact on all position matched sequences, were included in the image analysis. This initial image selection process was conducted by an independent operator not involved in the measurement analysis. An earlier pilot study validated the signal intensities of the individual plaque components on the different MR sequences with histology. From this earlier analysis the STIR sequence was determined to be the best to identify the fibrous cap and lipid core components. Any area of very high signal, adjacent to the lumen, on the STIR sequence was classified as fibrous cap. Haemorrhage infrequently gave similar appearance...
on this sequence but not the others therefore, the other sequence images were viewed to confirm that the high signal region on the STIR image was fibrous cap. Lipid core was seen as a low signal region on the STIR sequence but of intermediate intensity on the T1W and intermediate T2W sequences. These other sequences were reviewed to ensure that the low signal region on the STIR sequence was not calcium, as this can appear as a low signal region on both T1 and T2 weighted sequences. Following the initial characterization, only the STIR images depicting plaques free of haemorrhage or calcium were used in the quantitative component analysis.

**Measurement analysis**

Using the Analyze software (version 4; BIR, Mayo Clinic, Rochester, MN, USA) axial MR images were magnified 200% prior to localizing the carotid artery region, to maximize measurement accuracy and consistency. Image contrast settings were adjusted on all images to achieve standardized window/level settings. The following measurements were made using a manual tracing technique: the area of the fibrous cap (FC) and lipid core (LC) components and total plaque (TPA). Only areas within the vessel that the readers were confident to assign as fibrous cap or lipid core, based on the above algorithm were defined in the component analysis. The FC area was defined as the region of high signal intensity enclosed between the lumen and the low signal region below, by a manually traced contour (Fig. 1(a)). The area of the LC was defined as the region of low signal intensity (if present) below the fibrous cap, enclosed by the manually traced contour (Fig. 1(a)). The TPA was defined as the region enclosed by the manually traced contour, which included the fibrous cap and lipid core components, as well as regions that were not confidently assigned as either fibrous cap or lipid core. The radial extent approximated the vessel boundary and luminal edges (Fig. 1(a)); the vessel wall boundary appeared of slightly higher signal intensity compared to the surrounding tissue and was defined as the edge beyond which the region was no longer considered to be within the vessel but within the adjacent soft tissue. An experienced reader independently reviewed stained histological sections and identified the FC and LC components and similar area measurements (FC, LC and TPA) were made, again using a manual tracing contour using computerized planimetry (Leica Qwin, Milton Keynes, UK) from digitally captured images using a 1.6 magnification lens which allowed the entire vessel cross-section to be visualized in one field of view (Fig. 1(b)). Measures of plaque burden analysed *ex vivo* by others were not assessed in this study as from the earlier characterization it was evident that section deformation due to the relative thin nature of non-diseased vessel wall (Fig. 1(b)) would make assessment of both lumen and total vessel area difficult on histological sections.

**Statistical analysis**

The relative proportion of the plaque made up by the FC and LC components was quantified as a percentage of TPA, so that the amount of FC and LC were defined as the quotients of the cross-sectional area of FC and LC, respectively, divided by the cross-sectional TPA \( \times 100\% \). Inter-observer variability for measurements taken by the two MR readers was determined by computing the intra-class coefficient for both components. For the comparative analyses with histology, the mean values of the two MR measurements were used. The level of agreement between MR and histology based measurements for the component quantification was determined by generating Bland–Altman plots. All data were analyzed using SPSS for Windows (version 9).

**Results**

One hundred and eleven corresponding MR images and histological sections from 23 patients (average five corresponding image pairs per patient; range 3–6) were available for analysis. Corresponding MR and histological images from two patients (11 slices/sections) were excluded because of poor image quality or histological preparation. Seven sections from three plaques contained haemorrhage (Fig. 2); there were a
further 23 MR slices (from nine plaques) thought to depict calcium (Fig. 3). These 30 corresponding MR and histology pairs were excluded from the FC and LC measurement analysis. Consequently FC and LC area measurement analysis was performed on 81 corresponding MR and histological pairs.

There was excellent inter-observer agreement between MR readers for quantifying cross-sectional fibrous cap and lipid core areas, with an intra-class coefficient of 0.88 (95% confidence intervals: 0.73, 0.95) for LC measurements, 0.94 (95% confidence intervals: 0.89, 0.97) for FC measurements. Histological analysis revealed that the median fibrous cap content of plaques was 60.11%, and that of the lipid core was 32.5% in those plaques that did not contain either haemorrhage or calcium. The mean (± 2 SD) difference between MR and histology derived component quantification was −0.87% (±1.76%) for LC and −0.75% (±2.86%) for FC. Bland–Altman plots revealed strong agreement between MR and histology derived quantification of both components (Figs. 4 and 5). The level of agreement between MR and histology was stronger for quantifying fibrous cap content than for lipid core content but for both components, 95% of values were within two standard deviations of the mean difference.

