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DOI: [10.1159/000339984](https://doi.org/10.1159/000339984)**Evaluation of Ultrasmall Superparamagnetic Iron Oxide-Enhanced MRI of Carotid Atherosclerosis to Assess Risk of Cerebrovascular and Cardiovascular Events: Follow-Up of the ATHEROMA Trial**Andrew J. Degnan^a, Andrew J. Patterson^a, Tjun Y. Tang^b,
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Atherosclerosis is a major risk factor for cerebrovascular and cardiovascular disease and their related morbidity and mortality. Atherosclerosis is fundamentally an inflammatory disease involving macrophages and T cells. Pathological studies of carotid atherosclerosis implicate macrophages in increasing the risk of fibrous cap rupture and they appear with greater frequency in plaques taken from symptomatic patients. Recent studies of Toll-like receptors offer evidence to support that monocyte activation is associated with larger and symptomatic plaques, again reinforcing the concept of inflammation worsening plaque vulnerability [1, 2]. There is clear clinical utility and necessity for an inflammatory marker capable of identifying high-risk atherosclerotic plaque.

The ability to detect macrophage activity indicative of plaque inflammation by MRI is possible using ultrasmall superparamagnetic iron oxides (USPIOs). USPIOs are thought to enter the inflamed plaque through the dysfunctional endothelium incited by a multitude of insults including cholesterol deposition, free radical damage from superoxides eluted by macrophages, and biomechanical stress. Studies to date have principally detected USPIOs as localized signal loss on T₂*-weighted sequences due to superparamagnetic effects of the iron oxide particles [3].

One initial retrospective study, using the commercially available USPIO agent Ferumoxtran-10 (Sinerem[®], Guerbet, Roissy, France), reported signal loss in atherosclerotic arteries [4]. Subsequent studies confirmed that Sinerem induced MRI-detectable signal loss in macrophage-rich plaques [5–7]. USPIO hypo-intensity changes can differentiate symptomatic carotid arteries from the contralateral asymptomatic side [8] with greater relative signal loss seen in symptomatic patients [9].

In 2006–2007, the ATHEROMA trial (Atorvastatin Therapy: Effects on Reduction of Macrophage Activity; NCT00368589) assessed differences in USPIO-related signal change in patients with

moderate carotid stenosis. The study randomly assigned patients into two treatment arms, low- (10 mg) and high-dose (80 mg) atorvastatin therapy, over 12 weeks, and assessed the dose-response interaction [10]. The purpose of this preliminary evaluation of long-term follow-up is to report on the ability of initial USPIO-enhanced MRI to predict subsequent cerebrovascular and cardiovascular morbidity and mortality.

*Methods**Study Participants*

All study participants (n = 62) initially screened for recruitment into the ATHEROMA trial [10] were retrospectively examined for the occurrence of cerebrovascular events, cardiovascular events and mortality following initial imaging with USPIO. Symptomatic status was defined as the occurrence of a cerebrovascular event (transient ischaemic attack and stroke) or cardiovascular event (myocardial infarction) to encompass the systemic risk nature of atherosclerosis. Of the study participants, 40 patients received statin therapy as randomized in the ATHEROMA trial for 12 weeks and then were unblinded and returned to statin therapy as directed by their clinician; the remaining patients initially screened but not randomized into the ATHEROMA study generally received routine standard of care using lower-dose statins. Medical records were sourced from the study centre and study participants' general practitioners as well as official government-issued death certificates in accordance with written informed consent previously approved by the Institutional Review Board. Patients were excluded from the retrospective review if there was no follow-up data beyond the 6 months after study entry date or if records were incomplete.

Inclusion and exclusion criteria were as per the initial study [10]; patients in this follow-up study included those who were initially screened and imaged with USPIO regardless of whether they were randomized to receive statin in the ATHEROMA study. Briefly, patients were included if they had reported carotid atherosclerotic disease (both asymptomatic and previously symptomatic patients were included) and were excluded if they had a previous carotid endarterectomy, possessed any contraindication to MRI (e.g. pacemaker, metal implants), demonstrated statin intolerance in the past, were diagnosed with renal impairment or reported a history of malignancy. The patients were required to have a minimum degree of stenosis of 40% on at least one side as measured at the initial ultrasound evaluation.

