MULTIPLE SCLEROSIS JOURNAL | MSJ

Original Research Paper

Expansion of chronic lesions is linked to disease progression in relapsing–remitting multiple sclerosis patients

Samuel Klistorner, Michael H Barnett, Con Yiannikas, Joshua Barton, John Parratt, Yuyi You, Stuart L Graham and Alexander Klistorner

Abstract

Background: Slow-burning inflammation is putatively associated with lesion expansion and leads to progressive loss of axons and disability worsening.

Objective: To investigate the incidence and extent of chronic white matter lesion expansion in relapsing–remitting multiple sclerosis (RRMS) patients and to evaluate its relationship with biomarkers of disease progression.

Methods: Pre- and post-gadolinium T1, fluid-attenuated inversion recovery (FLAIR) and diffusion tensor images were acquired from 33 patients. Lesional activity were analysed between baseline and 48 months using custom-designed software.

Results: A total of 569 lesions were identified as chronic at baseline, of which 261 were expanding, 236 were stable and 72 were shrinking. In addition, 139 new lesions (both confluent and free-standing) were observed. Chronic lesion expansion was associated with patient's age and accounted for the bulk (67.3%) of total brain lesion volume increase, while only 32.7% was attributable to new lesion formation. Change in chronic lesion volume correlated with the rate of brain atrophy ($r=-0.57$, $p=0.001$), change of Expanded Disability Status Scale (EDSS; $r=0.38$, $p=0.03$) and an increase of isotropic diffusivity inside the lesions ($r=0.75$, $p<0.001$).

Conclusion: Expansion of chronic lesions in RRMS patients is the primary determinant of increased T2 total lesion load. It significantly contributes to disease progression and partially driving axonal loss inside the lesions and brain damage outside of lesional tissue.

Keywords: T2 lesions, MRI, progressive, axonal loss

Date received: 21 August 2020; revised: 8 October 2020; accepted: 22 October 2020.

Multiple Sclerosis Journal

1–10

DOI: 10.1177/1352458520974357

Correspondence to:
A Klistorner
Sydney Medical School, Save Sight Institute, The University of Sydney, 8 Macquarie Street, Sydney, NSW 2000, Australia.
sasha@eye.usyd.edu.au

Samuel Klistorner
Sydney Medical School, Save Sight Institute, The University of Sydney, Sydney, NSW, Australia

Michael H Barnett
Brain and Mind Centre, The University of Sydney, Sydney, NSW, Australia/ Sydney Neuroimaging Analysis Centre, Sydney, NSW, Australia

Con Yiannikas
John Parratt
Royal North Shore Hospital, Sydney, NSW, Australia

Joshua Barton
Brain and Mind Centre, The University of Sydney, Sydney, NSW, Australia

Yuyi You
Alexander Klistorner
Sydney Medical School, Save Sight Institute, The University of Sydney, Sydney, NSW, Australia/ Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia

Stuart L Graham
Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia

Introduction

Multiple sclerosis (MS) is a chronic inflammatory, neurodegenerative disease of the central nervous system (CNS). Axonal loss is accepted as the major cause of irreversible neurological disability in MS. Acute inflammatory demyelination typically manifests as new white matter lesions and is the principal cause of axonal transection and subsequent axonal degeneration.[1] As such, new lesion formation is likely to contribute significantly to disease progression, particularly in the relapsing–remitting stage of the disease.

New lesional activity is typically measured as a combined volume of new lesions and an enlargement of chronic (i.e. pre-existing) lesions. Recent studies, however, have demonstrated that the mechanisms responsible for the development of new lesions and the slow expansion of chronic lesions are likely to differ.[2,3] While formation of new acute lesions is associated with a disturbance of the blood–brain barrier, smouldering inflammation at the edge of some chronic lesions is dominated by microglial activation, the activation and proliferation of astrocytes and B-cell infiltration.[2,4] Such lesions, which are typically described as chronic active lesions,[5] reveal an inactive plaque core that is surrounded by a rim of profound microglial activation,[2] ongoing demyelination[6] and axonal injury.[7–9] This slow-burning inflammation at

the edge of MS lesions and associated gradual lesion expansion[3,10] may represent one pathological substrate of gradual worsening of pre-existing clinical deficits and disability progression.[11]

Therefore, in this study, we examined the incidence and extent of the expansion of chronic white matter lesions in a cohort of patients with relapsing–remitting MS (RRMS) followed for 5 years. Furthermore, we investigated the relationship between lesion expansion and patient demographics, the appearance of new lesions, the severity of progressive tissue damage inside pre-existing lesions and recognized clinical and imaging biomarkers of disease progression.

Method

Standard protocol approvals, registrations and patient consents

The study was approved by University of Sydney and Macquarie University Human Research Ethics Committees and followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Subjects

Fifty consecutive patients with established RRMS, defined according to the revised McDonald 2010 criteria,[12] were enrolled in a study from three MS clinics in the Sydney metropolitan area. Patients underwent magnetic resonance imaging (MRI) scans and clinical assessment at 0, 12 and 60 months. The main analysis was performed between 12 months (termed 'baseline') and 60 months (termed 'follow-up'), while scans performed at 0 months (termed 'pre-study scans') were used to identify newly developed lesions at the start of the study.

MRI protocol

MRI was performed using a 3T GE Discovery MR750 scanner (GE Medical Systems, Milwaukee, WI). The following sequences were acquired:

1. Pre- and post-contrast (gadolinium) Sagittal 3D T1;
2. Sagittal 3D (CUBE) FLAIR;
3. Diffusion-weighted MRI.

Specific parameters are presented in Supplementary Material 1.

