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Multiple sclerosis lesion formation and early evolution revisited: A weekly high-resolution magnetic resonance imaging study

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Abstract

Background: Several magnetic resonance imaging (MRI) studies investigated the evolution of multiple sclerosis (MS) lesions to understand the pathophysiological mechanisms leading to blood-brain barrier breakdown and lesion formation. Only a few assessed the early natural history of MS lesions using short-interval longitudinal MRI.

Objective: The purpose of this study was to characterize MS lesion occurrence and early evolution on high-resolution MRI acquired at weekly intervals.

Methods: Active lesions were characterized on 3D fluid attenuation inversion recovery (FLAIR) and gadolinium-enhanced 3D T1-weighted MRI performed weekly (seven weeks) on five untreated patients with relapsing–remitting MS (RRMS).

Results: Active lesions (n=212) were detected in all patients. All showed contrast-enhancement on at least one time-point. Most new lesions (83.5%) were visible on FLAIR and post-contrast T1-weighted images at first detection; 11.2% showed activity on FLAIR images, one or more weeks before the appearance of contrast-enhancement; 12.5% enhanced before being apparent on FLAIR.

Conclusion: Blood brain barrier disruption is a constant step in the natural history of active MS lesions, but does not always constitute the initial event. These findings are consistent with the existence of a subpopulation of lesions with an 'inside-out' genesis, where neurodegenerative processes might precede microglial activation, and a subsequent adaptive immune response.

Keywords: Magnetic resonance imaging, multiple sclerosis, relapsing–remitting, T2 lesions

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Introduction

Brain magnetic resonance imaging (MRI) contrast enhancement in multiple sclerosis (MS) is a consequence of blood-brain-barrier (BBB) breakdown, and reflects areas of active inflammation in MS lesions.[1,2] Several monthly brain MRI follow-ups of MS patients have demonstrated gadolinium (Gd)-chelate uptake as an early feature of most, but not all new lesions appearing on T2-weighted images.[3–8] Very few studies performed MRI more frequently than monthly to characterize MS lesion formation.[9,10] In a weekly study of three patients, all 16 new lesions showed contrast enhancement.[9] A larger natural history study with weekly MRI on 26 MS patients with relapsing–remitting (RR) or secondary progressive (SP) MS reported a median duration of lesion enhancement of two weeks with a non-normal distribution skewed towards shorter durations, but did not assess the frequency of enhancement in new T2 lesions.[11] Since enhancement duration correlated with lesion size, we hypothesized that many small lesions enhancing for less than a week were occurring without being detectable at the lower image resolution being used at the time.[11] That study was performed on a 1.5 Tesla system with older pulse sequences yielding image resolutions well below those achievable with current state-of-the-art MRI.

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Table 1. Summary of clinical and magnetic resonance imaging (MRI) data of the study subjects.

Patients ID	Gender	Age	Disease duration	EDSS	Number of active lesions
1	F	28	3	2	51
2	M	30	3	0	1
3	F	49	8	4	11
4	F	39	6	4	136
5	F	45	18	0	13

EDSS: Expanded Disability Status Scale; F: female; M: male.
Age and disease duration refer to the time of baseline MRI scan and are expressed in years.

This work was performed to characterize MS lesion activity, onset frequency, and early evolution on high-resolution 3D fluid attenuation inversion recovery (FLAIR) and Gd-enhanced 3D T1-weighted (3D-T1-Gd) images at 3T. In particular, we aimed to systematically explore whether BBB breakdown is an obligatory step in active MS lesions, and to assess its temporal relationship with lesion appearance on T2-weighted MRI.

Materials and methods

Patients

Frequent high-resolution 3T MRI consisting of a baseline MRI followed by seven consecutive weekly MRI exams was performed on five RRMS patients with no disease-modifying treatment. Five patients (four women and one man; mean age: 38.2 years old; mean disease duration: 6.6 years; mean Expanded Disability Status Scale (EDSS): 2) with definite RRMS[12] were enrolled in this study between March 2009–September 2010 (Neurological Hospital, Hospices Civils de Lyon, France) (Table 1). Inclusion criterion was the presence of at least one enhancing lesion during the six months preceding study enrolment. Exclusion criteria included administration of long-term immunotherapy or systemic corticosteroids in the month prior to inclusion and during the study. No patient presented clinical relapse that would have mandated treatment during the study. Further exclusion criteria were the presence of other brain pathologies (e.g. cerebral microangiopathy, stroke), contraindications for MRI (pacemaker, claustrophobia, contrast allergy, pregnancy) or contrast agent (renal insufficiency). To limit the risk of nephrogenic systemic fibrosis associated with Gd administration,[13] creatinine clearance was checked every 15 days and had to be higher than 60 ml/min using the Cockcroft-Gault formula. A pregnancy test was performed on all female patients of childbearing age before each MRI exam.

