

Title: A longitudinal study of cortical grey matter lesion subtypes in relapse-onset multiple sclerosis

Authors: Varun Sethi (1), Tarek Yousry (1,2), Nils Muhlert (3), Daniel J. Tozer (4), Daniel Altmann (1,5), Maria Ron (1), Claudia Wheeler-Kingshott (1), David H. Miller (1,6), Declan T. Chard (1,6)

Affiliations

1. Queen Square MS Centre, NMR Research Unit, Department of Neuroinflammation, UCL Institute of Neurology, London, UK
2. Lysholm Department of Neuroradiology, National Hospital for Neurology and Neurosurgery, London, United Kingdom
3. School of Psychology and Cardiff University Brain Research Imaging Centre, Cardiff University, Cardiff, UK
4. Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK
5. Medical Statistics Department, London School of Hygiene & Tropical Medicine, London, UK
6. National Institute for Health Research (NIHR) University College London Hospitals (UCLH) Biomedical Research Centre, UK

Corresponding author: Declan Chard

Address: Queen Square Multiple Sclerosis Centre, Department of Neuroinflammation, UCL Institute of Neurology, University College London, Queen Square, London, WC1N 3BG, UK.

Telephone number: +44 2078298771

Email: d.chard@ucl.ac.uk

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Abstract

Background: Cortical grey matter (GM) lesions are common in multiple sclerosis (MS), but little is known about their temporal evolution. We investigated this in people with relapsing-remitting (RR) and secondary progressive (SP) MS.

Methods: 27 people with RRMS, and 22 with SPMS were included in this study. Phase sensitive inversion recovery (PSIR) scans were acquired on two occasions. Cortical GM lesions were classified as intracortical (IC, only involving GM) and leucocortical (LC, mixed GM-white matter [WM]); WM lesions touching the cortex as juxtacortical (JC). On follow up scans, new IC, LC and JC lesions were identified, and any change in classification of lesions previously observed was noted. WM lesion counts in the whole brain were assessed on PD/T2-weighted scans.

Results: Over a mean (SD) of 21.0 (5.8) months, the number of new IC lesions per person per year was greater in SPMS (1.6 [1.9]) than RRMS (0.8 [1.9]) (Mann-Whitney $p=0.039$). All new LC lesions arose from previously seen IC lesions (SPMS 1.4 [1.8] per person per year, and RRMS 1.1 [1.0]), and none arose *de novo* or from previously seen JC lesions. Changes in cortical GM (either new IC or IC converting to LC) lesion counts did not correlate with the changes in WM lesion counts.

Conclusions: New cortical GM lesions rarely arise from the WM and the rate of new IC lesion formation is not closely linked with WM lesion accrual. IC lesion formation appears to be more common in SPMS than RRMS.

Introduction

Histopathological [1-4] and magnetic resonance imaging (MRI) studies [5,6] have established that cortical grey matter (GM) lesions can be extensive and are clinically relevant in multiple sclerosis (MS). Cortical GM lesions are seen in all MS subtypes [1,2], but are particularly apparent in people with progressive MS [1,7]. Cortical lesions play a significant role in the accumulation of irreversible disability [5].

Little is known about the development and evolution of cortical GM lesions [8-10]. It is uncertain whether lesions confined to cortical GM (intracortical [IC] lesions) accrue at the same rate as leucocortical (LC) lesions (those involving both GM and WM) or WM lesions, or whether the rate of cortical GM lesion formation is similar throughout the course of relapsing-remitting (RR) and secondary progressive (SP) MS. It is also not known if LC lesions form *de novo*, or through the extension of IC lesions into WM, or juxtacortical (JC) WM lesions into GM. Cortical GM demyelination has been linked with meningeal inflammation, providing a plausible mechanism for cortical GM lesion formation to occur independently of WM demyelination [11]. and so it cannot be assumed that an effect MS disease modifying treatments have on WM lesion accrual will be similar for GM lesions.

