Characterisation of Carotid Atherosclerotic Plaque in-vivo and ex-vivo using MRI, CTA and Histology

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Summary

Stroke is the third leading cause of death and the single largest cause of disability in the UK, its treatment consumes 4-6% of the total NHS budget[1]. Surgical excision of the atherosclerotic carotid plaque (carotid endarterectomy, CEA) reduces the risk of subsequent stroke. However, 70% of patients with severe stenosis remain stroke-free over the next 5 years with medical therapy alone. Outcomes from CEA could be improved by targeting treatment at high-risk subgroups[2].

It is crucial to identify high-risk subgroups that could benefit from CEA, as it has been reported that surgery within 14 days of transient ischaemic attack (TIA) could be prevented approximately 250 strokes a year, yielding saving to the NHS of around £4 million.

The aim of this work is to identify unstable or vulnerable plaque that defines higher risk by studying atherosclerotic plaque morphology and plaque composition.

Methods

In-vivo: Datasets were obtained from 30 selected symptomatic stroke patients (71±15 years) at the Institute of Neurological Sciences, Southern General Hospital, Glasgow. Subjects were imaged using 3T MR scanner (Sigma Excite, GE) with a four-channel prototypal surface coil. Black blood T1-weighted (T1-w), T2-weighted (T2-w), Proton Density-weighted (PD-w) and 3D time-of-flight (ToF) scans were carried out. T1-w, T2-w and PD-w scans were carried out with 0.27 x 0.27 x 2.8 mm^3 resolution and ToF 0.46 x 0.46 x 1.9 mm^3. The CTA studies were obtained on a CT scanner (Brilliance 64 slice, Philips) with 0.34 x 0.34 x 0.33 mm^3 resolution.

Ex-vivo: From the patients studied *in-vivo*, 16 of them underwent CEA. The specimens from these patients were imaged on a Bruker Biospec Avance system using a 7T horizontal 30 cm bore magnet. Carotid plaque specimens were imaged in a sealed 20 ml syringe filled with fomblin, to reduce susceptibility artefacts. A small phantom containing $MgCl_2$ was placed within the field of view as a standard. T1-w, T2-w (100 x 100 x 100 μm^3 isotropic resolution) and Diffusion weighted images (DWI) (181 x 181 x 181 μm^3 isotropic resolution) were carried out during the same weekend scanning session. Total scan time 40 hours.

Histopathology: After imaging, the endarterectomy specimens were embedded in paraffin, and then sectioned and stained with hematoxylin and eosin (H&E) and Miller's elastin/van Gieson (EVG) at 2mm intervals. Digital images of the histological preparations were acquired at 0.54 x 0.54 x 4 μm^3 resolution. Specimens were marked with ink and used as reference system.

Data Analysis

Datasets for each scan, MRI and CTA, were co-registered using a commercial package Analyze (Biomedical Imaging Resource, Mayo Foundation). We quantify the volume of the different plaque components by multiple thresholding of the MR signal and using a semi-automated analysis programmed in MatLab and freeware Image J. The total number of analysed MR slices were 334, each slice was review and excluded all the slices that either were noisy or did not have atherosclerotic plaque.

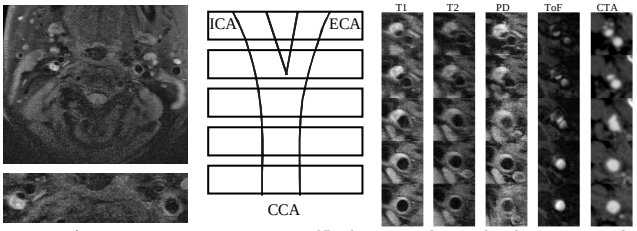

Figure 1: Left 2D T1-w image 512 x 512 matrix size. Middle schematic carotid artery, where the Common Carotid Artery (CCA) bifurcates into the Internal Carotid Artery (ICA) and External Carotid Artery (ECA). Right shows images of the carotid artery at different levels, T1-w, T2-w, PD-w, ToF and CTA.

Tissue classification

In this investigation we are using the tissue classification criteria publish by Saam et al. This criteria is for MRI data at 1.5 T, results have suggested that with identical automated tissue classification algorithms at 1.5 T give equivalent results at 3T [4].

Components	T1	T2	PD	ToF
Lipid-Rich/Necrotic Core: No/little, Fresh or recent haemorrhage	0/+ + +	-/0 -/0 +	0/+ -/0 +	0 + +
Calcification	-	-	-	-
Loose matrix	-/0	+	+	0
Dense (fibrous) tissue	0	0	0	-

Table 1: Summary of how to identify different plaque components depending on the MRI signal intensity of the different contrast images, T1-w, T2-w, PD-w and ToF. - hypointense, 0 isointense, and + hyperintense MRI signal intensity. Saam et al, 2005. All MRI signal intensities are relative to the adjacent sternocleidomastoid muscle.

In total 48 carotid arteries were segmented using the criteria in table 1 and classified according to the American Heart Association (AHA) classification table 2.