MRI image pre-processing

The baseline T1-weighted imaging was realigned to Anterior and Posterior Commissure (AC–PC) orientation. Using FLIRT (FSL, FMRIB Software Library), follow-up T1 images were co-registered to initial (month 0) AC–PC space by applying transformation matrices derived from linear co-registration between baseline AC–PC aligned brain and follow-up native T1 brain images (Figure 1(a)). In parallel, diffusion MRI was corrected for motion and eddy-current distortion in FSL, then echo planar imaging (EPI) susceptibility distortion was minimized by applying deformation maps generated from nonlinear co-registration between the diffusion-weighted imaging (DWI) b0 brain image and T1-weighted imaging at each time-point using ANTS (Advanced Normalization Tools). Subsequently, tensor reconstruction was performed in MRtrix3. Tensor and fluid-attenuated inversion recovery (FLAIR) images were then linearly co-registered to corresponding T1 AC–PC images at each time-point.

Lesion identification and analysis

Individual lesions were identified on the co-registered T2 FLAIR images and semi-automatically segmented using JIM 7 software (Xinapse Systems, Essex, UK) on all scans by a trained analyst (Figure 1(b)). Only lesions measuring larger than 50 mm^3 were selected for analysis, since it represents minimal size (2×2 voxels) of the lesional core used for diffusion tensor imaging (DTI) analysis.

Gadolinium enhancement usually does not persist beyond 2 months, after which newly formed T2 hyperintense lesions continue to shrink in size for another 3–5 months, reflecting resolution of edema and some tissue repair, including remyelination.[13] Therefore, to minimize lesion changes related to evolution of newly developed lesions, both baseline gadolinium-enhancing lesions and newly formed baseline non-enhancing T2 lesions (i.e. lesions visible on baseline images, but not present on pre-study images) were excluded from analyses.

Once selected, baseline and follow-up lesions were converted to a binary mask and analysed by a fully automated in-house lesion progression algorithm written in Python. The algorithm includes several steps:

1. Lesion matching: Individual lesions are matched between baseline and follow-up time-points based on spatial connectedness (i.e. lesion overlap) (red arrows, Figure 1(d) and (e)). All follow-up lesions that did not have a

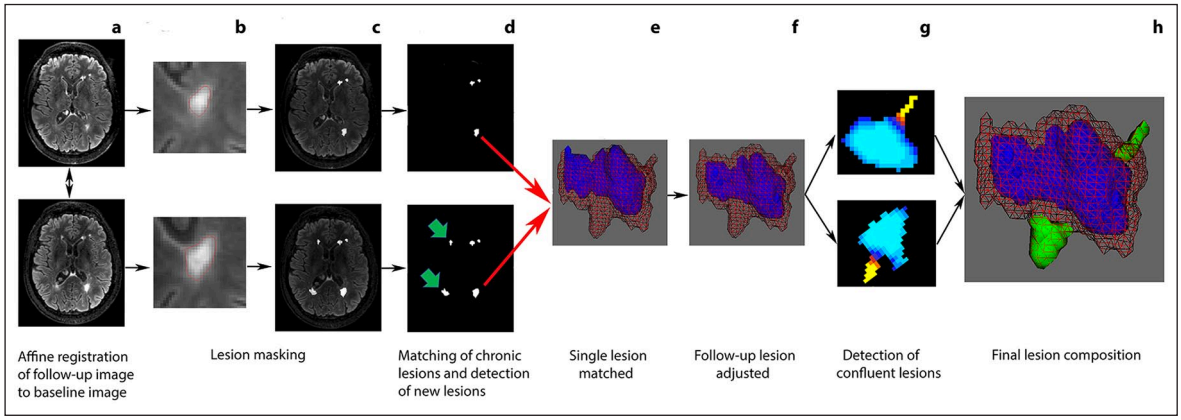

Figure 1. Lesion identification and analysis pipeline. (a) Follow-up T1 image was co-registered to a baseline T1 image. (b) Lesions were segmented on the co-registered T2 fluid-attenuated inversion recovery (FLAIR) images using JIM 7 software on pre-study, baseline and follow-up scans. (c) Lesions were converted to a binary mask. (d) and (e) Individual lesions were matched between baseline and follow-up time-points (red arrows, Figure 1(d) and (e)). All follow-up lesions that did not have a matched baseline counterpart were classified as 'new free-standing lesions' (green arrows). (f) Position of the baseline lesion mask was adjusted for brain atrophy by implementing affine co-registration between the two masks using 6 degrees of freedom (DOF) with limited rotation (5deg) (FLIRT) rigid body registration. (g) New confluent lesions (red-yellow scale) were identified by custom-designed algorithm detecting local areas of lesion expansions (Supplementary Figure 1). (h) Volumetric characteristics were calculated for following lesion components: baseline lesion (blue volume), change of chronic lesion volume (difference between mesh volume and blue volume), and new confluent lesions (green volume).

matched baseline counterpart are classified as 'new free-standing lesions' (Figure 1(d), green arrows).

