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Mult Scler published online 25 July 2011
DOI: 10.1177/1352458511415305

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MULTIPLE SCLEROSIS JOURNAL | MSJ

Multiple Sclerosis Journal
0(00) 1–11

DOI: 10.1177/1352458511415305
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Focal multiple sclerosis lesions abound in 'normal appearing white matter'

Niraj Mistry[1], Emma C Tallantyre[2], Jennifer E Dixon[3], Nicolas Galazis[1], Tim Jaspan[4], Paul S Morgan[5], Peter Morris[3] and Nikos Evangelou[1]

Abstract

Background: The 'normal appearing white matter' (NAWM) in multiple sclerosis (MS) is known to be abnormal using quantitative magnetic resonance (MR) techniques. The aetiology of the changes in NAWM remains debatable.

Objective: To investigate whether high-field and ultra high-field T_1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) MRI enables detection of MS white matter lesions in areas defined as NAWM using high-field T_2-weighted fluid attenuation inversion recovery (FLAIR) MRI; that is, to ascertain whether undetected lesions are likely contributors to the burden of abnormality in similarly defined NAWM.

Methods: Fourteen MS patients underwent MRI scans using 3T FLAIR and MPRAGE and 7 Tesla (7T) MPRAGE sequences. Independent observers identified lesions on 3T FLAIR and (7T and 3T) MPRAGE images. The detection of every individual lesion was then compared for each image type.

Results: We identified a total of 812 white matter lesions on 3T FLAIR. Using 3T MPRAGE, 186 additional lesions were detected that were not detected using 3T FLAIR. Using 7T MPRAGE, 231 additional lesions were detected that were not detected using 3T FLAIR.

Conclusions: MRI with 3T and 7T MPRAGE enables detection of MS lesions in areas defined as NAWM using 3T FLAIR. Focal MS lesions contribute to the abnormalities known to exist in the NAWM.

Keywords

FLAIR, MRI, multiple sclerosis, 'normal appearing white matter'

Date received: 16th February 2011; revised: 27th May 2011; accepted: 1st June 2011

Introduction

Quantitative magnetic resonance (MR) techniques have enabled in-vivo demonstration of alterations in multiple sclerosis (MS) 'normal appearing white matter' (NAWM). The aetiology and pathological substrate of processes affecting NAWM remain unclear, but three main explanations exist (Figure 1).[1] Macroscopic white matter lesions are known to exert effects on functionally connected NAWM via Wallerian degeneration[2] and trans-synaptically.[3] However, this fails to explain fully why marked NAWM abnormalities are also observed in primary progressive MS,[4,5] in which imaging typically reveals relatively few visible white matter lesions. Cytokines have been implicated in mediating MS symptoms without intermediary acute inflammatory plaque formation,[6] and up-regulation of both pro-inflammatory and anti-inflammatory genes has been shown in NAWM in

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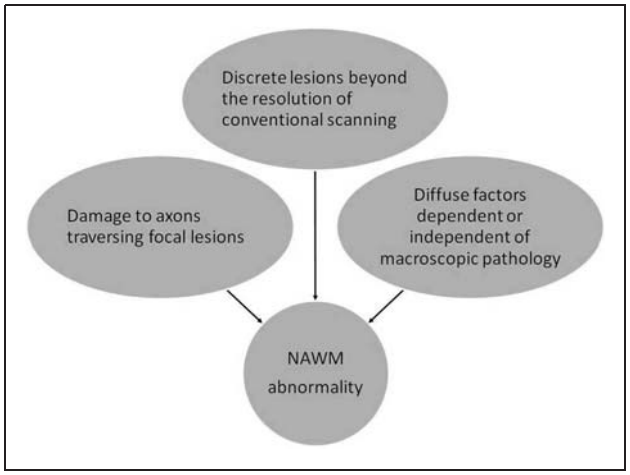

Figure 1. Proposed explanations for the abnormalities known to exist in NAWM. Whilst there is evidence supporting hypotheses that diffuse factors and damage to axons traversing lesions contribute to the abnormalities in NAWM, until now evidence has been lacking that NAWM contains substantial numbers of small focal lesions undetected using high field imaging (i.e. 3T FLAIR). FLAIR: fluid attenuation inversion recovery, NAWM: normal appearing white matter.