Conventional AHA Classification	Pathology findings	MR Imaging findings
Type 0: **No intimal thickening**		Normal
Type I: **Initial lesion**	Isolated macrophages contain oxidized lipid droplets (foam cells)	Normal / thin plaque
Type II: **Fatty streak**	Lesion grossly apparent with Sudan III staining, foam cells and smooth muscle cells contain lipid droplets	Thin plaque
Type III: **Preatheroma**	Raised fatty streak in gross morphology, multiple but small extracellular lipidic cores, foam cells contain lipid droplets, increasing number of smooth muscle cells	Small lipid core
Type IV: **Atheroma**	Single and massive extracellular lipid pool (lipid core), grossly visible, well delimited, covered by a proteoglycan-rich layer infiltrated with foam cells and smooth muscle cells with and without lipid droplet inclusion	Large lipid core
Type V: **Fibroatheroma**	Type IV with a cap rich in fibrosis (collagen), possible small calcifications	Large lipid core with a fibrous cap +/- small calcifications
Type VIa: **Complicated plaque**	Erosions or fissures on surface of lesion	Erosions or fissures on surface of lesion
Type VIb: **Complicated plaque**	Haematoma within lesion	Haematoma within lesion
Type VIc: **Complicated plaque**	Thrombosis on surface of lesion	Thrombosis on surface of lesion
Type VII: **Regressed plaque**	Calcification without lipid pools	Calcification without lipid pools
Type VIII: **Regressed plaque**	Fibrous tissue without lipid pools	Fibrous tissue without lipid pools

Table 2: Conventional AHA classification.

Results

In-vivo

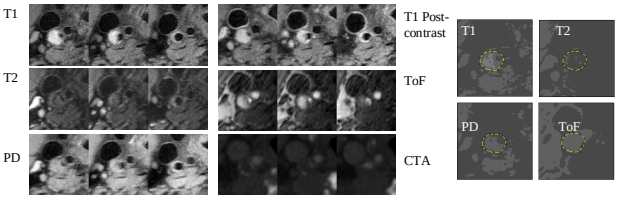

Figure 2: 61 years old male, on the left MRI scans T1-w, T2-w, PD-w, T1-w post-contrast, ToF MRI scans and CTA data. Right thresholded images using MatLab.

Figure 3 shows the result of classifying the 48 carotid plaques.

Figure 3: Left all the carotid plaques were graded using the AHA classification criteria. Right of 9640 mm^2 of tissue analysed 5.6% was calcification, 9.8% was LR/NC, 3.1% was haemohrrage and 81.5% of fibrous tissue.

Ex-vivo

After CEA the specimens were scanned in the 7T MR scanner, figure shows a female 76 years old.

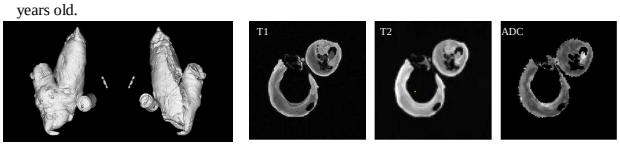

Figure 4: Carotid plaque specimen of a female 76 years old after CEA. Left 3D reconstruction of the specimen using the 7T MR T1-w image. Right 7T MR T1-w, T2-w and ADC images.

Carotid plaque specimens were dissected, embedded in paraffin wax and stained with H&E and EGV. The H&E sections were digitalised.

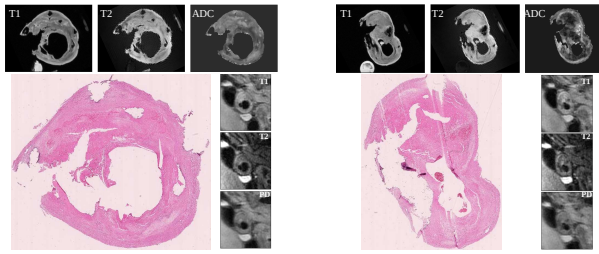

Figure 5: Comparison of the different weighted 7T MR images, H&E stained histology slices and 3T MR.

Correlation of 3T MRI and Histology

All the corresponding MR and digitised histology slices were compared, in total 29 images.

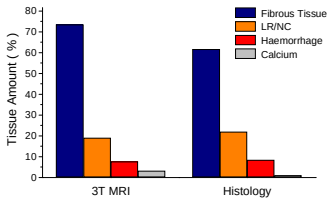

Figure 6: Comparison of 3T MRI and Histology. Left histograms showing percentages of the different tissue of the plaque, the p-values of the two datasets were 0.1 for LR/NC and calcium, 0.4 for Haemorrhage, and < 0.05 for fibrous tissue.

Conclusions

In this work we found that 3T MRI can distinguish between different carotid components such as LR/NC (with/without haemorrhage), calcification and fibrous tissue. Plaque composition calculated as percentage of the vessel wall was comparable for 3T MRI and histology for LR/NC (19 vs. 21.9%, p = 0.1), dense fibrous tissue (73.4 vs. 61.5%, p < 0.05), calcification (7.6 vs. 8.3%, p = 0.1). Haemorrhage was overestimated by MRI (3.2 vs. 1%, p = 0.4).

References

1. National Audit Office. *Reducing Brain Damage: Faster access to better stroke care* (2005).
2. Markus HS, King A, Shipley M, et al. *Lancet Neurol* 4422(10):70120-4 (2010).
3. Saam T et al, *Arterioscler. Thromb. Vasc. Biol.* 25:234-239 (2005).
4. Kerwin WS et al, *Journal of Magn. Reson. Imag.* 28:987-995 (2008).

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