2. Correction of follow-up lesions for brain atrophy: With relatively long periods of follow-up, brain atrophy frequently leads to a significant shift in the position of MS lesions,[14] which affects the accurate estimation of the lesion. Therefore, for each baseline-to-follow-up lesion pair, the position of the baseline lesion mask is adjusted for brain atrophy by implementing linear co-registration between the two masks using 6 degrees of freedom (rigid body) with a limited rotation of around 5 degrees (FLIRT)[15] (Figure 1(e) and (f) and in Supplementary Video 1).
3. Detection of new confluent lesions: performed by a custom-designed algorithm (see Supplementary Figure 1 and Figure 1(g)).
4. Calculation of chronic lesion volume change during follow-up: The final size of chronic lesions at follow-up is determined by subtracting confluent lesions (Figure 1(h), green area) from a follow-up lesion mask. Subsequently, the difference between the remainder of the follow-up lesion mask and baseline lesion mask is used to calculate change in chronic lesion volume (Figure 1(h)). Chronic lesions which demonstrate less than 10% volume change (both positive and negative) are classified as stable (10% represents test–retest variability of manual lesion masking). Lesions with more than 10% volume increase are classified as expanding, while lesions with more than 10% volume decrease are classified as shrinking.
5. New free-standing lesions and new confluent lesions that developed during the follow-up were combined together to compute the patient-wise volume of new acute lesions.

The degree of tissue damage within chronic lesions was assessed by mean diffusivity (MD), as previously suggested.[14] Progressive tissue damage in chronic lesions was measured as an increase of MD in lesional core between baseline and follow-up timepoints[14,16] using the baseline lesion mask, which was adjusted to correct for brain atrophy-related displacement at follow-up (Figure 1(e), blue area).

Brain atrophy

Percentage brain volume change (PBVC) between baseline and follow-up was calculated using the SIENA/FSL (v4.1 software; FMRIB, Oxford, UK) pipeline.[17] Briefly, T1 intensity inhomogeneity correction[18] was performed, followed by removal of non-brain tissue using the Brain Extraction Tool from the FMRIB Software Library (FSL) with manual QC. The pre-processed brain masks were then imported into SIENA with standard parameters (SIENA v. 2.6).

Statistics

Statistical analysis was performed using SPSS 22.0 (SPSS, Chicago, IL, USA). The principal analysis was performed on a patient-wise basis. The Pearson correlation coefficient was used to measure statistical dependence between two numerical variables. $p < 0.05$ was considered statistically significant. Comparisons between groups were made using Student's *t* test. Longitudinal changes were assessed using the paired two-sample *t* test. A Univariate General Linear Model was applied to analyse the potential effect of various factors on brain atrophy and Expanded Disability Status Scale (EDSS) progression.

Results

Thirty-three patients completed the study. Demographic data are presented in Table 1. Fifteen patients were treated with low-potency disease-modifying therapies (DMTs), while 17 patients received high-potency DMTs for the bulk of the study (one patient was treatment-free).

Table 1. Demographic data (one patient remained on the same DMT throughout the study duration).

Total number of patients	33
Age at enrolment, years	42.6 ± 10.3
Sex	13M/20F
Disease duration at enrolment, years	6.5 ± 2.8
EDSS at baseline	1.1 + 1.0
No. of patients on stable treatment	32
Low-potency DMT	
Betaferon	2
Avonex	5
Rebif	1
Glatiramer acetate	7
High-potency DMT	
Fingolimod	9
Natalizumab	6
Dimethyl fumarate	2

EDSS: Expanded Disability Status Scale.

There were 569 lesions identified as chronic at baseline (15 lesions were gadolinium (GAD)-enhancing or newly appearing compared to pre-study scans and, therefore, were excluded from analysis). Lesions were classified as follows: 261 (46%) chronic lesions were expanding, 236 (42%) chronic lesions were stable and 72 (12%) chronic lesions were shrinking (see examples in Figure 2). In addition, 139 new free-standing and confluent lesions were detected. Volumetric characteristics of each lesion group are presented in Table 2.

In all, 31 patients had at least one expanding lesions, while 22 patients had at least one shrinking lesion. Lesion expansion was a dominating pattern of lesion volume change, as shown in Figure 3, which demonstrates patient-wise proportion of expanding and shrinking lesions. Furthermore, average lesion volume in expanding lesions was significantly larger than average volume of lesion shrinking (120 mm^3 vs 37 mm^3). As a result, total volume of chronic lesion expansion exceeds total volume of lesion shrinking by more than tenfold (31,320 mm^3 vs 2664 mm^3). Patients with more expanding lesions tended to have less lesions that shrink, which is supported by a significant negative correlation between proportion of expanding and shrinking lesions in individual patients ($r = 0.53$, $p = 0.002$).

There was significant association between incidence of expanding lesions and patient's age ($r = 0.53$, $p = 0.002$), with older patients demonstrating larger proportion of expanding lesions. An equilibrium between expanding and non-expanding (i.e. stable and shrinking lesions pooled together) lesions was observed at 46 years of age (Supplementary Figure 2).

Lesion-based analysis demonstrated a trend towards a larger lesion size at baseline in the group of expanding lesions in comparison with shrinking lesions (analysis of variance (ANOVA): $p = 0.063$, post hoc Tukey analysis between expanding and shrinking groups: $p = 0.069$).

For the purpose of further analysis, all chronic lesions (including expanding, stable and shrinking lesions) were combined together into a 'chronic lesion' group, while new free-standing and confluent lesions were pooled into a 'new lesion' group.

