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Leptomeningeal enhancement at 7T in multiple sclerosis: frequency, morphology, and relationship to cortical volume

Daniel M. Harrison, MD¹, Kevin Y. Wang, MD², Julie Fiol, BSN³, Kerry Naunton, BSN¹, Walter Royal III, MD¹, Jun Hua, PhD^{4,5}, and Izlem Izbudak, MD⁵

¹Department of Neurology, University of Maryland School of Medicine, Baltimore, Maryland

²Department of Radiology, Baylor College of Medicine, Houston, Texas ³Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland ⁴Department of Radiology and Radiological Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland ⁵F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, Maryland

Abstract

Background and Purpose—Perform an investigation of the frequency and distribution of leptomeningeal enhancement on post-gadolinium magnetization-prepared FLAIR (MPFLAIR) in multiple sclerosis (MS) on 7 Tesla (7T) MRI and to relate this finding to measures of brain structure and lesion volumes.

Methods—Twenty-nine participants with MS underwent 7T MRI of the brain. Three healthy volunteers (HV) were scanned for comparison. Areas of post-contrast leptomeningeal enhancement were identified. Images were segmented for brain structure and lesion volumes. The relationship between leptomeningeal enhancement and clinical and volumetric data was explored.

Results—Two patterns of enhancement were found: “nodular” (discrete, spherical nodules at the pial surface or subarachnoid space) and “spread/fill” (appearance of contrast spread through the subarachnoid space). Twenty-six of 29 (90%) MS participants had at least one focus of leptomeningeal enhancement. Nodular foci were present in 15/29 (51%) MS participants. Spread/fill foci were present in 22/29 (76%) MS participants. Two HVs had examples of nodular foci, but none had spread/fill enhancement. MS participants with spread/fill foci were older (48.9 years (SD

Corresponding Author: Daniel M. Harrison, M.D., Department of Neurology, University of Maryland School of Medicine, 110 South Paca Street, 3rd Floor, Baltimore, Maryland 21201, Phone: 410-328-5605. Fax: 410-328-5425. dharrison@som.umaryland.edu.

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8.3)) than those without (33.3 years (SD 11.5), $p = 0.005$). MS participants with spread/fill foci had reduced cortical gray matter volume compared to those without ($p = 0.020$).

Conclusions—Leptomeningeal enhancement on post-contrast 7T MPFLAIR is more prevalent than prior reports at 3T – occurring at frequencies closer to histopathologic data. Spread/fill foci are associated with reduced cortical GM volumes and may represent blood-meningeal barrier breakdown near sites of meningeal inflammation, whereas nodular foci may be a normal variant.

Keywords

Multiple sclerosis; Ultra-high field MRI; 7 Tesla; meningeal; Meningeal enhancement; meningeal inflammation

Introduction

Meningeal inflammation is an increasingly recognized histopathologic finding in multiple sclerosis (MS).^{1–3} Meningeal inflammation is not just a bystander consequence of the long-term disease processes of MS. Evidence is mounting to support a direct pathophysiologic role for meningeal inflammation, including induction of widespread cortical demyelination, cortical neuronal loss, microglial activation, and oligodendrocyte pathology.^{4–6}

Previous attempts at visualization of meningeal inflammation in MS have led to variable and controversial results- indicating that a novel method is needed. Most prior work focused on gadolinium-enhanced fluid attenuated inversion recovery (FLAIR) MRI, given its high sensitivity for meningeal infections and carcinomatosis.^{7, 8} Initial attempts at visualization of meningeal pathology with contrast-enhanced FLAIR on lower field MRI scanners were mostly unsuccessful.^{9, 10} However, Absinta et al demonstrated leptomeningeal enhancement in 25% of patients with MS on post-contrast 3 Tesla (3T) FLAIR MRI, which correlated with meningeal inflammation in two autopsy cases.¹¹ This suggested that leakage of gadolinium into the cerebrospinal fluid (CSF) may be an indirect marker for meningeal inflammation in MS.¹² Although this report and subsequent confirmatory papers^{13, 14} were groundbreaking observations, it is likely that this approach visualizes only the ‘tip of the iceberg.’ Pathologic descriptions of meningeal inflammation in MS report widespread, multifocal meningeal inflammation when present, some element of meningeal inflammation in up to 89% of cases with MS, and ectopic lymphoid follicles in the meninges in nearly half of MS cases.^{2, 3, 15}

Multiple studies suggest that ultra-high field, 7 Tesla (7T) MRI provides imaging findings in MS more akin to histopathologic descriptions of this condition, including both white matter lesions and cortical gray matter pathology.^{16–18} In this study we investigated whether translation of the gadolinium-enhanced FLAIR approach to 7T MRI would yield findings of leptomeningeal enhancement more analogous to histopathologic studies in MS and allow probing of the relationship between meningeal inflammation and clinical and imaging outcomes in MS.

Methods

Standard protocol approvals and patient consents

Protocols were approved by the Institutional Review Boards at the University of Maryland School of Medicine, the Johns Hopkins University School of Medicine, and the Kennedy Krieger Institute. Written, informed consent was obtained from all participants.

Participants

Volunteers aged 18 to 65 with diagnoses of relapsing-remitting (RRMS), secondary progressive (SPMS), and primary progressive MS (PPMS) according to revised 2010 McDonald Criteria¹⁹ were recruited from the Johns Hopkins MS Center and the University of Maryland Center for Multiple Sclerosis Treatment and Research. Recruitment for this cohort began in September 2014 and concluded in July 2016. A small group of healthy volunteers without MS or any other known neurologic condition were also scanned for comparison. Participants were excluded for contraindications to MRI (i.e. metallic foreign bodies) or gadolinium contrast (i.e. previous allergy to contrast, renal failure).