MS.[7] T_2-weighted MRI sequences such as fluid attenuation inversion recovery (FLAIR) are accepted as having excellent sensitivity to MS white matter lesions;[8] they are therefore commonly used to segment-out areas of visible white matter abnormality and thus to define NAWM. Use of high-field and ultra high-field MRI has already uncovered small MS lesions undetected using 1.5T MRI.[9,10] In clinical settings 3T MRI employing a highly sensitive sequence such as FLAIR is regarded as the investigation of choice to detect demyelinating lesions. No prior studies have examined whether small discrete white matter lesions exist within areas classified as normal using 3T FLAIR MRI. This might have diagnostic implications in a few individuals and would also contribute to a better understanding of NAWM pathology.

In previous studies comparing lesion detection sensitivity by MRI and histopathology, white matter lesions were missed with imaging. However, those studies were performed using low field strength MRI scanners.[11] As formalin fixation reduces T_1 and T_2 relaxation times, any correlations between fixed tissue and in-vivo data are complicated.[12] Post-mortem histopathological confirmation of additional lesions (cf. FLAIR) found using in-vivo MRI sequences was beyond the scope of this study. Instead we sought histopathological equivalents of our in-vivo MRI findings, using pre-existing post-mortem tissue obtained from other patients with MS.

Currently 7T MRI remains a research tool and is not used in clinical practice. The increase in field strength leads to an increase in signal-to-noise ratio (SNR), allowing a combination of improvement in spatial resolution and shorter acquisition times. Ultra high-field imaging in MS has shown promise in the detailed characterization of cortical lesions[10,13–15] and also white matter lesions in MS.[16] However, there are also technical challenges, and optimization of inversion recovery spin echo sequences such as FLAIR remains challenging at 7T, principally due to inhomogeneities in the RF pulse flip angle across the head. While RF inhomogeneities also affect other pulse sequences, in our experience 3D MPRAGE at 7T does not reflect this inhomogeneity as dramatically, has the advantage of providing good contrast between lesions and the adjacent white matter, and offers rapid acquisition times with good anatomical coverage and high resolution isotropic voxels. Hence we elected to compare standard clinical 3T FLAIR and MPRAGE sequences with 7T MPRAGE.

Methods

Subjects

Fourteen patients were recruited for the study, from the Neurology outpatients department at Nottingham University Hospitals NHS Trust, UK. All of the patients had clinically definite MS. The mean age of the patients was 41 years (range 24–60), mean disease duration was 12.0 years (range 1.2–25.0), and median Expanded Disability Status Scale (EDSS) was 2.5 (range 0–6.5).[17] Disease course of the patients was as follows: 11 had relapsing–remitting MS, two had secondary progressive MS and one had primary progressive MS. Nine of the patients were receiving disease modification treatment (one on azathioprine, five on beta interferon and three on glatiramer acetate). None of the patients had received corticosteroid treatment within 6 months prior to this study. On the day of the MRI scan, disability was assessed using EDSS. Three healthy volunteers (one man aged 45 and two women aged 29 and 30) were recruited by local advertisement. Each participant provided prior written informed consent. This study was approved by Nottingham Local Research Ethics Committee.

Data acquisition

For each patient 3T and 7T scans were obtained on the same day. The healthy volunteers had 7T scans only. 7T images were acquired using a Philips Achieva system

(Philips Medical Systems, Best, The Netherlands) equipped with whole-body gradients, a 16-channel head-only parallel imaging SENSE receive coil and a head-only volume transmit coil (Nova Medical, Inc., Wilmington, MA). Images were acquired using a 3D T_1-weighted MPRAGE sequence ($192 \times 164 \times 100\,mm^3$ field of view; 0.5 mm isotropic voxels; TE = 6.5 ms; TR = 14 ms; TI = 1033 ms; inter-shot interval = 3000 ms; flip angle = 8°; acquisition time 11.9 min) and a 3D gradient-echo sequence with strong T_2*-weighting (200 transverse slices in four overlapping stacks, interleaved to decrease imaging time, giving a field of view of $192 \times 164 \times 85\,mm^3$; 0.5 mm isotropic voxels; TE = 20 ms; TR = 150 ms; flip angle = 14°; parallel imaging factor 2 (RL direction); EPI factor 3; acquisition time 8.8 min).