This longitudinal prospective study (ClinicalTrials.gov Identifier: NCT00861172) was approved by an ethics committee (CPP Lyon Sud-Est IV) and the French Health Products Safety Agency (AFSSAPS). All patients signed an informed consent form approved by the ethics committee (Institutional Review Board).

MRI acquisition

This work is part of a multimodal imaging study of anatomical, metabolic, diffusion tensor and perfusion MRI. To meet the objectives of this study, we only analysed 3D FLAIR and 3D-T1-Gd images. MRI acquisitions were performed on a 3T MRI system (Achieva 3T, Philips Medical Systems, the Netherlands) with a 16-channel head coil on the same day, at the same time (every Tuesday at 12:00) for all patients between March–December 2009. The MRI protocol included a sagittal 3D FLAIR sequence (TE/TR/TI: 356/8000/2400 ms; slice thickness: 1.2 mm; acquisition matrix: 228×226; reconstruction matrix of 576×576 for a 25 cm field-of-view (FOV) yielding a nominal in-plane pixel size of 0.434×0.434 mm), and an axial 3DT1 post-Gd contrast turbo field echo sequence, (TR/TE: 6.7/3 ms; slice thickness: 0.9 mm; acquisition matrix: 268×211; reconstruction matrix: 512×512; FOV: 24 cm; yielding a nominal in plane pixel size of 0.469×0.469 mm). A standard dose of 0.1 mmol/kg Gadobutrol (Gadovist) was administered during each MRI session 60 s before the 3D T1 acquisition.

MRI analysis

We identified all active lesions during a seven-week period monitored with eight weekly MRI exams. Active lesions were defined as those that showed change on FLAIR or 3D-T1-Gd MRI images, at any follow-up time point. This definition encompassed both new, as well as pre-existing reactivating lesions. Both enlarging and shrinking lesions were

considered active. Radiologists identified active lesions with the help of subtraction and Jacobian images, that were computed as follows. All 3DT1-Gd data and follow-up FLAIR data were first registered and resampled to the baseline FLAIR data using a rigid-body mutual information-based algorithm[14] and sinc interpolation. The effect of registration and resampling on small lesions was visually assessed before and after registration and no impact on the number of lesions or their size was observed. The intensity of each follow-up 3DT1-GD and FLAIR image-set was normalized to the intensity of the baseline 3DT1-Gd and FLAIR images, respectively. Intensity normalization was performed using the differential intensity inhomogeneity correction technique detailed by Lewis et al.[15] Subtraction images for each follow-up time-point were obtained by subtracting the baseline images from follow-up images for each patient. Jacobian images were computed by taking, at each voxel location, the determinant of the Jacobian matrix derived from the deformation field, which resulted from the non-linear registration of each follow-up image-set to the baseline data using the technique described by Vemuri et al.[16] The intensity morphing was achieved via evolving level-sets of one image into the level-sets of the other. If the determinant of the Jacobian matrix was higher than one, it represented an expansion; if it was lower than one, it represented a contraction.[17]

Finally, for each time-point in each patient, co-registered, intensity-normalized FLAIR and 3DT1-Gd images, as well as corresponding subtraction and Jacobian images were displayed side-by-side to enable identification of active lesions by the neuroradiologists.

Lesions in which only Gd-enhancement or FLAIR hyperintensity was detected at presentation, were carefully and separately reviewed by three independent radiologists to verify the sequence of events (MR, JAR, FC). Consensus between at least two out of the three experts determined the final classification in three groups (FLAIR activity preceding Gd-enhancement; Gd-enhancement preceding FLAIR activity; simultaneous presentation on FLAIR and 3D-T1-Gd).