MRI protocol

Participants underwent MRI of the brain in a 7T Philips Achieva scanner with a volume transmit/32-channel receive head coil (Novamedical). Dielectric padding was used to improve image homogeneity.²⁰ A 3-dimensional, multi-shot turbo spin echo magnetization-prepared FLAIR (MPFLAIR) sequence was obtained at 0.7mm isotropic resolution across the whole brain. Imaging parameters were: TR 8000ms, TI 2077ms, TE 400ms, SENSE factor 2×3 , flip angle 90 degrees, 10 minutes and 48 seconds duration. A 3-dimensional, multishot fast field echo magnetization prepared 2 rapid acquisition gradient echoes (MP2RAGE) was also acquired across the whole brain at 0.7mm isotropic resolution. Imaging parameters were: MP2RAGE_{TR} 8.25s, TR 6.9ms, TI1 1s, TI2 3.3s, TE 1.97ms, SENSE factor 2×2 , flip angle 1 = 7 degrees, flip angle 2 = 5 degrees, 9 minutes and 46 seconds duration. Images were acquired prior to administration of contrast and again after intravenous administration of 0.1mmol/kg of gadoteridol (ProHance®, Bracco Imaging). MP2RAGE images were initiated approximately 3 minutes after contrast administration and MPFLAIR images were initiated approximately 20 minutes after contrast administration. The contrast-delay timing for MPFLAIR was chosen based on prior work demonstrating the superiority of delayed post-contrast FLAIR over other sequences for imaging for meningeal disease.⁷

Image processing and analysis

MP2RAGE images were processed as per Marques et al²¹ to create a T1-weighted image and T1 map. Images were subsequently manipulated in MIPAV (version 7.2, <http://mipav.cit.nih.gov>) and the analysis pipeline was created using the Java Image Science Toolkit (JIST, version 3.0, <https://www.nitrc.org/projects/jist>).²² All images were registered to the pre-contrast T1 map. An MPFLAIR subtraction image was created by direct subtraction of the post-contrast and pre-contrast images (Figure 1).

The MPFLAIR subtraction images were reviewed alongside pre- and post-contrast MPFLAIR by two independent raters (DH and KW), who were blinded to participant identity and disease-state. Hyperintensities on the subtraction image were reviewed on anatomic images and only included if located within the leptomeningeal space (pia, subarachnoid, or subarachnoid space). This included elimination of regions of post-contrast hyperintensity in/near dural sinuses, large subarachnoid veins, and basal meninges, as in Absinta et al.¹¹ Hyperintense regions were viewed in 3 orthogonal planes for confirmation and for labelling according to the observed pattern of enhancement. Because the concordance coefficient on initial review between raters was poor (Lin's $\rho = 0.046$, $p = 0.782$), a consensus review was then performed under supervision of an experienced neuroradiologist (II). Concordance between raters and the consensus review were: Lin's $\rho = 0.778$, $p < 0.001$ for reviewer 1 (DH) and Lin's $\rho = 0.008$, $p = 0.966$ for reviewer 2 (KW).

A brain image processing and segmentation pipeline was built in the JIST environment, including tools from the CBS Tools processing package for high resolution, ultra-high field MRI²³ and the Lesion-TOADS segmentation algorithm²⁴. MPFLAIR images underwent N4 inhomogeneity correction²⁵ prior to segmentation. Lesion-TOADS segmentation masks were manually corrected for segmentation errors. Raw volumes for white matter (WM) lesions, cortical gray matter (GM), cerebral WM, thalamus, caudate, and putamen were normalized to intracranial volume and are expressed as fractions.

Disability measures

Disability was characterized by the Kurtzke Expanded Disability Status Scale (EDSS) score.²⁶ The symbol digits modalities test (SDMT) was used to assess cognitive functioning.²⁷ The modified fatigue impact scale (MFIS) was used to assess MS-related fatigue.²⁸

Statistical analysis

Statistical analysis was performed in Stata 10.1 IC (StataCorp, College Station, Texas). Lin's concordance coefficient was used to assess interrater reliability.²⁹ Group comparisons for demographic and clinical variables were performed using Fisher's exact test, except for EDSS, which was tested by Wilcoxon rank-sum. Group comparisons for imaging findings were tested by Wilcoxon rank-sum. Categorical analyses compared those with/without each of the enhancing foci subtypes. Since nearly all participants had enhancement of one type or another, an additional analysis was performed for those with 1 solitary focus versus multiple. Significant variables in the categorical comparisons for volumetric measurements were further interrogated in a multivariate regression model, with adjustment for demographic variables attaining significance in group comparisons. All statistical tests were performed with a significance threshold of $p = 0.05$. Adjustments for multiple comparisons were not performed given the exploratory nature of the study.

Results

Twenty-nine participants with MS were recruited. Fifteen participants (52%) were female and 14 (48%) were male. Their mean age was 45.1 (SD 11.2) years and their mean disease

duration was 10.1 (SD 7.0) years. Most of the participants were of the relapsing-remitting phenotype (n = 21, 72%). Four participants had SPMS (14%) and four participants had PPMS (14%). No participants had any co-morbid neuroinflammatory disorders. Most participants with MS were on disease modifying therapy (76%). This consisted of 4 (18%) on interferon- β , 4 (18%) on glatiramer acetate, 3 (14%) on natalizumab, 1 (5%) on teriflunomide, 2 (9%) on fingolimod, and 8 (36%) on dimethyl fumarate. This was a relatively stable and moderately disabled population, with a median of 0 relapses (range 0 to 1) in the year prior to the study visit and a median EDSS score of 3.0 (range 1 – 6.5). Furthermore, no contrast-enhancing white matter lesions were seen in any subject on review of T1-weighted images. Three healthy volunteers were scanned for comparison. There were two males and one female, with an average age of 37.3 (SD 8.1).