There was a significant increase in patient-wise total brain lesion volume during the follow-up period (average volume 6680 ± 5509 vs 7951 ± 6315 mm^3 at baseline and follow-up, respectively, $p < 0.0001$) (Figure 4(a)), the bulk of which was accounted for by an increase in chronic lesion volume (67.3% or 855 ± 1066 mm^3), while only 32.7% (or 420 ± 633 mm^3) was attributable to new lesions. No association was found between absolute and relative (percentage-wise) change in the volume of chronic lesions and the volume of new lesions ($p = 0.4$ and $p = 0.7$, respectively).

Change in chronic lesion volume in individual patients is presented in Figure 4(b). While there was significant heterogeneity in the rate of chronic lesion

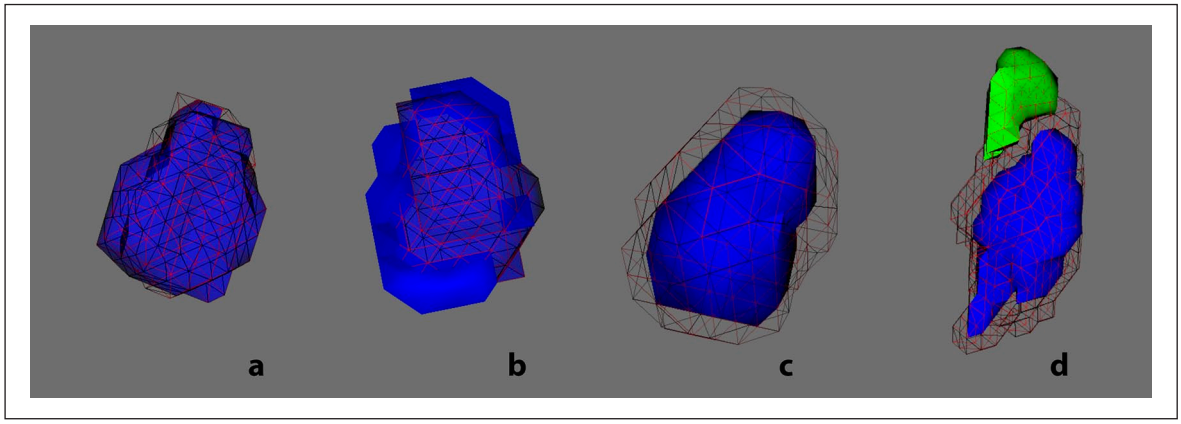

Figure 2. Examples of lesion evolution between baseline and follow-up visits. The baseline lesion mask is in blue. New confluent lesion is in green. The follow-up lesion mask is represented by the mesh: (a) stable lesion, (b) shrinking lesion, (c) expanding lesion and (d) expanding lesion with a new confluent lesion.

Table 2. Volumetric characteristics of expanding, shrinking, stable and new lesions.

Lesions	*n*	Average baseline volume (mm^3)	Average volume change (mm^3)	Total volume change (mm^3)
Expanding	261	462 ± 1106	120 ± 246	31,320
Stable	236	345 ± 724	4 ± 27	1380
Shrinking	72	201 ± 248	−37 ± 42	−2664
New lesions	139	–	100 ± 133	13,867

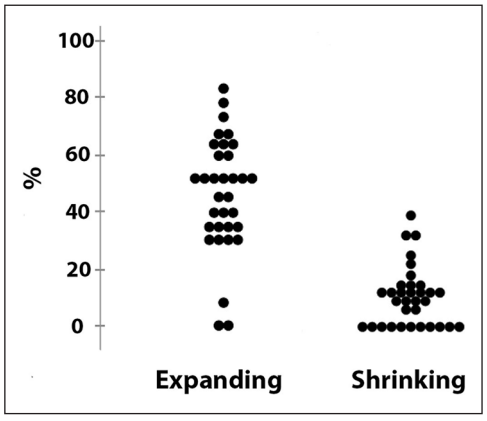

Figure 3. Percentage of expanding and shrinking lesions in individual patients.

volume change, which varied between −5% and 47% (12.1 ± 12%), 15 out of 33 patients demonstrated substantial (>10%) enlargement of chronic lesions volume.

The percentage of chronic lesion volume change correlated significantly with patient's age ($r=0.52$, $p=0.002$; while volume of new lesions was independent of age), but not with disease duration ($p=0.5$) or baseline lesion volume ($p=0.7$). There was no difference between the rate of lesion volume change in males versus females ($p=0.8$, *t* test) or between patients receiving high- and low-potency DMTs ($p=0.19$, *t* test).

There was, however, significant correlation of the volume of chronic lesion change with the degree of brain atrophy ($r=-0.57$, $p=0.001$) and change of EDSS during the follow-up period ($r=0.38$, $p=0.03$), indicating that expansion of chronic lesions is associated with both radiological and clinical markers of disease progression.