Since follow-up was limited to seven weeks, and a considerable fraction (111/212=52.4%) of all active lesions was either already enhancing at baseline and/or was still enhancing at week 7, we are unable to report a satisfyingly accurate and representative estimate of mean/median duration of enhancement.

In post-hoc analysis, we visually compared the temporal evolution profiles of normalized signal intensities within the region of interest (ROI) defined by the maximal size of the lesion on FLAIR and 3D-T1-Gd for each lesion that appeared on FLAIR before Gd-enhanced MRI (Figure 1). Normalized signal intensities were measured on subtraction images comparing a given time-point to the first time-point in that patient.

Results

All five patients had at least one active lesion over the seven-week follow-up, and lesion activity varied widely between patients (Table 1). Collectively, 212 active lesions were detected. All active lesions showed Gd-enhancement on at least one MRI scan. With the exception of one small lesion, all lesions were still detected on FLAIR on the final weekly scan. At baseline, 51 out of the 212 active lesions were already enhancing and nine lesions were already present on FLAIR but showed enhancement only at a later time-point. The remaining 152 active lesions were newly active lesions that activated during the follow-up period. Sixty of these lesions were enhancing on 3DT1-GD at the last time point. The vast majority of these lesions (115/152 or 75.7%) were visible both on contrast-enhanced and on FLAIR images at first detection. One small lesion was detected only on 3D-T1-Gd. Interestingly, 17/152 newly active lesions (11.2%) showed activity on T2-weighted FLAIR one or more weeks before the appearance of contrast-enhancement (only one of these lesions was a 'reactivating' lesion, while the rest (n=16) were newly appearing lesions) (Table 2). The size of these lesions was very small (smaller than 2 mm in most cases). Conversely, 19/152 lesions (12.5%) were visible only on contrast-enhanced images at first detection (Table 2). To validate the visual observation of the subset of 17 lesions presenting on FLAIR images prior to their detection on 3D-T1-Gd, we compared the time-course of average and maximum difference in signal intensities on these two contrasts within the ROI defined by the lesion's maximal size (Figure 1). Visual inspection of these plots largely confirmed the neuroradiologists' observations (Figures 1 and 2). Signal intensity changes were consistently higher on FLAIR images compared to 3DT1-GD, and changes on FLAIR preceded changes on 3DT1-GD for most lesions (Figure 1). Often FLAIR signal differences with respect to baseline were already detectable at time point one, even when visual detection occurred on a later time point. Plots of the maximum signal difference were expected to be more sensitive at detecting