3T images were acquired using a Philips Achieva system (Philips Medical Systems, Best, The Netherlands) equipped with whole-body gradients, an 8-channel head-only parallel imaging SENSE receive coil and a whole-body transmit coil. Images were acquired using a 3D T_1-weighted MPRAGE sequence ($164 \times 164 \times 118\,mm^3$ field of view; 0.8 mm isotropic voxels; TE = 2.3 ms; TR = 7.6 ms; TI = 960 ms; inter-shot interval = 3000 ms; flip angle = 8°; acquisition time 9.4 min) and a 2D multi-slice FLAIR sequence ($256 \times 204 \times 140\,mm^3$ field of view; $1 \times 1 \times 2.5\,mm^3$ voxels; echo train le\ngth 27; 120° refocusing pulse; TE = 125 ms; TR = 11 s; TI = 2800 ms; acquisition time 6 min). No interpolation was performed at acquisition for any of the sequences.

Data analysis

An experienced observer identified all visible white matter lesions on 3T FLAIR images. A second observer (blinded to FLAIR data) independently identified all visible white matter lesions on 3T MPRAGE and 7T MPRAGE images. Comparisons were then performed to ascertain whether each individual lesion was identified prospectively on 3T FLAIR, 3T MPRAGE or 7T MPRAGE. Ascertaining whether each individual lesion was detected on each sequence is more representative of lesion detection. Drawing conclusions based on simple lesion counts is fraught with difficulties, since lesion appearances differ between sequences (which can impact on the lesion counts), as illustrated in Figure 2. Additional (i.e. not seen on FLAIR) abnormalities found on 3T and 7T MPRAGE were reviewed by an experienced neuroradiologist; thus consensus was reached with the neuroradiologist that additional abnormalities identified using high-field MPRAGE were consistent with MS lesions. These additional abnormalities seen on MPRAGE were also