MRI Acquisition

Sinerem (Ferumoxtran-10) was diluted in normal saline and administered as a slow infusion through an indwelling large-bore intravenous cannula over 30 min with a total dose of 2.6 mg/kg. The patient received pre- and post-USPIO imaging, the post-USPIO imaging session was performed 36 h after the infusion (de-

tails describing the contrast agent administration and MRI protocol have previously been reported) [10].

All patients had both carotid arteries imaged using a custom-designed 4-channel phased-array surface coil (Flick Engineering Solutions BV, Winterswijk, the Netherlands) held close to the neck. Movement artefact was minimized using a vacuum-based head restraint system (VAC-LOK Cushion, Oncology Systems Ltd., UK). A quadruple inversion recovery 2-dimensional ECG-triggered T_2^* -weighted spiral sequence was performed with the following scan parameters: FOV 12 cm, NEX 2, TE 2.6 ms, TR 1 R-R, flip angle 60°, slice thickness 3 mm, slice spacing 0 mm.

Image Analysis

Pre- and post-USPIO infusion T_2^* -weighted images were manually coregistered on the basis of plaque morphology and position relative to carotid bifurcation; figure 1 depicts an example of matched pre- and post-USPIO contrast images. Two experienced, independent and blinded image readers manually segmented the artery wall and plaque into quadrants using predefined criteria. A horizontal line was placed through the lumen centre and a perpendicular line in the midpoint of the artery (CMR Tools, London, UK). Using these defined regions of interest, the mean signal changes between pre- and post-USPIO infusion were calculated for each quadrant following signal normalization to that of the adjacent sternocleidomastoid muscle. We report the percentage signal intensity decrease as a relative measure of USPIO uptake.

Statistical Analysis

Relative signal intensity change before and after USPIO infusion was computed within each quadrant. A Cox proportional hazard regression model was performed where the predictive variable was defined as the quadrant with maximum signal intensity change. The model outcome variable was defined as 'true' if patients experienced myocardial infarctions, transient ischaemic attacks or strokes, which resulted in either morbidity or mortality. Alternatively, the outcome variable was defined as 'false'. Time to event was defined based on the time which elapsed following the baseline MRI examination. Not experiencing an event before 2011 was defined for censorship for event occurrence.

Univariate analyses were also performed to compare differences in patients that had subsequent events. Fisher's exact test was used to compare differences in proportions and Wilcoxon's non-parametric test was used to compare continuous distributions.

A subjective evaluation of the predictive value of USPIO uptake was performed by generating Kaplan-Meier survival curves. The predictive variable, maximum percentage change within a quadrant, was stratified in two groups: 'USPIO positive' and 'USPIO negative'. The threshold was defined by the median.

An additional analysis was performed to test if signal intensity differences before and after USPIO infusion were associated with an early clinical event within 1 year of follow-up. Wilcoxon's non-parametric test was used to compare distributions.

Distributions which were not normally distributed were reported using median and interquartile ranges (IQs). The analysis was performed using the statistical programming language R version 2.12.2 (R Foundation for Statistical Computing, Vienna, Austria) using the survival package version 2.36-2.

Fig. 1. The T_2^* -weighted effect of USPIO on conventional spin echo MRI. Pre- (a) and post- (b) USPIO MRI of a symptomatic carotid plaque. Focal USPIO uptake can be seen at 24 h (arrow).

Fig. 2. Kaplan-Meier survival curves showing subsequent cerebrovascular and cardiovascular morbidity and mortality events. USPIO uptake is defined based on the difference in the relative signal intensity change between plaque and sternocleidomastoid muscle before and after USPIO infusion. Patients were stratified into the following two groups: USPIO+ is defined as $\geq$ median USPIO uptake; USPIO- is defined as $<$ median USPIO uptake.