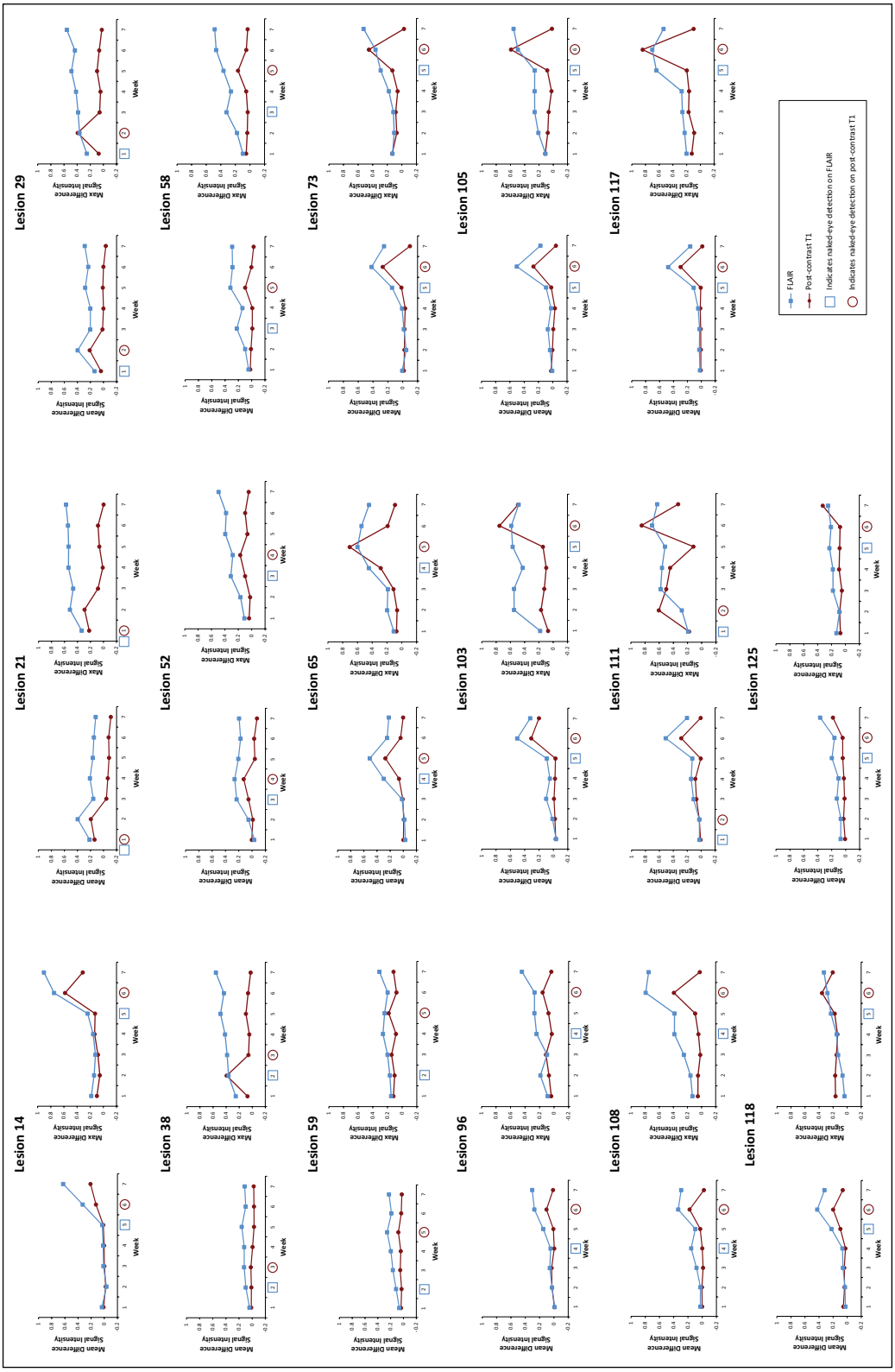

Figure 1. Temporal evolution profiles of signal intensities of the 17 lesions appearing on fluid attenuation inversion recovery (FLAIR) prior to post-contrast T1-weighted images. Signal intensities within the region of interest (ROI) defined by the maximal size of the lesion on FLAIR and post-contrast T1-weighted images were calculated at each time point. The signal intensity values on the *y* axis refer to the mean and maximum normalized difference in ROI signal intensity from subtraction images between the current and the previous time-point. For each lesion, FLAIR signal intensity profiles are represented in blue, while post-contrast T1-weighted profiles are represented in red. The markers on the *x* axis indicate the time point of naked-eye detection of the lesions on FLAIR (blue square) and post-contrast T1-weighted images (red circle).

Table 2. Chronology of new active lesion occurrence on fluid attenuation inversion recovery (FLAIR) relative to post-contrast T1-weighted images in all patients pooled together and in each patient separately.

Time of FLAIR occurrence relative to post-contrast T1 (weeks)	–3	–2	–1	0	+1	+2	+3
All patients							
Number of lesions	1	3	13	115	15	2	2
%	0.7	2.0	8.6	76.2	9.9	1.3	1.3
Patient 1							
Number of lesions	0	0	1	6	2	1	0
%	0.0	0.0	10.0	60.0	30.0	0.0	0.0
Patient 2							
Number of lesions	0	0	0	0	0	0	0
%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Patient 3[a]							
Number of lesions	1	3	12	83	8	1	1
%	0.9	2.8	11.0	76.1	7.3	0.9	0.9
Patient 4							
Number of lesions	0	0	0	20	3	0	1
%	0.0	0.0	0.0	83.3	12.5	0.0	4.2
Patient 5							
Number of lesions	0	0	0	6	2	0	0
%	0.0	0.0	0.0	75.0	25.0	0.0	0.0

[a]One new lesion in patient 3 was detected on gadolinium (Gd)-enhanced 3D T1-weighted images, but was never visible on FLAIR, and is not included in this table.