The combined volume of new lesions also correlated significantly with the degree of brain atrophy ($r=-0.45$, $p=0.01$), but not with EDSS progression ($p=0.6$). Therefore, to estimate relative contribution of both lesion-related factors to brain atrophy, multiple linear regression analysis including the volume of chronic lesion expansion and the volume of new lesions was performed. The model demonstrated that while both factors contributed significantly (model predictive value: $r^2=0.46$, $p<0.001$, standardized coefficients beta: −0.52 and −0.38, $p=0.001$ and

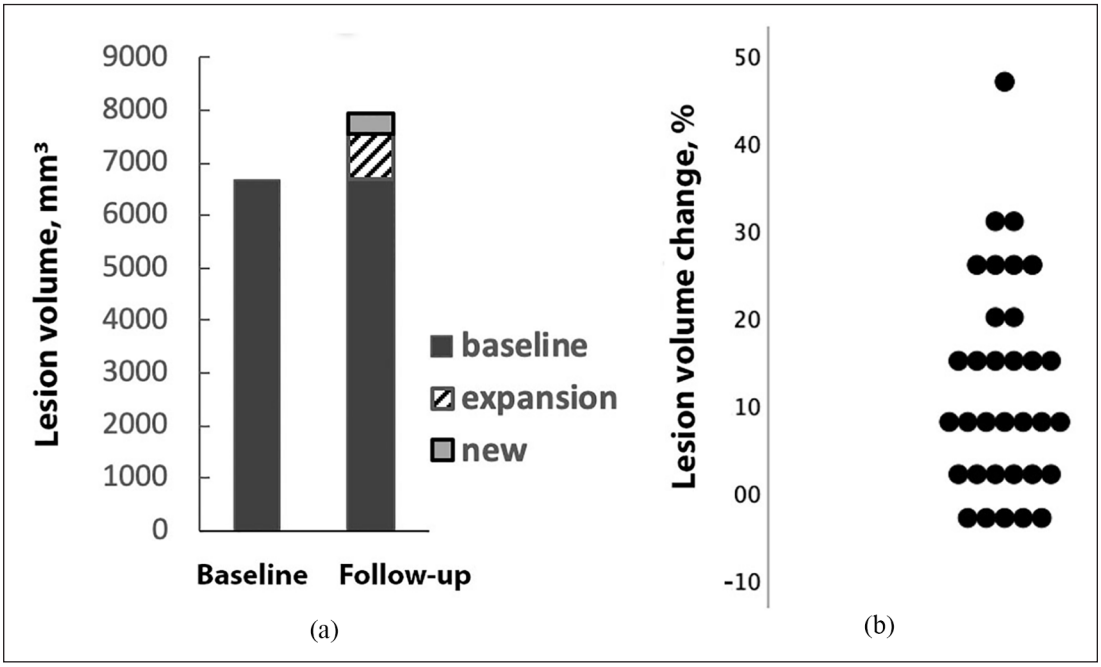

Figure 4. Patient-based lesion analysis. (a) Lesion composition at baseline and follow-up. (b) Rate of chronic lesion volume change. Dots grouped by 5**%.**

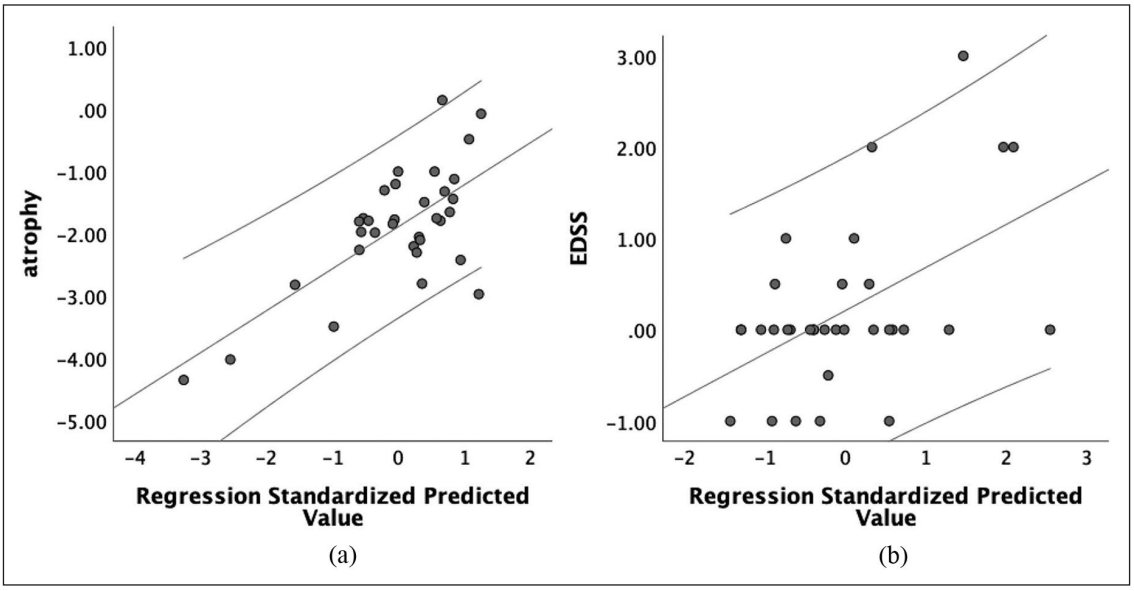

Figure 5. Linear regression analysis, which demonstrates relationship of brain atrophy (a) and EDSS (b) with the volume of chronic lesion expansion, the volume of new lesions and patient's age. Lines represent 95% confidence intervals.

0.009 for volume of chronic lesion change and volume of new lesions, respectively) (Figure 5(a)), the effect of the chronic lesions expansion was more apparent.

Similar analysis using EDSS progression as the dependent variable (model predictive value: $r^2 = 0.26$, $p = 0.03$), however, demonstrated a significant contribution only of chronic lesion volume change to the model (standardized coefficient beta = 0.5, $p = 0.006$) (Figure 5(b)).

