Correlation of Carotid Atheromatous Plaque Inflammation Using USPIO-Enhanced MR Imaging With Degree of Luminal Stenosis

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Background and Purpose—Inflammation is a recognized risk factor for the vulnerable atherosclerotic plaque. The study explores the relationship between the degree of Magnetic Resonance (MR)–defined inflammation using Ultra Small Super-Paramagnetic Iron Oxide (USPIO) particles and the severity of luminal stenosis in asymptomatic carotid plaques.

Methods—Seventy-one patients with an asymptomatic carotid stenosis of ≥40% underwent multi-sequence USPIO-enhanced MR imaging. Stenosis severity was measured according to the NASCET and ECST methods.

Results—No demonstrable relationship between inflammation as measured by USPIO-enhanced signal change and the degree of luminal stenosis was found.

Conclusions—Inflammation and stenosis are likely to be independent risk factors, although this needs to be further validated. (Stroke. 2008;39:2144-2147.)

Key Words: asymptomatic carotid stenosis ▪ atherosclerosis ▪ USPIO ▪ MRI ▪ inflammation ▪ plaque vulnerability

Inflammation within atherosclerotic lesions increases the risk for plaque rupture and subsequent thromboembolism.1 It is possible to identify carotid inflammation in humans with Ultra small Super-Paramagnetic Iron Oxide (USPIO) enhanced magnetic resonance (MR) imaging.2 The use of a USPIO agent, Sinerem (Guerbet), has allowed the direct visualization of macrophage infiltration of carotid atheroma in vivo.2,3 USPIO particles are absorbed by activated macrophages in vulnerable plaques and when clumped produce a T2* susceptibility effect.

Recently published data suggest that the relationship between the degree of MR-defined inflammation using USPIO particles and the severity of luminal stenosis in asymptomatic carotid plaques is complex if not tenuous, and that plaques causing only a moderate asymptomatic carotid stenosis can have a high inflammatory load.3 This study was designed to explore the nature of any relationship which might exist and to evaluate any correlation (correlations below 0.3 are unlikely to be useful in clinical practice). We also evaluated correlations with a more established measure, the normalized wall index (NWI), which would a priori be expected to show a correlation with stenosis.

Materials and Methods

Patients
This observational cohort study consisted of 71 patients with asymptomatic carotid stenosis. The conduct of the MR studies did not cause a delay in surgical intervention. This study was approved by the institution’s internal review board. All patients gave written informed consent.

Duplex ultrasonography showing at least 40% carotid stenosis and sufficient MR image quality to identify the lumen wall and the outer boundary of the arterial wall were necessary requirements for inclusion.

Exclusion criteria were prior carotid endarterectomy and contraindication to MRI.

High-Resolution MR Imaging
Mult-contrast imaging of both internal carotid arteries (ICA) was acquired before and 36 hours after USPIO infusion, using a 1.5T MRI system (GE Diagnostic Imaging) and a 4-channel phased array neck coil (PACC, Machnet BV). The imaging protocol has been previously described in detail4 and included ECG-gated, black blood (BB), fast spin-echo sequences with imaging parameters modified to yield T1-weighted and intermediate and heavily T2-weighted images for characterizing plaque structure. A T2*-weighted gradient-echo based spiral sequence with a quadruple inversion recovery preparation was used to null the signal from blood pre- and post-USPIO.

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Quantitative Image Analysis

Pre- and post-USPIO MR images were manually coregistered according to plaque morphology and distance from the carotid bifurcation at the time of imaging. Images were manually segmented into quadrants, and the luminal blood pool was excluded using predefined rules in an effort to reduce interobserver error (CMR Tools). Signal intensities in each quadrant were normalized to the adjacent sternocleidomastoid muscle pre- and post-USPIO infusion.

Figure 1. a. Trellis plot showing correlation between 4 plaque assessment measures. b. Scatter plot showing luminal stenosis (NASCET) against USPIO signal change measured in each quadrant.
Signal change was then calculated as the difference in these normalized quantities. This was accomplished by manual delineation of each quadrant as a region of interest.

Two experienced readers independently reviewed each MR study. Image quality was rated per artery for each contrast weighting on a 5-point scale (1 = poor, 5 = excellent) dependent on the overall signal-to-noise ratio and clarity of the vessel wall boundaries. Slices with image quality of ≤2 were excluded from the study. Electronic calipers were used to measure percentage stenosis on the axial BB MRI images according to both the NASCET and ECST methods. The final reading used for luminal stenosis was defined as the mean of the 2 independent reviewers for both methods.

Carotid wall area was calculated as the difference between total vessel area and lumen area. NWI was calculated by dividing the wall area by the total vessel area.

Statistical Methods

Power
The cohort of 71 evaluable patients was considered sufficient to investigate the questions of interest, because this provides 80% power to detect a correlation of 0.32.