Twenty-six of 29 (90%) participants had at least 1 focus of enhancement in the leptomeningeal space on post-contrast MPFLAIR. Participants had a median of 2 foci per subject (range 0 – 8). Two distinct patterns of leptomeningeal enhancement were noted and termed “nodular” and “spread/fill.” Nodular foci (examples in Figure 2) appeared as small, discrete nodules of contrast either at the pial surface or in the subarachnoid space. Nodular foci were quite small, usually 1–2 voxels (0.7 – 1.4 mm) in diameter, and were spherical-shaped. This pattern was seen in 15 of 29 (51%) subjects. The overall median number of nodular foci per subject was 1 (range 0 – 7). Amongst the 15 subjects who had nodular foci, a median of 1 (range 1 – 7) nodular foci were seen. Spread/fill foci (examples in Figure 3) appeared as larger, nebulous areas of contrast in the subarachnoid space. Spread/fill foci often had a visual appearance reminiscent of contrast leakage from one or multifocal sites, with amorphous spreading throughout the local subarachnoid space through fluid dynamics and diffusion, filling and settling into local sulci. Twenty-two of 29 (76%) participants had spread/fill foci, with an overall median of 1 (range 0 – 6) per subject. Amongst the 20 MS subjects who had spread/fill foci present only, the median number of spread/fill foci seen was 2 (range 1 – 6). Nodular and spread/fill foci occurred simultaneously in 11 (38%) MS participants. None of the observed foci had any clear signs of parenchymal cortical or white matter involvement.

No instances of spread/fill foci were seen in any of the three healthy volunteer subjects. However, 2 of the 3 healthy volunteers were found to have nodular foci (Figure 4). One subject’s images demonstrated 2 and the other demonstrated 3 nodular foci.

Regions of leptomeningeal contrast enhancement on MPFLAIR were reviewed on co-registered T1-weighted MP2RAGE images, and no analogous regions of contrast in the leptomeningeal space were seen. Co-registered T1-weighted MP2RAGE images were also reviewed for the presence of vasculature at sites where contrast leakage was noted on MPFLAIR (cerebral vessels are hyperintense on MP2RAGE images). Although vessels at times could be noted near sites where contrast had been seen on MPFLAIR, no vessels were at the exact co-registered coordinates or of the same dimensions and shape as areas of contrast enhancement on MPFLAIR images. Thus, none of the regions of leptomeningeal contrast enhancement on MPFLAIR could be explained simply as contrast flowing through a leptomeningeal vessel.

Demographic and clinical characteristics were compared between those with multiple enhancing foci (> 1) and a single ($= 1$) enhancing focus and those with or without spread/fill and nodular foci (Table 2). MS subjects with spread/fill foci were significantly older than those without ($p = 0.005$), whereas those with nodular foci present were significantly younger than those without ($p = 0.047$). No other significant demographic or clinical differences were found, including no significant differences in levels of disability.

Brain structure volumes and WM lesion volume were compared between those with multiple enhancing foci (> 1) and a single ($= 1$) enhancing focus and those with or without spread/fill and nodular foci (Table 3). Participants with spread/fill enhancing foci had a smaller normalized cortical GM volume (median 0.267, range 0.174 – 0.306) than those without (median 0.296, range 0.239 – 0.309) ($p = 0.020$). Since spread/fill foci were more common in older participants in this cohort, a regression analysis was utilized to determine if the association of spread/fill foci and reduced cortical volume was independent of age. The presence of spread/fill foci predicted reduced cortical volume in a univariate model (coefficient = -0.025 [95% confidence interval (CI): $-0.041, -0.01$], $p = 0.003$). The same relationship present with adjustment for age in a multivariate model, however, it was reduced to a non-significant trend (coefficient = -0.019 [95% CI: $-0.040, 0.002$], $p = 0.073$). There were no significant differences in WM lesion, cerebral WM, thalamus, caudate, or putamen volume in those with and without spread/fill foci. No significant differences were found for any segmented volumes based on no or solitary foci versus multiple foci or for nodular versus without nodular foci present.

Discussion

This study demonstrates that the clear majority of patients with MS (90%) have signs of leptomeningeal enhancement when scanned with a delayed-acquisition, high resolution, post-gadolinium 7T MPFLAIR approach. To our knowledge, this is the first prospective description of the frequency of meningeal inflammation at ultra-high field in MS. This data supports the notion that meningeal inflammation is highly prevalent in MS and suggests that its presence was hidden from previous study due to limitations in imaging technology. Although this was an *in vivo* study and thus no histopathologic confirmation could be performed, prior data has confirmed a link between leptomeningeal enhancement on FLAIR MRI and meningeal inflammation in MS.¹¹ Compared to prior data, the proportion of patients with leptomeningeal enhancement in this study more closely approximates findings from autopsy studies in MS. For example, Howell et al² found at least one example of moderate meningeal inflammatory infiltrate in hemispheric sections in 107/123 (87%) autopsies of patients with SPMS. The same authors subsequently described at least mild inflammatory infiltrate into the cerebellar meninges of 24/27 (89%) of progressive MS cases.¹⁵ These data suggest that the imaging approach used in this study may be a closer approximation of the *in vivo* presence of meningeal inflammation in MS.