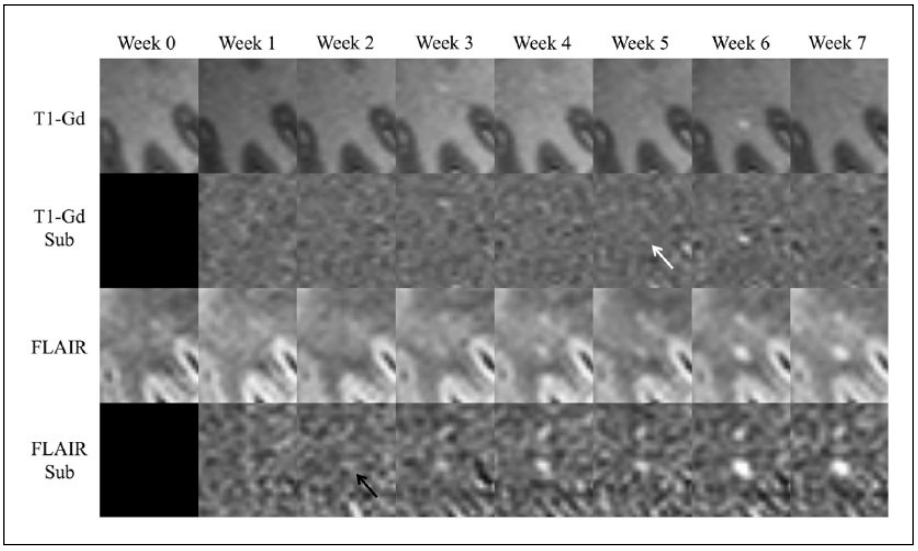

Figure 2. Example of a lesion in which fluid attenuation inversion recovery (FLAIR) activity precedes contrast-enhancement. Gd: gadolinium. Black arrow indicates appearance of lesion on FLAIR subtraction imaging at week 2; white arrow indicates appearance of the same lesion on post-Gd subtraction imaging at week 5.

changes around lesion occurrence when lesions are small, and occupy a small fraction of the maximal lesion extent. Indeed, these plots most frequently appeared to be more sensitive than those of mean signal differences.

New lesions were detected at all time-points, and their occurrence showed relatively spread-out distribution over the seven time-points for the four most active patients (Figure 3). All except one of the lesions presenting on FLAIR prior to 3D-T1-Gd were found in

the patient with most active lesions, and represented 14.5% of this patient's new lesions (Table 2). This lesion pattern was seen by the neuroradiologists in one lesion of one other patient, but could not be confirmed on closer scrutiny by signal intensity time course analysis (lesion 14 in Figure 1).

Table 3 shows the number of new lesions presenting on 3DT1-GD at each time-point in weeks 1–5, as well as the incidence of lesions enhancing at one or two weekly measurements. The average incidence of lesions enhancing for one or two time-points, between weeks 1–5 was 49.3%.

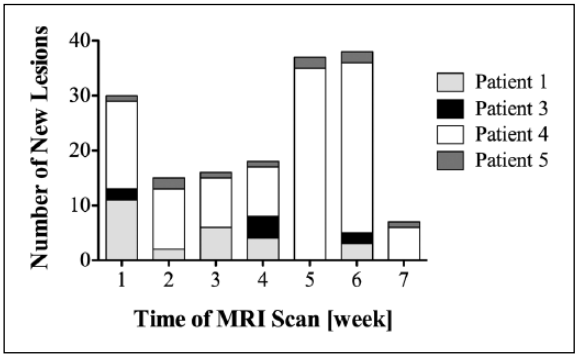

Figure 3. Time of occurrence of new lesions. The histograms represent the cumulative number of new lesions occurring at each follow-up time point (weeks 1–7), distinguished for four out of five relapsing–remitting multiple sclerosis (RRMS) patients. Patient 2 is not depicted because he had only one new lesion at baseline. Time of occurrence was defined as the earliest lesion detection on fluid attenuation inversion recovery (FLAIR) or on post-contrast T1-weighted images. Lesions already enhancing at baseline are not depicted, since their time of occurrence can not be precisely defined. MRI: magnetic resonance imaging.