Investigating the evolution of cortical GM lesions in histopathological studies is not possible, as serial tissue sampling cannot be undertaken. Investigating lesion evolution *in vivo* with magnetic resonance imaging (MRI) has been hampered by limited detection of GM lesions using conventional scanning methods. However, with the development of MRI techniques that improve the detection of GM lesions, such as double inversion recovery [12,13] and more recently phase sensitive inversion recovery (PSIR) [14,15], it is now possible to see GM lesions in nearly everyone with MS. Compared with DIR, PSIR appears to increase GM lesion detection 2 to 3 times and,

importantly, allows cortical GM lesions to be more robustly distinguished from JC WM lesions, and sub-classified into IC and LC lesions [15,16]. Using a high resolution PSIR sequence, we studied the formation and evolution of cortical GM lesions on a lesion-by-lesion basis, and examined their relationship with WM lesion accrual.

Methods

Sixty five people took part in this study; all gave a written informed consent. This study had approval from our local institutional ethics committee. Data from three people with RRMS had to be excluded due to MRI motion artefacts; of the remaining 62 people, 27 had RRMS, 22 SPMS, and 13 were healthy controls [See Table 1]. For the MS groups, a detailed clinical history was obtained and neurological examination undertaken. Disease duration, number of relapses since the baseline visit, and change in disease modifying therapy were all noted. Expanded disability status scale scores were estimated [17].

PSIR (0.5x0.5x2mm) and PD/T2-weighted (1x1x3mm) scans were acquired (as per our previous work [15]) on a 3T Philips Achieva system, on two occasions at least one year apart. Follow-up images were registered to baseline scans using NiftyReg (<http://sourceforge.net/projects/niftyreg>) [18] with an affine transformation. On baseline scans, cortical GM lesions were classified as IC or LC; WM lesions touching but not entering the cortex were classified as JC lesions. As in our previous work [15], a cortical GM lesion was defined as a focal hypointensity relative to the surrounding normal cortex, that involved the cortex in part or whole. When small or ill-defined on a single slice, a lesion was only counted if it was present on at least one other adjacent slice. If a lesion was only seen to involve cortical GM it was classified as being IC, and if it also involved WM it was classified as LC. Total cortical GM lesion counts were calculated as the sum of IC and LC lesion counts. On follow up scans, new cortical GM lesions were counted, and on a lesion-by-lesion basis any change in classification of lesions previously identified in or adjacent to the cortex (e.g. from IC or JC to LC) was noted. The intra-operator

[intra-class correlation coefficient, using this PSIR sequence and these criteria, was 0.995 for total cortical GM lesion counts \(IC and LC\), and for IC and LC lesions separately, 0.990 and 0.984 respectively \[15\].](#) The PD/T2-weighted scans were used to count new WM lesions appearing over the follow up interval. All lesion marking was undertaken by VS under the supervision of an experienced neuroradiologist (TY) using guidelines previously reported [15]. Marking of all scans was blind to clinical details.

Statistical analyses were performed using SPSS version 21 (SPSS, Chicago, IL, USA). Differences in demographic and clinical variables between RR and SPMS were examined using independent sample t-tests. Since lesion numbers tend not to be normally distributed, changes in lesion numbers were compared between patient groups using the Mann-Whitney test. Spearman rank correlation was used to investigate associations between GM and WM lesion accrual. The threshold for statistical significance was $p \leq 0.05$.

Results

The demographic and clinical details of the participants are shown in Table 1. As expected people with SPMS were older (mean 53.4 (SD 7.3) compared with 41.7 (10.9) years; $p < 0.001$) and had longer disease durations than RRMS (25.4 (10.0) compared with 13.1 (9.5) years; $p < 0.001$). The mean (SD) follow up period for the RR group was 22.2 (6.3) months and for SP 19.5 (4.8) months, which was not significantly different. The median EDSS for people with RRMS was 1.0 at baseline (range 1.0-6.0) and 1.5 (range 1.0-6.0) at follow up, and for SPMS was 6.5 (range 4.0-8.5) at both time points. At baseline 23 RRMS and 9 of the SPMS group were on a disease modifying treatment, and during follow up disease modifying therapy was started in one person and changed in five people with RRMS.