Post-gadolinium FLAIR is ideally suited as a surrogate biomarker of meningeal inflammation. The noted exchange of B-cell clones between the meninges and peripheral circulation in MS patients would require disruption of the blood-meningeal and blood-CSF barrier surrounding leptomeningeal vessels,^{30, 31} leading to gadolinium leakage. Cytokine

mediated barrier disruption likely occurs close to regions of meningeal inflammation, especially near meningeal lymphoid follicles (seen in ~40% of autopsy cases in MS), where inflammation is highly concentrated and coordinated release of cell-recruitment cytokines occurs.^{1, 32} Blood-meningeal and blood-CSF barrier disruption in this setting is likely subtle, resulting in minor leakage of gadolinium. The concentration of any gadolinium leaked into the CSF compartment would also be immediately reduced through passive diffusion into the CSF and active washing away by CSF flow dynamics. These properties explain the lack of leptomeningeal enhancement seen on gadolinium-enhanced T1 MRI in MS.¹⁰ Extravasation of very low concentrations of gadolinium into the CSF, however, shorten T1 relaxation times and result in reduced fluid signal suppression on FLAIR.³³ This results in a nearly 10-fold increase in the sensitivity of FLAIR over T1-weighted MRI for gadolinium in CSF.³⁴ Furthermore, the T2-weighting of FLAIR suppresses signal from high flow structures, removing cortical/meningeal vessels from view and ensuring that any visualized gadolinium must be outside of a vessel.⁸

The association between spread/fill foci and reduced cortical GM volumes in this cohort warrants further study. It is unclear if this relationship is independent of age, given the association of spread/fill foci and advanced age in this cohort and progressive brain atrophy with both normal and diseased brains with time. Although statistical significance was lost for this association when considering age as a co-variate, this adjustment did not alter the magnitude or direction of the observed relationship, and it is quite possible that this non-significant trend would remain if the sample size were higher. The link between spread/fill foci and reduced cortical GM volume without a similar link found to WM lesion volume or deep gray nuclei in this report is consistent with pathologic data suggesting a direct pathophysiologic link between meningeal inflammation and cortical pathology.²⁻⁴ MS patients with meningeal inflammation at autopsy are described to have widespread cortical demyelination and neuronal loss, but the relationship between such pathology and white matter lesion burden is weak. Furthermore, histopathologic studies describe gradients of cortical demyelination, loss of cortical neurons and oligodendrocytes, and cortical microglial activation emanating outwards from adjacent meningeal lymphoid follicles.⁴ This suggests that meningeal inflammation in MS may be directly causative of cortical pathology, perhaps resulting in a more disabling phenotype. Indeed, MS patients with meningeal lymphoid follicles at autopsy are reported to have had more rapid disability accumulation and faster time to death than those without.^{3, 35} Our data confirms similar results from an analysis of leptomeningeal enhancement and cortical atrophy in MS at 3T¹³ and both studies put together help make a case for the pathologic significance of meningeal enhancement on MRI in MS.

It is unclear from this study if the two patterns of leptomeningeal enhancement (“nodular” and “spread/fill”) represent different physiologic entities or if they are on a continuum. The presence of nodular foci in 2 of 3 healthy volunteers may suggest that this pattern of enhancement may be a normal variant. Spread/fill foci, on the other hand, were not seen in any healthy volunteers, and their presence was associated with reduced cortical volumes in MS participants- all supporting the notion that this pattern is indeed pathologic. However, full determination of the pathologic/non-pathologic nature of these observations will require further study with larger cohorts, including larger numbers of healthy volunteers.

The two types of foci, at least in the MS population, may also exist on a continuum. The association found in this cohort between spread/fill foci and older aged participants and nodular foci and younger participants suggests the possibility that nodular foci represent an earlier stage in the process of the development of meningeal inflammation in MS. With only minimal, focal meningeal inflammation, the disruption of the blood-meningeal barrier may be small, only allowing a minimal or slower amount of gadolinium leakage, which may appear as small nodules. As the MS disease process advances, and more widespread meningeal inflammation develops, perhaps aggregating into meningeal follicles, the blood-meningeal barrier disruptions may become more extensive (either multifocal, wider, or both), allowing for more extensive gadolinium leakage into the CSF space- visualized as spread/fill foci. Further study will be required to better sort out these differences, including dynamic contrast-enhancement protocols and longitudinal follow up of this and similar cohorts.

There are other, alternate explanations, including the possibility that nodular foci represent gadolinium restricted in flow by meningeal fibrosis, arachnoid trabeculae, or lymphatic structures, whereas spread/fill foci may simply occur in areas where CSF flow is less obstructed. Although the signal from higher flow vessels is suppressed on FLAIR, and thus nodular foci cannot represent contrast flowing through arteries or higher-flow veins,^{8, 10} nodular foci could also alternately represent a small region of venous stasis or turbulence in a small cerebral vein.