Discussion

We performed weekly MRI with new state-of-the-art MRI technology to revisit results obtained from a similar experiment performed with a much less sensitive MRI approach in the early 1990s.[11] Multiple aspects of our current MRI protocol contributed to increase the sensitivity of MS lesion detection. In comparison to the previous protocol, the current one was performed on a high-field system (3T vs 1.5T), using high resolution acquisitions (near-isotropic millimetric for FLAIR and contrast-enhanced T1-weighted imaging vs the anisotropic 3×1×1 mm (for T2-weighted imaging) and 5 (including 1 mm gap)×1×1 mm (for contrast-enhanced T1-weighted imaging) applied in the Cotton et al.[11] study). Furthermore, the new protocol relied on 3D (as opposed to 2D-multi-slice) acquisitions. To further increase the yield of active lesions, inclusion criteria were designed to select active patients by requiring the presence of at least one contrast-enhancing lesion in the six months preceding recruitment, and by considering the use of disease-modifying treatment as an exclusion criterion.

In the present study, active lesions were detected in all five patients, while our previous study[11] detected contrast-enhancing lesions only in half of the 26 studied patients. We detected approximately five times as many new lesions per patient and time-point in the current vs the older study (4.34 versus 0.87 lesions). The occurrence of new lesions was spread-out throughout the seven-week follow-up period, suggesting that disease activity on MRI is more evenly

Table 3. Count and incidence of new lesions enhancing for less than three weeks (appearing on one or two consecutive post-contrast T1-weighted images) at week 1–5.

	Week 1	Week 2	Week 3	Week 4	Week 5	Average
Total new enhancing lesions (*n*)	25	14	14	13	32	19.6
New lesions enhancing at one time point						
Count (*n*)	3	2	4	2	7	3.6
Incidence (%)	12.0	14.3	28.6	15.4	21.9	18.4
New lesions enhancing at two time points						
Count (*n*)	13	4	1	3	14	7.0
Incidence (%)	52.0	28.6	7.1	23.1	44.8	31.1
New lesions enhancing at one or two time points						
Count (*n*)	16	6	5	5	21	10.6
Incidence (%)	64.0	42.9	35.7	38.5	65.6	49.3

distributed over time, than could be assumed by the frequency of clinical attacks or of new lesions shown in previous work. The finding that close to 50% of new lesions enhanced only at one or two weekly measurements is consistent with our previous findings in a much larger group of patients.[11]

Seventeen out of 152 newly active lesions (11.2%) were present on FLAIR images before enhancing on T1-weighted images. Signal intensity profiles (Figure 1) confirmed this visual observation and suggested that this pattern might be more sensitively and more frequently detected. Future studies will be required to extend these findings. We speculate that this rarely, yet convincingly, observed lesion behaviour might hold true for a significant subset of lesions. In other parts of the body, inflammatory responses follow an insult at intervals much shorter than a week, more likely in the minutes to hours range. If, as we speculate, enhancement were frequently to occur minutes or hours after first lesion detection on FLAIR images, weekly MRI sampling would detect this sequence only in a minuscule proportion of cases, while the majority of lesions with this evolution pattern would appear to occur simultaneously. Up to 87.5% of lesions in our study could fit this model. The fact that we also encountered the opposite sequence (Gd-enhancement before FLAIR) in 19/152 (12.5%) new lesions is not contradictory. In fact, these two distinct lesion evolution patterns (FLAIR before Gd-enhancement vs Gd-enhancement before FLAIR) might be the MRI expression of histopathologically defined Type III vs Type I/II lesions described by Lucchinetti et al.[18]

Our findings of T2 FLAIR changes preceding Gd-enhancement in the genesis of new MS lesions are consistent with studies that demonstrated abnormalities in magnetization transfer and diffusion imaging preceding contrast-enhancement.[19,20]

Our speculation that contrast-enhancement might follow T2-weighted changes in a large sub-set of new lesions, while another sub-set might demonstrate the classically described pattern of contrast enhancement marking lesion onset is provocative, but consistent with histopathological evidence. Multiple histopathological studies suggest that in a significant subset of MS patients, oligodendrocyte damage and associated microglial activation precede and/or are disconnected from the classically described adaptive immune-reaction, characterized by lymphocytic infiltration in perivascular spaces.