Baseline lesion characteristics

Baseline and follow up lesion counts are given in Table 2. While the total number of cortical GM lesions ([IC and LC combined](#)) was higher in the SP compared with RRMS groups (46.6 [19.4] compared with 36.8 [14.8] respectively), this did not reach statistical significance. IC, LC and JC lesion

counts (Table 2) also did not differ significantly between the RR and SP groups.

Lesion accrual

At follow up the SPMS group had a statistically higher [total](#) number of cortical GM lesions when compared with the RRMS group (mean 49.0 (19.7) and 38.0 (15.7) respectively; Mann-Whitney $p=0.043$), but again there was no statistically significant difference between the groups for IC, LC or JC lesion counts. All lesions seen at baseline remained visible at follow up. The accrual of new IC lesions per person per year was greater in SPMS (1.6 (1.9)) than RRMS (0.8 (1.9)) (Mann-Whitney $p=0.039$). No *de novo* new JC or LC lesions were seen, and new LC lesions were seen to evolve from IC lesions but not JC lesions (Table 2). An example of an IC lesion converting to LC, is shown in Figure 1.

The mean number of lesions converting from IC (at baseline) to LC lesions (at follow up) per person per year was not significantly greater in SPMS than RRMS (1.4 [1.8] compared with 1.1 [1.0] respectively). No new cortical lesions were seen in controls.

WM lesion accrual per year was not significantly different in RRMS and SPMS groups (Mean [SD]: 0.7 [1.3] compared with 0.2 [0.4] respectively). No new WM lesions were seen in controls. In the entire MS group, there was no significant correlation between the number of new WM lesions and the number of lesions converting from IC to LC or the number of new IC lesions. Similar results were seen in the RRMS and SPMS subgroups, and so are not shown.

Discussion

The most striking finding in our study was that new LC lesions (lesions that involve both cortical GM and adjacent [JC] WM) evolved from pre-existing IC lesions (which are confined to GM), and not from JC WM lesions or *de novo*. We also found significantly more new IC lesions in SPMS than RRMS

and, consistent with previous work [10] we found that changes in IC lesion numbers did not correlate with changes in WM lesion counts. The latter finding suggests that cortical GM lesion accrual is not closely linked with WM lesion formation.

In a previous study using DIR scans to identify and count cortical GM lesions, Calabrese and colleagues followed up a mixed cohort of people with MS over 6 years [5]. When assessed at 3 years [10], the average rate of new cortical lesions was ~0.6 per person per year in RRMS (n=76) and ~0.7 in SPMS (n=31). At 5 years [5], the new cortical GM lesion accrual was 0.6 per person per year in both RRMS (n=157) and SPMS (n=31), and the rate of new WM lesions was ~0.8 and ~0.3 per person per year in RRMS and SPMS groups respectively. At 6 years [10], in RRMS the mean rate of cortical lesion accrual was 0.9 per person per year (n=95), but SPMS figures were not reported. Roosendaal and colleagues also assessed cortical GM lesion formation in 13 people with RRMS (=9) and SPMS (n=4) over 3 years, but did not report on lesion accrual in these groups separately [9].

In the present study, new cortical GM lesions formed at a rate of ~0.8 in RRMS and ~1.6 per person per year in SPMS, and WM lesions at a rate of ~0.7 and ~0.2 per person per year respectively. With the exception of new cortical GM lesion accrual in SPMS, our results are very similar to those of Calabrese *et al.* With regard to the rate of cortical GM lesion accumulation in SPMS, the discrepancy in results between studies may be due to chance alone. However, the SPMS group included in the present work also had a much longer mean disease duration (25.4 years compared with 11.6 years) and greater disability as measured by EDSS scores (median 6.5 compared with 4.5) than the previous study [5], and so these two studies may actually be looking different phases of progressive MS and it is possible that with longer disease duration there is an increase in formation of new IC lesions in SPMS. It is also possible that the difference reflects the use of different MRI sequences to detect cortical lesions in the studies (PSIR in ours and DIR in Calabrese *et al.*)

When we tracked individual lesions we found that LC lesions did not occur *de novo*, but instead arose from previously seen IC lesions, and the rate of conversion from IC to LC lesions was matched by the rate of new IC lesion formation. As such, the apparently static total IC lesion counts (Table 2) gives a false impression of stability, when actually new IC lesions did occur (more so in SPMS), and other IC lesions extended into WM so increasing LC counts. This has important implications when assessing treatment effects, as counting either total IC or LC lesions alone may overlook substantial increases in the number of LC lesions and significantly underestimate the rate of new IC lesion formation.