There was a striking difference in the rate of leptomeningeal enhancement seen in this study (90%) compared to prior 3T approaches (25% in Absinta et al, 50% in a more recent study¹³). Even if one removes nodular foci from consideration, this increased sensitivity is still seen for spread/fill foci (76% in this study). If leptomeningeal enhancement does indeed represent meningeal inflammation (as suggested by prior imaging-pathologic correlation¹¹), our 7T imaging protocol may be a much more accurate *in vivo* measure of the true extent of leptomeningeal inflammation in MS. Indeed, compared to prior work at 3T, our findings are much closer to rates of meningeal inflammation (nearly 90%) and inflammatory meningeal follicles (40 – 50%) described in pathologic literature.^{2, 3}

A true conclusion as to whether 7T MRI provides increased sensitivity for leptomeningeal enhancement in MS cannot be made without a direct comparison between 7T and 3T scans in the same subjects. This is therefore a limitation of this study. However, there are several reasons to suspect that this is indeed the case. Multiple prior studies in MS have demonstrated increased sensitivity for all aspects of MS pathology at 7T compared to lower fields.^{16–18} Further, the physical properties of a 7T scanner are particularly suited towards imaging the type of pathology being investigated here. Although there is a mild decrease in the r1 relaxivity of gadolinium at 7T compared to 3T, this effect is likely offset by the increased signal-to-noise and contrast-to-noise ratios achievable at 7T.³⁶ These properties allow creation of imaging protocols at fine resolution (0.7mm³ in this study) with excellent discrimination of physiologic detail and suppression of background noise- all essential for visualization of subtle pathology.

Alternately, the differences between our study and prior 3T evaluations may be protocol-driven, as previous studies had shorter or variable delays between contrast administration and scanning.^{10, 11} Delayed scanning is likely necessary, as this provides time for gadolinium to leave the arterial vascular pool, leak through areas of barrier breakdown, and collect in regions of slow CSF flow.³⁷ Our analysis methodology, utilizing both a pre- and post-contrast image, with review of co-registered subtraction images may also have increased visual sensitivity to subtle regions of enhancement. This was not performed in previous studies due to lack of acquisition of pre-contrast images in all cases.^{11, 13, 14} Without utilization of a pre-contrast comparator, it is also possible that the true rate of leptomeningeal contrast enhancement at 3T may be less than previously reported.

The conclusions of this study should be taken with caution given the small sample size. This small sample size particularly limits our conclusions about the relationship between our data and the clinical status of patients (i.e. disability levels, progressive versus relapsing phenotypes, etc.). The findings of this pilot study will hopefully lead to the ability to perform similar evaluations with a larger cohort and perhaps at multiple sites in the near future. Further, caution should be taken when reviewing our findings considering the small number of healthy volunteers in the control population – a clear limitation of this work. It should be noted, however, that comparisons to larger numbers of healthy volunteers at 3T have already established leptomeningeal enhancement on FLAIR as a pathologically specific finding in prior work,¹¹ enough so that subsequent reports did not see the need for inclusion of control subjects.¹³ Future work should also concentrate on evaluating the rates of similar enhancement seen in other inflammatory neurologic disorders, given recent observations of similar rates of leptomeningeal enhancement in other infectious/inflammatory neurologic disorders on 3T MRI.¹⁴ Further, the links seen in our study between leptomeningeal enhancement and reduced cortical volume are both consistent with both prior imaging and pathologic data, indicating that our findings are indeed real and pathologically significant. However, this report is intended as a preliminary description of the findings of a pilot investigation of this unique imaging protocol. Future work will concentrate on accumulation of higher numbers of MS subjects, inclusion of more controls, and longitudinal follow up, allowing more accurate assessments of the relationship between leptomeningeal enhancement on contrast-enhanced 7T MRI, clinical outcomes, and traditional MRI measures of disease burden. Longitudinal follow up for the evolution of enhancing foci will also allow for clarification if nodular and spread/fill foci are on a longitudinal continuum or represent distinct processes. Furthermore, our use of 7T imaging will allow future analyses to interrogate the relationship between meningeal inflammation and cortical pathology, such as cortical lesions and focal cortical atrophy.

This study demonstrates that further work is necessary to elucidate the ideal protocol for imaging of meningeal inflammation in MS. This will likely require 3T vs. 7T comparisons and varying contrast acquisition times and analysis methodologies. The importance of developing an ideal and consistent method is clear, however. Meningeal inflammation has pathologic significance, highlighted by the topological association of meningeal inflammation with cortical demyelination and atrophy and the cytotoxic effects of the secretory products of inflammatory meningeal follicles.^{6, 22, 38} The presence of meningeal enhancement in this study despite the use of immunomodulatory therapies by the vast

majority of subjects also suggests that current disease modifying therapies may not adequately control meningeal inflammation. Until an *in vivo* marker for meningeal inflammation is validated, this cannot be confirmed, nor can new therapies targeting meningeal inflammation be adequately screened. Although our study has limitations, and thus conclusions about its clinical significance should be tempered, this work will contribute to the eventual development of a validated and accurate surrogate biomarker of meningeal inflammation in MS, sparking future study and development of therapeutics.