The degree of chronic lesion volume change during the follow-up period correlated significantly with the level of MD within lesions at baseline ($r = 0.52$, $p = 0.002$) (Figure 5(a)). This association was

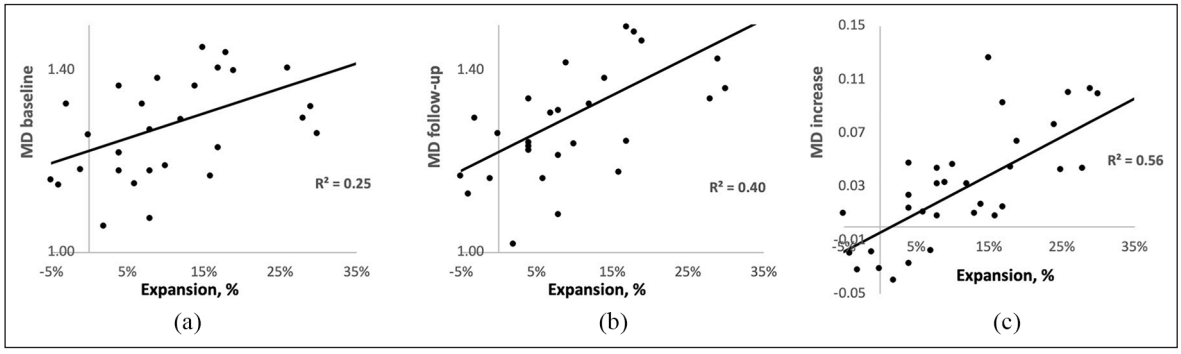

Figure 6. Correlation between rate of lesion volume change and mean diffusivity. (a) Correlation between the rate of lesion volume change and mean diffusivity in chronic lesions at baseline. (b) Correlation between the rate of lesion volume change and mean diffusivity in chronic lesions at 48 months. (c) Correlation between the rate of lesion volume change and change of mean diffusivity in chronic lesions between baseline and 48 months.

Table 3. Lesion-wise analysis of baseline MD and MD change in shrinking, stable and expanding lesions.

Lesions	MD baseline	Tukey multiple comparison test (vs expanding lesions)	MD progression	Tukey multiple comparison test (vs expanding lesions)
Shrinking	1.043 ± 0.137	$p = 0.03$	-0.014 ± 0.047	$p < 0.001$
Stable	1.073 ± 0.141	n/s	0.004 ± 0.054	$p < 0.001$
Expanding	1.102 ± 0.144	–	0.040 ± 0.077	–
ANOVA	$p = 0.025$	–	$p < 0.001$	–

MD: mean diffusivity; ANOVA: analysis of variance; MS: multiple sclerosis.

strengthened by the end of the follow-up period ($r = 0.64$, $p < 0.001$) (Figure 6(b)), suggesting that the degree of progressive tissue loss inside chronic lesions is associated with ongoing inflammation at the lesion rim. This was further supported by the patient-based observation of a strong positive correlation between the rate of chronic lesion volume change and an increase of MD inside lesions during the follow-up period ($r = 0.75$, $p < 0.001$) (Figure 6(c)). Of note, there was significant increase in MD within chronic lesions during the 4 year follow-up period (1.29 vs 1.33, $p < 0.001$, paired t test), consistent with previously reported data.[14] However, there was no correlation between the volume of new lesions and MD progression inside chronic lesions ($p = 0.2$).

Lesion-wise analysis demonstrated that baseline MD and MD change within the lesion core was largely driven by expanding lesions (Table 3).

Discussion

Slow-burning inflammation is putatively associated with lesion expansion and leads to progressive loss of axons and disability worsening.[2,3,19] However, in vivo evidence linking lesion expansion with biomarkers of disease progression, particularly during the relapsing–remitting stage of the disease, is lacking. Therefore, this study was conducted to evaluate the evolution of chronic lesions in RRMS patients and its potential association with biomarkers of disease progression.

Almost half (46%) of the chronic lesions identified in our RRMS cohort significantly enlarged over 48 months of follow-up. Conversely, only a small proportion (12%) of chronic lesions displayed shrinking, usually by a smaller volume. Lesion expansion, therefore, was the principal determinant of chronic lesion volume change over the course of the study.

Patient-wise analysis revealed that the increase in volume of chronic lesions reached as high as 30%–40% in some individuals. Enlarging chronic lesions were found in majority of the patients, and a substantial ($> 10\%$) increase in total chronic lesion volume was observed in about one-third of our patient cohort, which is in line with the rate of smouldering lesions detected pathologically.[20,21]

The study showed that the expansion of chronic lesions is driving, at least partially, disease progression by predicting the evolution of traditional imaging (brain

atrophy) and clinical (EDSS increase) biomarkers. This is not totally unexpected, considering that expansion of chronic white matter lesions reflects a low-grade inflammatory demyelination at the lesion edge, which is known to cause axonal transection.[11] The following Wallerian and retrograde degeneration leads to the elimination of the axolemma and myelin sheath of damaged axons from the white matter tissue and finally to neuronal deaths, which results in white and grey matter shrinkage and ultimately in brain atrophy.

While the rate of axonal loss caused by smouldering inflammation at the lesion edge is considerably smaller than the axonal damage triggered by acute lesions, chronic lesion expansion dominated lesional activity during the follow-up period (comprising a staggering 67% of total increase in T2 lesion load), presumably magnifying its impact on global neurodegeneration and disease progression. This is further supported by the higher contribution of lesion expansion to brain atrophy and EDSS progression compared to the impact of new lesions, as demonstrated by linear regression analysis. Thus, standardized coefficient beta was higher and more significant for expansion of chronic lesions when both brain atrophy and EDSS progression were used as independent variables.