Results

The study demographic included 62 patients (56 males; median age 68.5 years, IQ 61.3–74.0 years). The median carotid stenosis was 60% (IQ 50–65). The median follow-up period was 4.0 years (IQ 3.9–4.4). The results from the univariate analysis comparing both traditional vascular risk factors and relative signal intensity change due to USPIO infusion are summarized in table 1. The table stratifies the patients into two groups depending on whether they experienced a subsequent cardiovascular or cerebrovascular morbidity or mortality event. With the exception of the risk factor diabetes (p value = 0.020) there was no significant difference between groups.

In total 17 patients reported subsequent events; there were a total of 8 myocardial infarctions (3 fatal), 6 transient ischaemic attacks and 5 strokes (3 fatal) over the follow-up period. The timing of these events is graphically illustrated in figure 2. In total,

Table 1. Differences in traditional risk factors and macrophage activity (as measured by USPIO contrast-enhanced MRI)

	Cerebro/cardiovascular events		p value
	yes (n = 17)	no (n = 45)	
Symptomatic/asymptomatic	6/11 (54.5%)	17/28 (60.7%)	1.00 ¹
Female/male	0/17 (0.0%)	6/39 (15.4%)	0.176 ¹
Diabetes, yes/no	6/11 (54.5%)	4/41 (9.8%)	0.020 ¹
Hyperlipidaemic/normolipidaemic	7/10 (70.0%)	16/29 (55.2%)	0.771 ¹
Normotensive/hypertensive	1/16 (6.3%)	4/41 (9.8%)	1.00 ¹
Smoker, never/previous and current	4/13 (30.8%)	10/35 (28.6%)	1.00 ¹
Age, years	73 [65–76]	67 [60–73]	0.081 ²
Carotid endarterectomy, yes/no	12/17 (70.5%)	26/45 (57.7%)	0.37 ²
Mean percentage signal intensity decrease per plaque	15.0 [9.7–20.5]	8.9 [1.4–20.7]	0.279 ²
Max. percentage signal intensity decrease within slice	25.8 [20.7–35.6]	21.8 [11.2–32.6]	0.234 ²
Max. percentage signal intensity decrease within quadrant	48.0 [30.6–61.7]	41.8 [19.6–50.0]	0.172 ²

The population of 62 patients with moderate stenosis was stratified as having subsequent cerebrovascular and cardiovascular morbidity and mortality events over a median follow-up time of 4.0 years. Hyperlipidaemia was defined as LDL >130. IQs are given in square brackets. Max. = Maximum.

¹ Fisher's exact test compares differences between proportions.

² Wilcoxon's non-parametric test compares differences in distributions.

Table 2. Differences in the maximum signal intensity change within a quadrant before and after infusion after 1 year of follow-up comparing initially asymptomatic and previously symptomatic patients

	Cerebro/cardiovascular events after 1 year				p value
	n	yes	n	no	
Symptomatic + asymptomatic patients	9	57.7 [35.3–65.2]	53	41.8 [20.4–50.0]	0.074
Symptomatic patients only	3	57.7 [39.7–59.6]	20	37.1 [18.3–49.0]	0.355
Asymptomatic patients only	6	56.6 [38.5–125.5]	33	41.9 [21.9–53.1]	0.139

Wilcoxon's non-parametric test compares difference in distributions. Interquartile ranges are given in square brackets.

38 patients (61%) underwent carotid endarterectomy during the follow-up period with no difference between groups. Subjective evaluation of the Kaplan-Meier curves (with an arbitrarily defined threshold for USPIO positive and negative) suggests that the USPIO-positive patients had more events, which tended to occur within the first 10 months.

The Cox proportional hazard regression model found that the variable maximum percentage change within a quadrant was predictive of outcome ($b = 0.01134$, $se_b = 0.00626$) with a p value of 0.07.

An analysis of maximum signal intensity change within a quadrant reported the differences in patient groups who have subsequent events within 1 year of follow-up, and this is reported in table 2. The analysis observes a trend to having a higher relative change in signal intensity before and after USPIO infusion in those patients who had early clinical events within 1 year of follow-up; however, the difference is not statistically significant.