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References

1. Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Aloisi F. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathol.* 2004; 14(2):164–74. [PubMed: 15193029]
2. Howell OW, Reeves CA, Nicholas R, Carassiti D, Radotra B, Gentleman SM, et al. Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. *Brain.* 2011; 134(Pt 9):2755–71. [PubMed: 21840891]
3. Magliozzi R, Howell O, Vora A, Serafini B, Nicholas R, Puopolo M, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain.* 2007; 130(Pt 4):1089–104. [PubMed: 17438020]
4. Magliozzi R, Howell OW, Reeves C, Roncaroli F, Nicholas R, Serafini B, et al. A Gradient of neuronal loss and meningeal inflammation in multiple sclerosis. *Ann Neurol.* 2010; 68(4):477–93. [PubMed: 20976767]
5. Blauth K, Soltys J, Matschulat A, Reiter CR, Ritchie A, Baird NL, et al. Antibodies produced by clonally expanded plasma cells in multiple sclerosis cerebrospinal fluid cause demyelination of spinal cord explants. *Acta Neuropathol.* 2015; 130(6):765–81. [PubMed: 26511623]
6. Gardner C, Magliozzi R, Durrenberger PF, Howell OW, Rundle J, Reynolds R. Cortical grey matter demyelination can be induced by elevated pro-inflammatory cytokines in the subarachnoid space of MOG-immunized rats. *Brain.* 2013; 136(Pt 12):3596–608. [PubMed: 24176976]
7. Kremer S, Abu Eid M, Bierry G, Bogorin A, Koob M, Dietemann JL, et al. Accuracy of delayed post-contrast FLAIR MR imaging for the diagnosis of leptomeningeal infectious or tumoral diseases. *J Neuroradiol.* 2006; 33(5):285–91. [PubMed: 17213756]
8. Fukuoka H, Hirai T, Okuda T, Shigematsu Y, Sasao A, Kimura E, et al. Comparison of the added value of contrast-enhanced 3D fluid-attenuated inversion recovery and magnetization-prepared rapid acquisition of gradient echo sequences in relation to conventional postcontrast T1-weighted images for the evaluation of leptomeningeal diseases at 3T. *AJNR Am J Neuroradiol.* 2010; 31(5):868–73. [PubMed: 20037130]
9. Bozzao A, Floris R, Fasoli F, Fantozzi LM, Colonnese C, Simonetti G. Cerebrospinal fluid changes after intravenous injection of gadolinium chelate: assessment by FLAIR MR imaging. *Eur Radiol.* 2003; 13(3):592–7. [PubMed: 12594563]
10. Eisele P, Griebel M, Szabo K, Wolf ME, Alonso A, Engelhardt B, et al. Investigation of leptomeningeal enhancement in MS: a postcontrast FLAIR MRI study. *Neurology.* 2015; 84(8):770–5. [PubMed: 25616480]
11. Absinta M, Vuolo L, Rao A, Nair G, Sati P, Cortese IC, et al. Gadolinium-based MRI characterization of leptomeningeal inflammation in multiple sclerosis. *Neurology.* 2015; 85(1):18–28. [PubMed: 25888557]

12. Zurawski J, Lassmann H, Bakshi R. Use of Magnetic Resonance Imaging to Visualize Leptomeningeal Inflammation in Patients With Multiple Sclerosis: A Review. *JAMA Neurol.* 2017; 74(1):100–9. [PubMed: 27893883]
13. Zivadinov R, Ramasamy DP, Vaneckova M, Gandhi S, Chandra A, Hagemeyer J, et al. Leptomeningeal contrast enhancement is associated with progression of cortical atrophy in MS: A retrospective, pilot, observational longitudinal study. *Mult Scler.* 2016 Epub ahead of print.
14. Absinta M, Cortese IC, Vuolo L, Nair G, de Alwis MP, Ohayon J, et al. Leptomeningeal gadolinium enhancement across the spectrum of chronic neuroinflammatory diseases. *Neurology.* 2017 Epub ahead of print.
15. Howell OW, Schulz-Trieglaff EK, Carassiti D, Gentleman SM, Nicholas R, Roncaroli F, et al. Extensive grey matter pathology in the cerebellum in multiple sclerosis is linked to inflammation in the subarachnoid space. *Neuropathol Appl Neurobiol.* 2015; 41(6):798–813. [PubMed: 25421634]
16. Kollika K, Maderwald S, Putzki N, Schlamann M, Theysohn JM, Kraff O, et al. First clinical study on ultra-high-field MR imaging in patients with multiple sclerosis: comparison of 1.5T and 7T. *AJNR Am J Neuroradiol.* 2009; 30(4):699–702. [PubMed: 19147714]
17. Tallantyre EC, Morgan PS, Dixon JE, Al-Radaideh A, Brookes MJ, Morris PG, et al. 3 Tesla and 7 Tesla MRI of multiple sclerosis cortical lesions. *J Magn Reson Imaging.* 2010; 32(4):971–7. [PubMed: 20882628]
18. Sinnecker T, Mittelstaedt P, Dorr J, Pfueller CF, Harms L, Niendorf T, et al. Multiple sclerosis lesions and irreversible brain tissue damage: a comparative ultrahigh-field strength magnetic resonance imaging study. *Arch Neurol.* 2012; 69(6):739–45. [PubMed: 22351849]
19. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol.* 2011; 69(2):292–302. [PubMed: 21387374]
20. Haines K, Smith NB, Webb AG. New high dielectric constant materials for tailoring the B1+ distribution at high magnetic fields. *J Magn Reson.* 2010; 203(2):323–7. [PubMed: 20122862]
21. Marques JP, Kober T, Krueger G, van der Zwaag W, Van de Moortele PF, Gruetter R. MP2RAGE, a self bias-field corrected sequence for improved segmentation and T1-mapping at high field. *Neuroimage.* 2010; 49(2):1271–81. [PubMed: 19819338]
22. Lucas BC, Bogovic JA, Carass A, Bazin PL, Prince JL, Pham DL, et al. The Java Image Science Toolkit (JIST) for rapid prototyping and publishing of neuroimaging software. *Neuroinformatics.* 2010; 8(1):5–17. [PubMed: 20077162]
23. Bazin PL, Weiss M, Dinse J, Schafer A, Trampel R, Turner R. A computational framework for ultra-high resolution cortical segmentation at 7Tesla. *Neuroimage.* 2014; 93(Pt 2):201–9. [PubMed: 23623972]
24. Shiee N, Bazin PL, Ozturk A, Reich DS, Calabresi PA, Pham DL. A topology-preserving approach to the segmentation of brain images with multiple sclerosis lesions. *Neuroimage.* 2010; 49(2): 1524–35. [PubMed: 19766196]
25. Tustison NJ, Avants BB, Cook PA, Zheng Y, Egan A, Yushkevich PA, et al. N4ITK: improved N3 bias correction. *IEEE Trans Med Imaging.* 2010; 29(6):1310–20. [PubMed: 20378467]
26. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology.* 1983; 33(11):1444–52. [PubMed: 6685237]
27. Bever CT Jr, Grattan L, Panitch HS, Johnson KP. The Brief Repeatable Battery of Neuropsychological Tests for Multiple Sclerosis: a preliminary serial study. *Mult Scler.* 1995; 1(3): 165–9. [PubMed: 9345448]
28. Flachenecker P, Kumpfel T, Kallmann B, Gottschalk M, Grauer O, Rieckmann P, et al. Fatigue in multiple sclerosis: a comparison of different rating scales and correlation to clinical parameters. *Mult Scler.* 2002; 8(6):523–6. [PubMed: 12474995]
29. Lin LI. A concordance correlation coefficient to evaluate reproducibility. *Biometrics.* 1989; 45(1): 255–68. [PubMed: 2720055]
30. Palanichamy A, Apeltsin L, Kuo TC, Sirota M, Wang S, Pitts SJ, et al. Immunoglobulin class-switched B cells form an active immune axis between CNS and periphery in multiple sclerosis. *Sci Transl Med.* 2014; 6(248):248ra106.