Discussion

Until recently assessment of carotid athero-thrombosis following ischaemic stroke or transient ischaemic attacks (TIA), has been confined to quantitative assessment of luminal patency of the internal carotid artery. However, only selected symptomatic patients with severe stenosis have benefited from disease eradicating surgery.3,4 Additionally asymptomatic patients have been assessed by similar standards and although as a group have been shown to benefit from surgery, this has not been universally offered.17 Consequently, the current method of risk stratification may not be sufficient enough to accurately identify the high-risk patient. High-risk patients are thought to harbour vulnerable plaques18 and these vulnerable plaques can be visualized in a number of different ways. Ultrasound techniques are able to distinguish echolucent (predominant lipid core) from echo opaque plaques (predominant fibrous tissue/calcium), but are limited by resolution and are prone to inter-observer variability.19–23 More recent reports, however, have
extended the repertoire of characterization with promise of ultrasonographic correlates of cellular plaque components. Several studies have demonstrated the accuracy of multi-sequence high resolution MRI to identify individual plaque components, and furthermore carotid MRI is able to monitor plaque progression. Although some of these studies have used surrogates of plaque burden, such as total vessel wall thickness and relative size of lumen, there have been no previous studies that have attempted to quantify the individual plaque components. The latter is of considerable importance, in terms of risk stratification as histological studies have suggested that the relative amounts of the individual plaque components are important in determining plaque vulnerability. In this study we have shown that both the fibrous cap and lipid core components can be quantified using multi-sequence high resolution MRI with little inter-observer variability. For this validation study we chose to quantify the fibrous cap and lipid core components as an area percentage of total cross-sectional plaque area as this was thought to provide a better measure of burden than the absolute amount, particularly as critical thresholds have not yet been established for either of these plaque components. Secondly, the process of specimen fixation would cause plaque shrinkage and make comparison of the absolute values futile. We would, however, expect the area percentage determined by histology to still be proportionately representative as the fixative process causes shrinkage in the FC and LC components to similar extents. A recent report further showed that there is a considerable variation in the amount of plaque shrinkage between ex vivo specimens from different patients and so any relationship derived from absolute component measurements would not necessarily be widely applicable to all patients. Although we had little difficulty in identifying the fibrous cap and lipid core on MR images using our algorithm, it was more difficult to determine the full extent of either component in the shoulder regions, especially of eccentric shaped plaques and this might explain both the spread of values and the consistent over estimation of MR for the FC and LC area percentage measurements, as histological analysis was not subject to the same difficulties. We found that agreement between MR and histology was stronger for FC measurements than LC measurements, suggesting that the fibrous cap was easier to distinguish and delineate than the lipid core. It is, therefore, likely that with better tissue characterization, the agreement between MRI and histology for LC area measurements could potentially be improved.

We accept that by only attempting to validate the FC and LC burden with histology, the present MR study has some limitations. Intra-plaque haemorrhage is likely to alter the haemodynamic profile of plaques and as such the relative amount of haemorrhage would be an important parameter to quantify. Due to the heterogeneity of MR appearance both within any particular MR sequence series as well as between different sequences, haemorrhage is not reliably distinguishable. This most probably reflects the varying stages of haemoglobin degradation and consequently its ferromagnetic nature. Studies undertaken to determine the characterization of haemorrhage have shown this was only reliably identified when coupled with identification of the necrotic lipid core, which has overlapping signal intensity characteristics. The role that calcium plays in plaque stability is also not fully established, with some authors suggesting a protective role and others indicating that calcified plaques may alter the haemodynamic stress that the plaque can withstand, and may in fact increase the risk of rupture. Calcium has a low signal intensity appearance on T1W and T2W sequences and its presence is suggested by this fact. As the signal intensity characteristics overlap with lipid as well as haemorrhage on T2W sequences, MRI based characterization is reliant on multi-sequence review. Consequently, difficulties arise in MRI quantification when either the lipid core itself is partially calcified or is adjacent to an area of calcification. Plaque calcification, however, is better identified with CT angiography, where quantitative measurements are more reliable, and consequently a combined MR/CT approach to plaque characterization may overcome this problem.

It is likely that there exists a critical percentage area

![Bland–Altman analysis plot of MR and histology derived lipid core content measurements. 95% of values lie within 2 SD of the mean difference (–0.86 ± 1.74%).](image_url)
of FC below which and of LC above which, perhaps as a result of haemodynamic stress, that risk of plaque rupture is particularly high. The present study included only recently symptomatic patients and as such is not representative of the breadth of atherosclerotic plaques. Furthermore, as can be seen from the histology derived quantification, that the plaques in this study had a predominant fibrous cap content, most akin to the advanced but ‘stable’ fibroatheroma subtype described by the American Heart Association and others. In order to determine whether thresholds exist for the contribution of FC and LC to plaque burden, larger prospective studies need to be conducted, to include symptom free people with and without cardiovascular risk factors, which might yield a broader range of atherosclerotic plaques to include early lesions.

We have demonstrated the validity of MR in plaque quantification, and as such this technique has the potential for prospective, longitudinal plaque characterization and quantification studies in those asymptomatic patients who are deemed at high risk of stroke, as well as offering itself as a novel means to assess the impact of various therapeutic interventions.

References


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