Discussion

An ultimate aim of developing modern carotid imaging protocols is to identify vulnerable plaques corresponding with an enhanced risk of cerebrovascular and cardiovascular events. This study investigated the association between the magnitude of maximal USPIO-induced signal intensity loss within carotid plaques and morbidity and mortality in a largely asymptomatic population. There was an association, albeit non-significant ($p = 0.07$), between USPIO-defined plaque inflammation and developing subsequent vascular events.

Several key limitations may explain why this study failed to identify a significant association. This study included only 62 individuals with initial USPIO imaging and follow-up, and our study cohort was largely an asymptomatic population ($n = 39$) and most patients received medical therapy and carotid endarterectomy during the follow-up period; therefore these patients had a

relatively low baseline risk of subsequent vascular events. Patients with asymptomatic, severe carotid stenosis have an annual risk of ischaemic stroke of only 3–4% whereas patients similar to ours have an even lower risk of stroke closer to 2% per annum [11]. Others have suggested that positive imaging findings are only predictive of subsequent events in patients with prior neurological symptoms [12]. We grouped cardiovascular and cerebrovascular events together in this study to reflect the observation that atherosclerosis, particularly inflammation within the vasculature, is frequently present in both coronary and carotid arteries [13]. With only 17 aggregated cardiovascular and cerebrovascular events in the span of this long-term follow-up, the ability to detect differences between risks of events based on USPIO imaging was severely hindered by the low event rate, hence increasing the likelihood of type II error.

This study is the largest long-term follow-up of patients imaged with USPIO to ascertain the risk of subsequent clinical events, yet it is nevertheless inadequately powered to make a definitive conclusion about the utility of USPIO for the detection of carotid plaque inflammation. Future studies need to implement alternate ways of examining USPIO uptake. Future investigations using newer USPIO agents in development may offer better evaluation of inflammation in atherosclerosis; any new study should incorporate a prospective long-term clinical follow-up of patients.

The key attributes of a successful USPIO contrast agent for atherosclerosis imaging are fast blood clearance, rapid uptake into plaque, sufficient concentration within plaque and specific uptake of USPIO by inflammatory cells. However, there remains the problematic need for 2 scans a day apart which necessitates the patient to return and prevents the use of this technique for acutely symptomatic patients. In requiring 2 scans with a substantial time interval, coregistration of images to ensure the slices match between studies is necessary and thus, signal intensity changes related to USPIO-induced signal loss may be confused by subtle partial volume effects or slice mismatch. Quantitative techniques may mitigate these issues. Previous USPIO studies have derived quantitative T_2^* -weighted measurements and suggested that the pre-infusion scan might be omitted.

Other high-resolution MRI methods may aid in stratifying the vascular risk of carotid atherosclerosis; gadolinium contrast use has been proposed as a means of indicating the presence of neovascularization within the carotid plaque. One group recently reported a retrospective relationship between plaque enhancement following contrast and risk of combined events ($p = 0.032$), but failed to find such an association in a prospective cohort [14, 15]. Though contrast enhancement may offer some benefit in risk stratification, there are some limitations as in our study that limit the findings of these authors, and better, prospective trials are necessary to ascertain diagnostic utility.

In the coming years, the use of novel imaging methods such as USPIO-enhanced MRI as employed in this study must be tempered with evidence-based practices prior to widespread clinical adoption. While our study failed to draw a conclusive association between USPIO uptake and risk of future events, this finding was most likely due to inadequate statistical power, and further investigation of USPIOs is warranted to improve risk assessment in individuals with asymptomatic carotid stenosis whose risk of stroke is still poorly characterized by stenosis measurement.

Conclusion

Our study failed to note a statistically significant association of maximum USPIO-induced signal change by plaque quadrant and risk of subsequent cerebrovascular and cardiovascular events ($p = 0.07$); however, this lack of association is most likely reflective of inadequate statistical power. Prospective investigation of novel USPIO agents in carotid atherosclerosis will better ascertain the usefulness of USPIO-enhanced MRI in the risk stratification of asymptomatic carotid atherosclerosis.

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Disclosure Statement

We declare that we have no conflict of interest. J.H.G. is a consultant to GlaxoSmithKline.

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