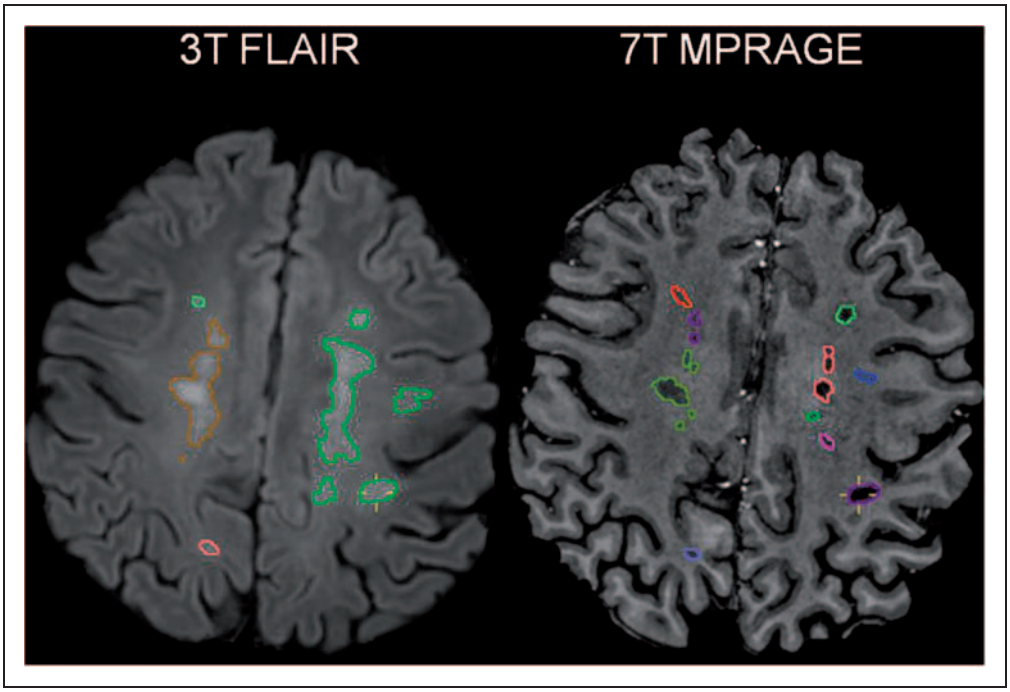

Figure 2. Differences in lesion counting when using MPRAGE and FLAIR images. On FLAIR images neighbouring lesions appeared as confluent hyperintense regions. On MPRAGE images the corresponding lesions appeared as more numerous discrete hypointensities. FLAIR: fluid attenuation inversion recovery, MPRAGE: magnetization prepared rapid acquisition gradient echo.

cross-checked against 7T T_2*-weighted images (and against 3T FLAIR); this allowed exclusion of MPRAGE 'false positives' caused by misinterpretation of hypointensities due to blood vessels or Virchow–Robin (or other CSF) spaces.

Image analysis comprised: image co-registration, drawing 'regions of interest' around visible abnormalities (using a semi-automatic intensity threshold seeding technique) and calculation of lesion map overlaps. All of the acquired images were registered to the same space using FLIRT in FSL (www.fmrib.ox.ac.uk/fsl/ and Jenkinson et al[18]). The other steps were all performed using an in-house software package. Analysis was restricted to supratentorial volumes because the existing 7T receive coil yielded poor SNR infratentorially. All lesions without overlapping counterparts in the other sequences were visually checked to eliminate potential errors in the event of imperfect co-registration of the images. The volume of each lesion detected on MPRAGE images was calculated using the lesion maps.

If a lesion was not identified on one or two of the three (3T FLAIR, 3T MPRAGE, 7T MPRAGE) sequences, its anatomical location was categorized. The location categories were: periventricular (lesion edge touching or within 2 mm of ventricular border), subcortical (lesion edge within 2 mm of cortex, but not touching cortex) or juxtacortical (white matter lesion which touches cortex), deep white matter (white matter lesions not encroaching within 2 mm of cortex or ventricle) and deep grey matter.

Statistical analysis

The 'GenStat' software package (http://www.vsni.co.uk/software/genstat/) was used for statistical analysis. A paired sample *t*-test was applied to the null hypothesis that the difference between the numbers of additional lesions found for each patient on 7T MPRAGE compared with the numbers of additional lesions found on 3T MPRAGE was equal to 0. To test whether the volume of the lesions seen on both FLAIR and MPRAGE was different from the volume of lesions seen only on MPRAGE, we applied a two sample *t*-test with the null hypothesis that there was no significant difference between the volumes of lesions that were also seen on 3T FLAIR and those that were not.

Histopathological comparison

Human cerebral tissue was obtained at autopsy by the UK MS Tissue Bank, Imperial College London, following donor or next-of-kin consent for its use in research. In total, 124 tissue blocks were available from 25 ms patients (seven secondary progressive MS patients and 18 primary progressive). In addition, 25 tissue blocks were available from five patients who had no clinical or pathological evidence of neurological disease. Tissue had been fixed in 4% paraformaldehyde for 14 days, cryoprotected using 30% sucrose for 7 days and then frozen by immersing in isopentane pre-cooled on a bed of dry ice. Blocks were later thawed and embedded in paraffin and sectioned at a thickness of 5 μm. Immunohistochemical staining was performed using a primary antibody targeting myelin basic protein, CD3 and CD163 using the sABC method. Stained sections were used to create digital images of the entire section, which could be viewed electronically with up to ×40 magnification (using Nanozoomer NDP, Hamamatsu, Japan).

Results

Total lesion counts according to the sequence used were: 7T MPRAGE, 1075 lesions; 3T MPRAGE, 967 lesions; 3T FLAIR, 812 lesions. However, these totals misrepresent lesion detection using FLAIR imaging, for the reasons discussed in the data analysis methods section and as depicted in Figure 2. Examples of lesions found on MPRAGE and not on FLAIR are shown in Figure 3.