MRI-guided sampling of post-mortem pathological specimens from progressive MS patients demonstrated a preponderance of (p)reactive lesions in T2-hyperintense lesions with mild T1-hypointensity.[21] These (p)reactive lesions are characterized by intraparenchymal clusters of microglia showing strong expression of CD68, CD45 and HLA-DR, as well as CD45- and HLA-DR positive peri- and intra-vascular leukocytes, in the absence of apparent demyelination.[21] Nodular microglial activation was also found in a study targeting pre-demyelinating tissue alterations both in autopsy tissue from acute (Marburg's) MS patients and from patients deceased during a fulminant exacerbation of RRMS.[22] In that study, nodules of activated microglia were found at the non-demyelinated edge of demyelinating lesions, but only in patients with type III lesions, which constituted only half the studied patient population (four out of eight patients).[22] Findings by Henderson et al. showed that areas outside the leading edge of demyelinating lesions demonstrate apoptotic oligodendrocytes and activated microglia, while lymphocyte infiltrates are largely absent.[23] In a post-mortem study comparing histological findings between areas corresponding to normal appearing white matter (WM), 'dirty WM', and focal lesions on MRI, Sewann and coworkers observed mild activation of antigen-presenting cells in diffusely hyperintense ('dirty') WM compared to the other two tissue compartments, and suggested that these findings were consistent with diffuse microglial activation.[24]

Taken together with these histological observations, our findings support the existence of two distinct MRI lesion initiation patterns (MRILIPs) in brain WM of MS patients: (a) MRILIP I lesions in which Gd-enhancement and BBB impairment precede or are concomitant with T2-visible changes; and (b) MRILIP II lesions in which T2-weighted focal changes precede MRI-visible BBB disruption. We speculate that MRILIP II lesions might be much more frequent than currently observed, and are only rarely evident due to the relatively long interval between MRIs (weekly in our work) compared to the rapidity of immunological changes. We postulate that the genesis of MRILIP II lesions might follow a sequence of events in which parenchymal (oligodendrocyte and axonal) degeneration accompanied by microglial activation precedes vasogenic, adaptive inflammation, consistent with histological Type III lesions described by Lucchinetti et al.[18]

While we met our primary target to characterize a significant number of untreated active MS lesions, we recognize that the patient sample was limited and therefore generalizability of the findings is not warranted. Of note, the MRILIP II pattern was convincingly demonstrated only in one patient, with a particularly large

number of new lesions. It is conceivable that this lesion pattern may be restricted to a subset of MS patients. Furthermore, our inclusion criteria required that patients had at least one enhancing lesion in the six months preceding enrollment, thereby biasing our sample towards the more active part of the spectrum of MS patients. Nevertheless, these criteria enabled us to study the natural history of a reasonable sample size of individual, newly occurring lesions with reasonable resources, in spite of the paucity of untreated patients in the current era of disease-modifying treatments in MS. A further potential limitation of our study was the relatively short (one-minute) interval between contrast agent injection and image acquisition, compared with the current clinically applied standard of five minutes. This might have led to an underestimation of the frequency of new lesion occurrence. Also, it is conceivable that higher Gd dosage and/or longer interval post-injection might have unveiled earlier enhancement than that detected in this experiment. However, it also should be noted that we went to extensive lengths in the post-processing of our images, and used both subtraction and Jacobian images comparing each Gd-enhanced images at each time-point to corresponding baseline images. Both of these techniques should yield significant increases in the sensitivity of detection of contrast-enhanced lesions. It is also worthwhile noting that subtle, yet widespread contrast enhancement has been shown using ultra-long post-contrast intervals in lesions not visible to the naked eye, and that such changes appeared more severe in, presumably chronic, T1-hypointense lesions.[25] Whether such techniques would unveil subtle enhancement also at very early, 'pre-naked-eye enhancement' stages of newly appearing lesions remains an open question.

In conclusion, the occurrence of new focal lesions is more frequent than previously assumed. Nevertheless, weekly, high-resolution, near-millimetric imaging at 3T might still underestimate actual disease activity in RRMS. In a significant number of active focal lesions, BBB disruption appears to follow focal activity on T2-weighted MRI rather than constitute the initial event. These findings are consistent with the existence of a sub-population of lesions (MRILIP II) with an 'inside-out' genesis, where neurodegenerative processes might precede microglial activation, and a subsequent adaptive immune response.

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Conflict of interest

S Vukusic reports receiving consulting and lecture fees, travel grants and research support from Bayer-Schering, Biogen Idec, Genzyme, Novartis, Merck Serono, Sanofi Aventis and Teva Pharma. F Cotton reports receiving consulting fees from Biogen-Idec. All the other authors report no disclosure.

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