31. von Budingen HC, Kuo TC, Sirota M, van Belle CJ, Apeltsin L, Glanville J, et al. B cell exchange across the blood-brain barrier in multiple sclerosis. *J Clin Invest*. 2012; 122(12):4533–43. [PubMed: 23160197]
32. Kowarik MC, Cepok S, Sellner J, Grummel V, Weber MS, Korn T, et al. CXCL13 is the major determinant for B cell recruitment to the CSF during neuroinflammation. *J Neuroinflammation*. 2012; 9:93. [PubMed: 22591862]
33. Bonzano L, Roccatagliata L, Levrero F, Mancardi GL, Sardanelli F. In vitro investigation of poor cerebrospinal fluid suppression on fluid-attenuated inversion recovery images in the presence of a gadolinium-based contrast agent. *Magn Reson Med*. 2008; 60(1):220–3. [PubMed: 18581384]
34. Mamourian AC, Hoopes PJ, Lewis LD. Visualization of intravenously administered contrast material in the CSF on fluid-attenuated inversion-recovery MR images: an in vitro and animal-model investigation. *AJNR Am J Neuroradiol*. 2000; 21(1):105–11. [PubMed: 10669233]
35. Choi SR, Howell OW, Carassiti D, Magliozzi R, Gveric D, Muraro PA, et al. Meningeal inflammation plays a role in the pathology of primary progressive multiple sclerosis. *Brain*. 2012; 135(Pt 10):2925–37. [PubMed: 22907116]
36. Fries P, Morelli JN, Lux F, Tillement O, Schneider G, Buecker A. The issues and tentative solutions for contrast-enhanced magnetic resonance imaging at ultra-high field strength. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2014; 6(6):559–73. [PubMed: 25196203]
37. Essig M, Knopp MV, Schoenberg SO, Hawighorst H, Wenz F, Debus J, et al. Cerebral gliomas and metastases: assessment with contrast-enhanced fast fluid-attenuated inversion-recovery MR imaging. *Radiology*. 1999; 210(2):551–7. [PubMed: 10207443]
38. Haider L, Zrzavy T, Hametner S, Hoftberger R, Bagnato F, Grabner G, et al. The topography of demyelination and neurodegeneration in the multiple sclerosis brain. *Brain*. 2016; 139(Pt 3):807–15. [PubMed: 26912645]

Figure 1. Method for identification of leptomeningeal enhancement

Images from a 38-year-old woman with secondary progressive multiple sclerosis treated with dimethyl fumarate. Shown are magnetization prepared fluid attenuated inversion recovery (MPFLAIR) pre-contrast (A) and post-contrast (B) images. The pre-contrast image was digitally subtracted from the post-contrast image to create the subtraction image (C). Areas of hyperintensity on the subtraction image were thus present on post-contrast images and not on the pre-contrast image. An example is highlighted by the red circle.

Figure 2. Examples of “nodular” type enhancing foci in multiple sclerosis

Images from a 49-year-old man with relapsing-remitting multiple sclerosis (RRMS) treated with fingolimod (A–D) and a 51 year old man (E–H), both with RRMS treated with dimethyl fumarate. Shown are post-contrast T1-weighted magnetization prepared 2 rapid acquisition gradient echo (MP2RAGE) (A, E) images, along with axial (B, F), sagittal (C, G), and coronal (D, H) slices from post-gadolinium magnetization prepared fluid attenuated inversion recovery (MPFLAIR) images. The yellow arrows indicate the location of a nodular focus of leptomeningeal contrast enhancement in all 3 planes and its expected location on MP2RAGE.

Figure 3. Examples of “spread/fill” type enhancing foci in multiple sclerosis

Images from a 53-year-old man with relapsing remitting multiple sclerosis (RRMS) treated with dimethyl fumarate (A–D) and a 58-year-old man with RRMS treated with glatiramer acetate (E–H). Shown are post-contrast T1-weighted magnetization prepared 2 rapid acquisition gradient echo (MP2RAGE) images (A, E), along with coronal (B, H) axial (C, G), sagittal (D, F), and coronal (D, F) slices from post-gadolinium magnetization prepared fluid attenuated inversion recovery (MPFLAIR) images. The yellow arrows indicate the location of an spread/fill focus of leptomeningeal contrast enhancement in all 3 planes and its expected location on MP2RAGE images. Note the spread of contrast to fill nearly the entire sulcal space as visualized in panels F and H. Panel G is an axial cut, in-plane with the horizontal component of this sulcus, demonstrating the horizontal spread of contrast in this profound example of spread/fill foci.