Although the majority of lesions were detected on both FLAIR and MPRAGE images, a substantial proportion were only detected using 3T MPRAGE (186 lesions, i.e. 19% of the total number of lesions detected) and 7T MPRAGE (231 lesions, i.e. 22% of the total number of lesions detected, of which 94 lesions were also detected using 3T MPRAGE). Of those additional (i.e. not found on 3T FLAIR) lesions detected using 3T and 7T MPRAGE, over half were juxtacortical or subcortical (see Figure 4). Significantly more additional lesions were detected using 7T MPRAGE compared with 3T MPRAGE ($p = 0.012$), as shown in Figure 5. Direct comparison of lesion detection using 3T MPRAGE vs. 7T MPRAGE is shown in Figure 6. In the lower half of the brain the SNR of the 7T images started to become suboptimal; this partly explains why some lesions were missed on 7T MPRAGE compared with 3T FLAIR/MPRAGE (see Table 1).

In two of the three healthy volunteers no lesions were seen on 7T MPRAGE images. The 29-year-old female healthy volunteer had three small discrete white matter lesions, not accounted for by veins, Virchow–Robin spaces or other CSF spaces when checked against T_2*-weighted and FLAIR imaging.

Considering lesion volumes calculated from 3T MPRAGE and 7T MPRAGE lesion maps, those lesions not found using FLAIR were significantly