The total number of cortical GM lesions was significantly greater in the SPMS compared with RRMS group at follow-up. However, when we subdivided cortical GM lesions into IC and LC, while both lesion types were more numerous in SPMS compared with RRMS, and the difference was about twice as great for LC compared with IC lesions (Table 2), the differences did not reach statistical significance. We think that this relates to the size and heterogeneity of the cohorts, which while large enough to demonstrate a difference in total cortical GM lesion counts were too small to distinguish subtypes of cortical GM lesions.

Consistent with previous work [10,19], we did not find a significant correlation between the formation of new cortical GM lesions and new WM lesions. There are a couple of possible explanations for this. First, that cortical GM and WM lesion genesis have at least partially different underlying mechanisms. As noted above, meningeal inflammation has been seen in tissue sample from people with SPMS, and linked with both the extent of cortical GM lesions [20], and the magnitude of neuronal loss [21], providing a plausible mechanism for GM lesions to form independently of WM lesions. Second, that similar mechanisms underlie both WM and GM lesion formation but have their greatest effect on WM and GM at different times. WM lesion accrual occurs most rapidly earlier in the clinical course of MS [10,22]. In contrast, the results of our study, and those from previous work [5], show that the rate of GM lesion formation is similar, if not higher, in SPMS compared

with RRMS. With improved discrimination between IC and LC lesions using the PSIR sequence [16], our study also suggests that new IC lesion formation is probably higher in SPMS than RRMS.

In recent work, Rinaldi *et al.* [23] looked at the effects of natalizumab, interferons and glatiramer acetate, on the rate of IC lesion formation in people with RRMS. Over the two years of their study, the average number of new IC lesions per person per year was ~1.5 in the untreated group (n=35), ~0.7 in those taking interferon or glatiramer acetate (n=50) and ~0.1 in those receiving natalizumab. This demonstrates a potentially useful role for cortical GM lesion measures in treatment trials. Our results suggest that not only are new IC lesion counts of interest, but the expansion of IC lesions into LC may also provide additional information about treatment efficacy.

A remaining limitation of our *in vivo* study is the sensitivity of cortical lesion detection on MRI. While using PSIR about 3 times more lesions IC are identified when compared with DIR [15], using DIR *in vitro* less than 10% of histopathologically confirmed IC lesions are detected [4]. It is therefore likely that many IC lesions are still not identified on the PSIR sequence. This is particularly the case for subpial lesions, which are the most common type of cortical GM lesion seen in histopathological studies [1], but are rarely seen on DIR or PSIR [12,15]. While this does not negate the results of the present study, it would be of great interest to know if the new IC lesions seen in this study began in a subpial location. It may be possible to address using ultra-high field MRI: Recent work at 7T acquiring T2*-weighted scans has demonstrated that a substantial number of subpial lesions can be seen [24]. In addition, we registered the follow-up to baseline scans, which allowed us to more readily match lesions seen at baseline with those seen at follow-up. However, this registration step will have subtly blurred the follow-up images and so reduced contrast between lesions and surrounding tissues, and between GM and WM. Given that the contrast between GM lesions and normal-appearing GM is lower than that between WM lesions and normal-appearing WM, and between GM and WM, slight blurring of the follow-up images will tend to make it relatively more difficult to detect IC lesions when

compared LC or JC lesions. As we only identified new IC, but no new LC or new JC lesions, we do not think this can be explained by registration-associated methodological bias, but we may have underestimated slightly the number of new IC lesions

In conclusion, we have found that lesions that involve cortical GM rarely arise from the WM, the rate of cortical lesion formation is not closely linked with WM lesion accrual, and that new IC lesions probably arise more often in SPMS than RRMS. These results suggest that measuring WM lesion accrual alone is not a sufficient marker of MS disease activity and progression, and should encourage further efforts to develop MRI techniques that improve the *in vivo* detection of cortical lesions in MS.