Figure 4. Example of a “nodular” focus in a healthy volunteer

Images from a 37-year-old healthy volunteer. Shown are axial T1-weighted post-contrast magnetization prepared 2 rapid acquisition gradient echo (MP2RAGE) (A) and axial (B), coronal (C), and sagittal (D) images from post-gadolinium magnetization prepared fluid attenuated inversion recovery (MPFLAIR). The yellow arrows indicate a location of leptomeningeal contrast enhancement in all 3 planes and its expected location on MP2RAGE.

Table 1

Contrast enhancement patterns.

MS cases (n = 29)	
Enhancing leptomeningeal foci present, n (%)	26 (90%)
Spread/fill foci present, n (%)	22 (76%)
Nodular foci present, n (%)	15 (51%)
Number of enhancing foci (any type), median (range)	2.0 (0 – 8)
Number of spread/fill foci, median (range)	1 (0 – 6)
Number of nodular foci, median (range)	1 (0 – 7)

MS = multiple sclerosis, n = number

Table 2

Comparison of demographic and clinical characteristics between those with/without leptomeningeal enhancement.

	> 1 Enhancing focus (n = 20)	1 Enhancing Focus (n = 9)	Spread/fill Foci Present (n = 22)	Spread/fill Foci Absent (n = 7)	Nodular Foci Present (n = 15)	Nodular Foci Absent (n = 14)
Age	46.6 (9.2)	41.9 (14.9)	48.9 (8.3)	33.3 (11.5) *	41.1 (10.7)	49.4 (10.5) *
Disease duration	11.2 (8.0)	7.8 (2.6)	10.3 (6.7)	9.6 (8.2)	10.4 (7.5)	9.9 (6.6)
% Male	55.0% (51.0)	33.3% (50.0)	55.0% (51.0)	28.6% (48.8)	53.3% (51.6)	42.9% (51.4)
% on MS treatment	80.0% (41.0)	67.0% (50.0)	77.2% (42.9)	71.4% (48.8)	73.3% (45.8)	78.6% (42.6)
% Progressive phenotype	25.0% (44.4)	33.3% (50.0)	31.8%, (47.7)	14.3% (37.8)	20.0% (41.4)	35.7% (49.7)
EDSS (median, range)	3.0 (1.0 – 6.5)	3.0 (1.0 – 4.5)	3.0 (1.0 – 6.0)	2.0 (1.0 – 6.5)	2.5 (1.0 – 6.5)	3.0 (1.0 – 6.0)
SDMT score	50.9 (10.6)	54.6 (15.4)	50.4 (9.9)	57.0 (17.5)	53.4 (10.6)	50.5 (13.8)
MFIS score	35.6 (21.1)	40.6 (18.8)	36.1 (20.3)	40.2 (21.6)	37.5 (18.8)	36.4 (22.6)

EDSS = expanded disability status scale, SDMT = symbol digits modalities test, MFIS = modified fatigue impact scale, n = number

Mean values shown with standard deviation in parentheses. EDSS expressed as median with range in parentheses.

* p < 0.05

Table 3

Comparison of normalized lesion and brain volumes between those with/without leptomeningeal enhancement

Normalized Volume	> 1 Enhancing focus (n = 20)	1 Enhancing Focus (n = 9)	Spread/fill Foci Present (n = 22)	Spread/fill Foci Absent (n = 7)	Nodular Foci Present (n = 15)	Nodular Foci Absent (n = 14)
WM Lesion	0.006 (0.001 – 0.014)	0.007 (0.003 – 0.029)	0.007 (0.001 – 0.029)	0.006 (0.003 – 0.020)	0.005 (0.001 – 0.014)	0.008 (0.003 – 0.029)
Cortical GM	0.267 (0.174 – 0.302)	0.276 (0.239 – 0.309)	0.267 (0.174 – 0.306)	0.296* (0.239 – 0.309)	0.280 (0.190 – 0.309)	0.267 (0.174 – 0.306)
Cerebral WM	0.281 (0.231 – 0.339)	0.298 (0.253 – 0.312)	0.296 (0.231 – 0.339)	0.281 (0.253 – 0.301)	0.287 (0.231 – 0.339)	0.286 (0.253 – 0.333)
Thalamus	0.013 (0.006 – 0.032)	0.017 (0.008 – 0.026)	0.014 (0.006 – 0.026)	0.010 (0.008 – 0.032)	0.014 (0.006 – 0.032)	0.013 (0.008 – 0.026)
Caudate	0.004 (0.001 – 0.008)	0.005 (0.003 – 0.007)	0.004 (0.001 – 0.008)	0.004 (0.003 – 0.007)	0.004 (0.001 – 0.008)	0.004 (0.001 – 0.005)
Putamen	0.010 (0.006 – 0.015)	0.001 (0.005 – 0.014)	0.010 (0.005 – 0.015)	0.009 (0.008 – 0.013)	0.010 (0.006 – 0.015)	0.011 (0.005 – 0.014)

WM = white matter, GM = gray matter, n = number,

* p < 0.05

All volumes are normalized to intracranial volume and are thus expressed as fractions (without units). Median values are shown with range in parentheses. This data shows a significant reduction in cortical GM volume in those with spread/fill enhancing foci, indicating a link between reduced cortical GM volume and spread/fill leptomeningeal enhancement.