Agreement
To ensure robustness of the quantification of stenosis and quadrant analysis for USPIO signal change interreader agreement was determined by calculation of the intraclass correlation coefficient (ICC) and using the Bland Altman method for calculation of limits of agreement.

Correlation
Spearman rank correlation coefficient was calculated for the association between each of the following 4 measurements (Figure 1a):

1. \( y = a_1 + b_1 x \) Inflammation increases linearly with degree of stenosis;
2. \( y = a_2 + b_2 x^2 \) Inflammation increases with degree of stenosis, more rapidly so at higher degrees of stenosis;
3. \( y = a_3 \) if \( x < c \) Inflammation remains constant for degrees of stenosis below a threshold, then increases linearly with stenosis above this threshold.

Estimates of model parameters were calculated with appropriate 95% confidence intervals. In all three cases, evidence that the “b” parameter differed from zero would provide evidence of a relationship. Model assumptions regarding homogeneity of variance were verified by inspection of residual plots. Distributional assumptions regarding normality were verified by assessment of normal probability plots.

The three modelling approaches provided the following parameter estimates (with 95% confidence intervals):

1. \( a_1 = -0.072 (-0.171, 0.027), b_1 \times 10^3 = 0.79 (-0.71, 2.29) \)
2. \( a_2 = -0.050 (-0.102, 0.001), b_2 \times 10^6 = 6.9 (-4.3, 18.0) \)
3. \( a_3 = -0.031 (-0.053, -0.009), b_3 \times 10^3 = 1.8 (-0.3, 4.0), c = 63 (33, 87) \)

Figure 2. Exploring the relationship between USPIO signal change and luminal stenosis.

Signal change was then calculated as the difference in these normalized quantities. This was accomplished by manual delineation of each quadrant as a region of interest.

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- Stenosis % (NASCET)
- Stenosis % (ECST)
- Normalized wall index (NWI)
- Mean USPIO signal change across all quadrants within the plaque

A statistical model was previously developed which models the correlation between signal loss in different quadrants within a slice, and different slices within a plaque, allowing all data points to be included in the analysis but adjusting the degrees of freedom appropriately. This model was applied to the USPIO-enhanced signal changes and the percent stenosis by NASCET to investigate 3 possible simple relationships (Figure 2).

Results

Patients
Of 75 patients recruited to the study, 4 (5.3%) were excluded because MR image quality was ≤2. This yielded 81 arteries for analysis. Patient characteristics are presented in the Table.

Agreement
Interobserver agreement was high for USPIO signal change calculations. The ICC was 0.91. 95% limits of agreement were −0.155 to 0.170. Similarly, agreement of the MRI-based luminal stenosis measurements was substantial: ICC = 0.86 (±0.12; NASCET) and 0.82 (±0.09; ECST). Thus variability in the measurement is not attributable in any major extent to interrater differences.

Correlation
A trellis plot showing the relationships between the plaque assessments visually, with the corresponding nonparametric
correlation coefficient, is shown in Figure 1a. As expected, the correlation between the 2 methods for measuring stenosis was very high, and there was a small correlation observed between NWI and stenosis. However, there was no evidence of correlation between USPIO-enhanced signal change and either stenosis or NWI.

Figure 1b shows luminal stenosis (NASCET) and USPIO-enhanced signal change, plotting signal change as measured in each quadrant. There is no evidence to suggest any plausible relationship between USPIO signal change and luminal stenosis (Figure 2).

Discussion
This is to our knowledge the first study to explore whether there is a correlation between the degree of MR-defined inflammation using USPIO and the severity of luminal stenosis. We have demonstrated that there is no clear relationship between luminal stenosis using 2 established measurement techniques (i.e., NASCET and ECST) and the degree of USPIO enhanced MR-defined inflammation in asymptomatic carotid disease. If there were some correlation or relationship, it would reduce its value as an alternative surrogate risk marker to luminal stenosis as it would not be a completely independent risk factor.

Although the cohort of patients we have studied may appear relatively small, the fact that we demonstrated a small but significant correlation between luminal stenosis and NWI indicates that this study should have been able to detect a similar degree of correlation with USPIO if it had existed.

Limitations to our study include the fact that we only used asymptomatic patients, and it might therefore be argued that our population did not cover the whole spectrum of disease severity, which could question the generalizability of our results. However, patients included in this study are those for whom the benefit of CEA is still controversial and for whom much is to be expected from noninvasive imaging techniques. Another shortcoming is the use of axial BB MRI to measure luminal narrowing, although a preliminary study showed that it can provide an alternative and accurate method of measuring stenosis.6

Conclusions
We have shown that the relationship between USPIO-enhanced MR-defined inflammation and the degree of luminal stenosis is weak. This generates the interesting hypothesis that both measures are likely to be independent risk factors, although this needs to be investigated further in a larger cohort of patients.

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Disclosures
S.R.M. is an employee of GlaxoSmithKline (GSK). J.H.G. is a consultant to GSK.

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