Tables

Table 1: Participant demographics

Group	Age (years)	M:F	Baseline EDSS Median (Range)	Disease duration in years	Scan interval in months
Controls (n=13)	35.1 (12.2)	7 : 6	NA	NA	24.6 (6.8)
RRMS (n=27)	41.7 (11.0)	6 : 21	1.0 (1.0-6.0)	13.1 (9.5)	22.2 (6.3)
SPMS (n=22)	53.4 (7.3)	9 : 13	6.5 (4.0-8.5)	25.4 (10.0)	19.5 (4.8)

Mean (SD) shown unless noted otherwise; RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

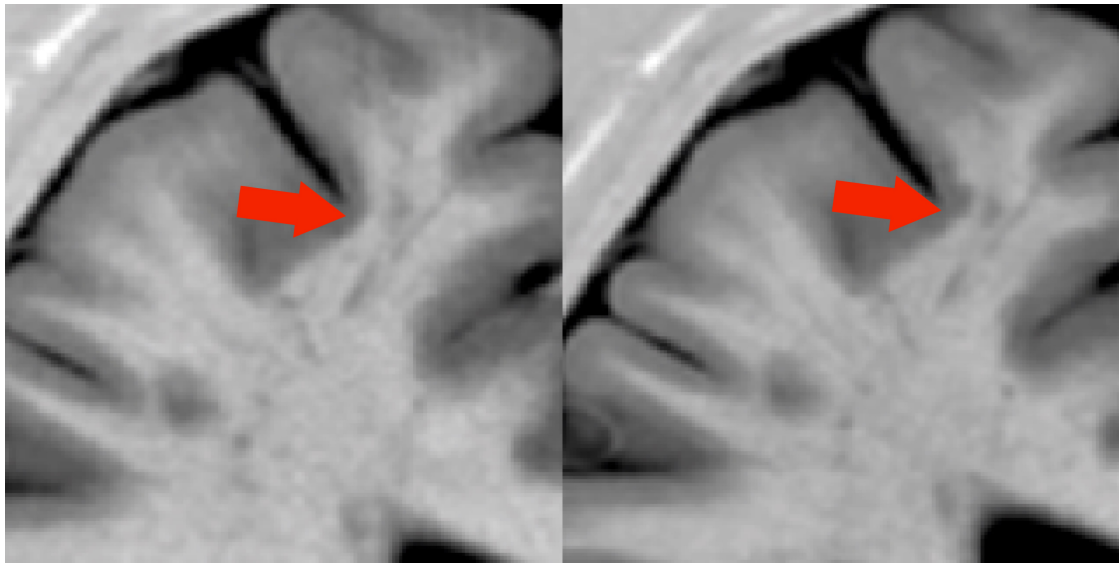
Table 2: Number of cortical lesions seen at baseline and follow up

Group	Mean (standard deviation) lesion counts							
	Baseline			Follow up				
	IC	LC	JC	New IC	IC to LC	IC	LC	JC
Controls	0.0	1.6 (2.6)	0.0	0.0	0.0	0.0	1.6 (2.6)	0.0
RRMS	20.3 (9.9)	16.4 (12.0)	7.7 (9.3)	1.1 (2.0)	1.7 (1.5)	19.7 (10.2)	18.2 (12.2)	7.6 (9.2)
SPMS	23.5 (10.6)	23.1 (15.3)	11.6 (12.5)	2.4 (2.6)	2.1 (1.9)	23.8 (10.6)	25.2 (16.0)	11.6 (12.5)

RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; IC = intracortical; LC = leucocortical; JC = juxtacortical.

Figures

Figure 1: An intracortical lesion evolving into a leucocortical lesion



Corresponding PSIR images at baseline (left) and follow up (right) showing an intracortical lesion at baseline that extends into white matter on follow up, so becoming a leucocortical lesion.

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