This is in agreement with the outcome of a recent study,[22] which demonstrated that longitudinal activity of chronic lesions, but not the formation of acute lesions, significantly predicts clinical progression in patients with primary progressive MS. It also aligns with the conclusion reached in another study,[11] which demonstrated that in a mixed MS cohort, the incidence of the smouldering lesions is associated with more aggressive disease, higher motor and cognitive disability and earlier transition to progressive disease.

Similar to primary progressive disease,[22] in our RRMS cohort, the evolution of chronic and acute white matter lesions was independent, implying that different mechanisms are likely to be responsible for smouldering and acute inflammation.[2–4,23] This is further supported by the fact that the rate of lesion expansion was independent of treatment type.

Another novel finding of this study is an observed correlation between the degree of chronic lesion volume change and increase in isotropic water diffusion within chronic lesions. Elevated MD, previously demonstrated in chronic white matter MS lesions, has been shown to reflect an enlargement of the extracellular space[24–28] (caused by combination of ongoing tissue loss and relatively rigid lesional structure, which is due to fibrillary gliosis developed during the acute stage of lesion formation),[29] and its gradual increase has been associated with on-going axonal loss.[14,16] Therefore, a close association observed between the rate of lesion expansion and an increase of MD inside of chronic MS lesions during the follow-up period suggests that ongoing low-grade inflammation at the lesion edge promotes progressive tissue damage within the lesion. These findings are in agreement with pathological studies demonstrating that active demyelination in mixed active/inactive lesions correlates with axonal injury in these lesions.[19,21] While both chronic inflammatory changes at the lesion rim and new acute lesions can potentially contribute to white matter loss inside chronic lesions, the strong association of MD increase with lesion expansion and a lack of correlation with new lesions suggest that slow-burning inflammation is a main factor responsible for the progressive damage of demyelinated axons.

Our study also revealed significant association between lesion expansion and patient's age. Both the incidence of expanding lesions and the degree of lesion expansion were higher in older patients. This finding is in line with accelerated disability progression in older patients.[30–32] While systemic low-grade inflammation and associated synaptopathy and synaptic plasticity impairments are evident in normal aging brains, they accelerate in the MS disease (see the work by Musella et al.[33] for review). Furthermore, chronic active lesions, which can be seen throughout the entire course of the disease, have been more frequently detected in patients with long-standing multiple sclerosis.[19] Therefore, considering significant correlation between chronic lesion enlargement and disability progression, it is plausible to assume that activation of smouldering inflammation with age contributes, at least partially, to acceleration of disability in older individuals. This is also supported by the relatively old age of our MS cohort, which approximates the average age at which smouldering plaques peak[19] and the average age-of-progression onset in patients with RRMS.[34]

This study has several limitations. First, a single measure of clinical disability (EDSS) was utilized in this study. More comprehensive physical, cognitive or composite assessments of clinical change, which may better predict disease progression,[22] were not used. The lack of inclusion of susceptibility-weighted imaging (SWI) in our MRI acquisition is a further limitation of the study. Since chronic inflammatory activity at the edge of slowly expanding lesions is sustained by activated macrophages and microglia, it is often seen as a hypointense rim on non-contrast SWI. In fact, several very recent studies demonstrated a close association between lesion expansion and the presence of a

perilesional paramagnetic rim.[3,11] However, since our study was initiated more than 7 years ago, SWI was not included in the original study protocol. Third, all but one patient was treated with DMT, with potentially differential effects on lesion dynamics. It is possible, for instance, that a particular DMT may reduce the appearance of new lesions, but have little or no effect on lesion expansion.

In summary, the expansion of chronic white matter lesions in patients with RRMS is the primary determinant of the increased T2 total lesion load and significantly contributes to disease progression. Low-grade inflammation at the lesion rim is driving, at least partially, axonal loss inside the chronic lesions and brain damage outside of lesional tissue. In addition, expansion of chronic lesions is more evident in older patients.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Supported by the National Multiple Sclerosis Society (NMSS), Novartis Save Neuron Grant, Sydney Eye Hospital foundation grant, Sydney Medical School Foundation, and National Health and Medical Research Council (NHMRC).

ORCID iD

Michael H Barnett https://orcid.org/0000-0002-2156-8864

Supplemental material

Supplemental material for this article is available online.

References

1. Compston A and Coles A. Multiple sclerosis. *Lancet* 2008; 372: 1502–1511.
2. Prineas JW, Kwon EE, Cho ES, et al. Immunopathology of secondary-progressive multiple sclerosis. *Ann Neurol* 2001; 50(5): 646–657.
3. Dal-Bianco A, Grabner G, Kronnerwetter C, et al. Slow expansion of multiple sclerosis iron rim lesions: Pathology and 7 T magnetic resonance imaging. *Acta Neuropathol* 2017; 133(1): 25–42.
4. Lassmann H. New concepts on progressive multiple sclerosis. *Curr Neurol Neurosci Rep* 2007; 7(3): 239–244.
5. Van Der Valk P and De Groot CJA. Staging of multiple sclerosis (MS) lesions: Pathology of the time frame of MS. *Neuropathol Appl Neurobiol* 2000; 26(1): 2–10.
6. Bramow S, Frischer JM, Lassmann H, et al. Demyelination versus remyelination in progressive multiple sclerosis. *Brain* 2010; 133(10): 2983–2998.
7. Trapp BD, Peterson J, Ransohoff RM, et al. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998; 338(5): 278–285.
8. Kutzelnigg A, Lucchinetti CF, Stadelmann C, et al. Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* 2005; 128(Pt 11): 2705–2712.
9. Henderson APD, Barnett MH, Parratt JDE, et al. Distribution of inflammatory cells in newly forming lesions. *Ann Neurol* 2009; 66(6): 739–753.
10. Filippi M, Brück W, Chard D, et al. Association between pathological and MRI findings in multiple sclerosis. *Lancet Neurol* 2019; 18(2): 198–210.
11. Absinta M, Sati P, Masuzzo F, et al. Association of chronic active multiple sclerosis lesions with disability in vivo. *JAMA Neurol* 2019; 76(12): 1474–1483.
12. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011; 69(2): 292–302.
13. Rovira A, Auger C and Alonso J. Magnetic resonance monitoring of lesion evolution in multiple sclerosis. *Ther Adv Neurol Disord* 2013; 6(5): 298–310.
14. Klistorner A, Wang C, Yiannikas C, et al. Evidence of progressive tissue loss in the core of chronic MS lesions: A longitudinal DTI Study. *Neuroimage Clin* 2018; 17: 1028–1035.
15. Jenkinson M, Bannister PR, Brady JM, et al. Improved optimisation for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* 2002; 17: 825–841.
16. Wang C, Barnett MH, Yiannikas C, et al. Lesion activity and chronic demyelination are the major determinants of brain atrophy in MS. *Neurol Neuroimmunol Neuroinflamm* 2019; 6(5): 1–9.
17. Smith SM, Zhang Y, Jenkinson M, et al. Accurate, robust and automated longitudinal and cross-sectional brain change analysis. *Neuroimage* 2002; 17: 479–489.
18. Tustison NJ, Avants BB, Cook PA, et al. N4ITK: Improved N3 bias correction. *IEEE Trans Med Imaging* 2010; 29(6): 1310–1320.
19. Frischer JM, Weigand SD, Yong G, et al. Clinical and pathological insights into the dynamic nature of the white matter multiple sclerosis plaque. *Ann Neurol* 2016; 78(5): 710–721.

20. Luchetti S, Fransen NL, Van Eden CG, et al. Progressive multiple sclerosis patients show substantial lesion activity that correlates with clinical disease severity and sex: A retrospective autopsy cohort analysis. *Acta Neuropathol* 2018; 135(4): 511–528.

21. Frischer JM, Bramow S, Del-Bianco A, et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain* 2010; 132: 1175–1189.

22. Elliott C, Belachew S, Wolinsky JS, et al. Chronic white matter lesion activity predicts clinical progression in primary progressive multiple sclerosis. *Brain* 2019; 142(9): 2787–2799.

23. Correale J, Gaitán MI, Yssraelit M, et al. Progressive multiple sclerosis: From pathogenic mechanisms to treatment multiple sclerosis. *Brain* 2017; 140: 527–546.

24. Klistorner A, Wang C, Yiannikas C, et al. Diffusivity in the core of chronic multiple sclerosis lesions. *PLoS ONE* 2018; 13(4): e0194142.

25. Castriota Scanderbeg A, Tomaiuolo F, Sabatini U, et al. Demyelinating plaques in relapsing-remitting and secondary-progressive multiple sclerosis: Assessment with diffusion MR imaging. *AJNR Am J Neuroradiol* 2000; 21(5): 862–868.

26. Filippi M, Iannucci G, Cercignani M, et al. A quantitative study of water diffusion in multiple sclerosis lesions and normal-appearing white matter using echo-planar imaging. *Arch Neurol* 2000; 57(7): 1017–1021.

27. Droogan AG, Clark CA, Werring DJ, et al. Comparison of multiple sclerosis clinical subgroups using navigated spin echo diffusion-weighted imaging. *Magn Reson Imaging* 1999; 17(5): 653–661.

28. Bammer R, Augustin M, Strasser-Fuchs S, et al. Magnetic resonance diffusion tensor imaging for characterizing diffuse and focal white matter abnormalities in multiple sclerosis. *Magn Reson Med* 2000; 44(4): 583–591.

29. Miller DH, Barkhof F, Frank JA, et al. Measurement of atrophy in multiple sclerosis: Pathological basis, methodological aspects and clinical relevance. *Brain* 2002; 125(Pt 8): 1676–1695.

30. Debouverie M, Pittion-Vouyovitch S, Louis S, et al. Natural history of multiple sclerosis in a population-based cohort. *Eur J Neurol* 2008; 15(9): 916–921.

31. Trojano M, Liguori M, Bosco Zimatore G, et al. Age-related disability in multiple sclerosis. *Ann Neurol* 2002; 51(4): 475–480.

32. Scalfari A, Neuhaus A, Daumer M, et al. Age and disability accumulation in multiple sclerosis. *Neurology* 2011; 77(13): 1246–1252.

33. Musella A, Gentile A, Rizzo FR, et al. Interplay between age and neuroinflammation in multiple sclerosis: Effects on motor and cognitive functions. *Front Aging Neurosci* 2018; 10: 1–13.

34. Tutuncu M, Tang J, Zeid NA, et al. Onset of progressive phase is an age dependent clinical milestone in multiple sclerosis. *Mult Scer* 2014; 19(2): 188–198.