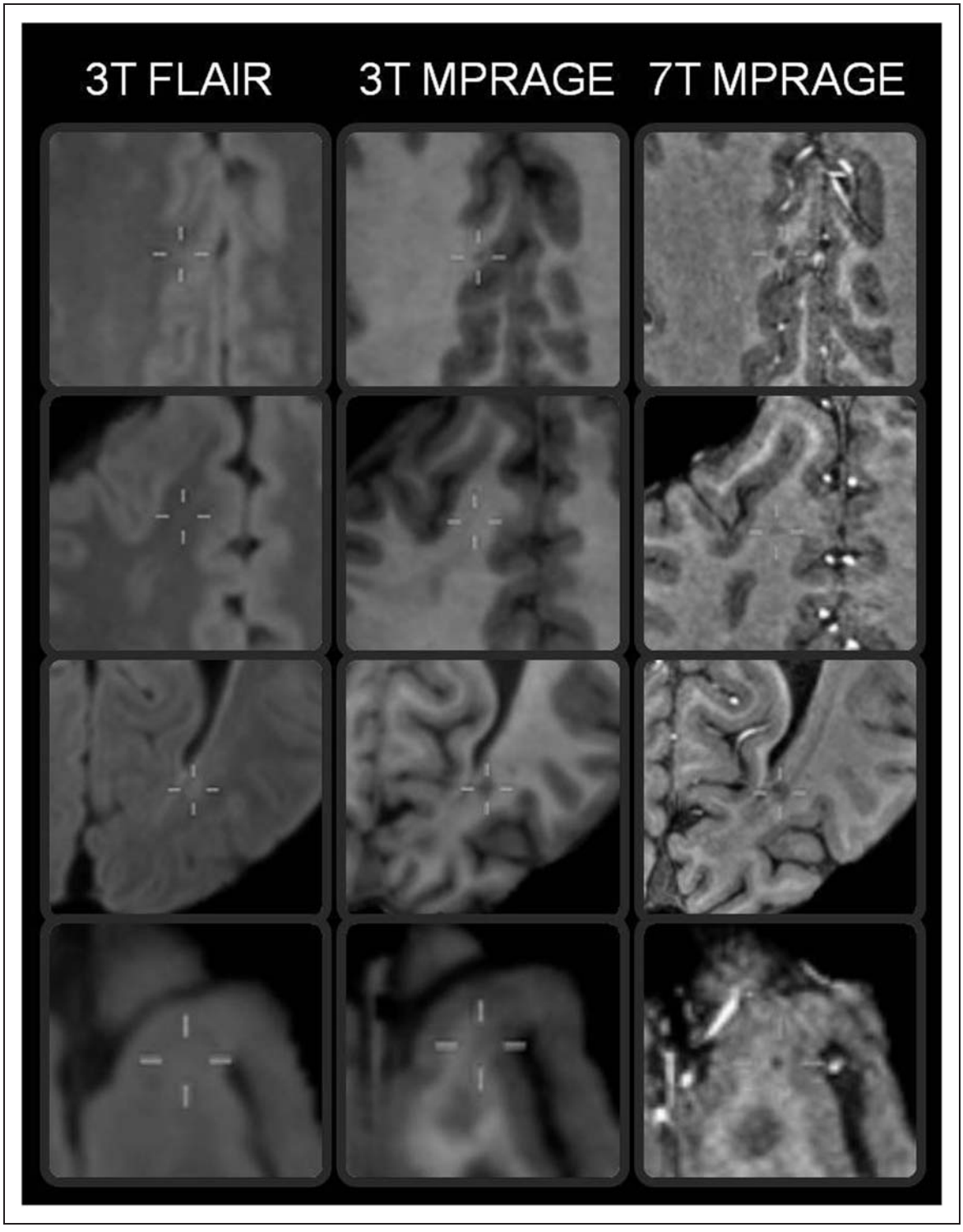

Figure 3. Examples of typical lesions detected by the observer using MPRAGE but not by the observer using 3T FLAIR images. The example in the third row was detected using 3T MPRAGE and 7T MPRAGE. The other examples were only detected using 7T MPRAGE. FLAIR: fluid attenuation inversion recovery, MPRAGE: magnetization prepared rapid acquisition gradient echo.

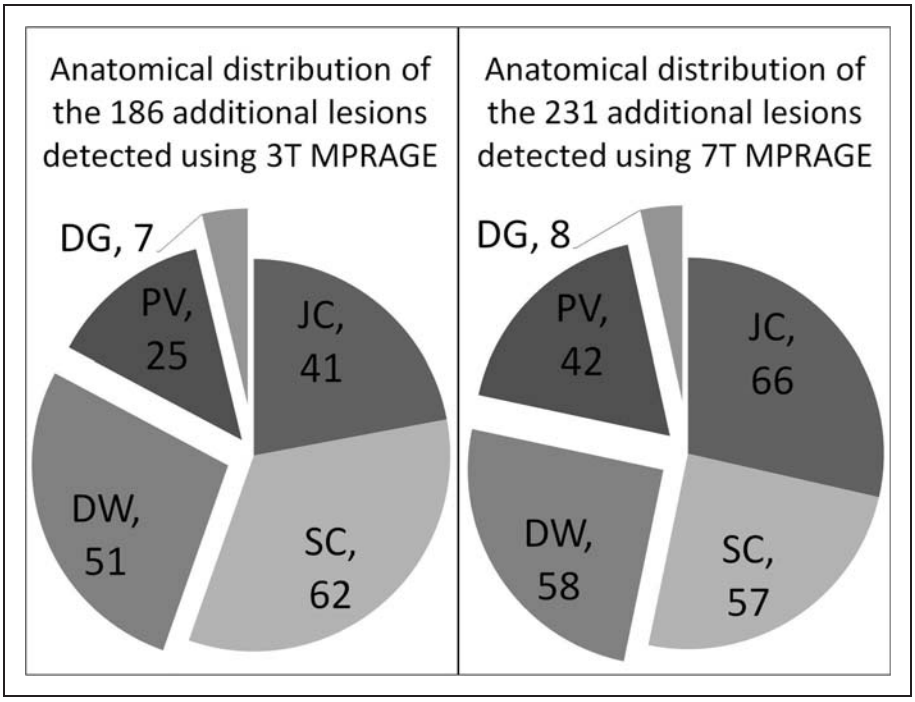

Figure 4. Anatomical distribution of additional lesions detected using MPRAGE. Pie chart labels: DG: deep grey matter, DW: deep white matter, JC: juxtacortical, PV: periventricular, SC: subcortical.

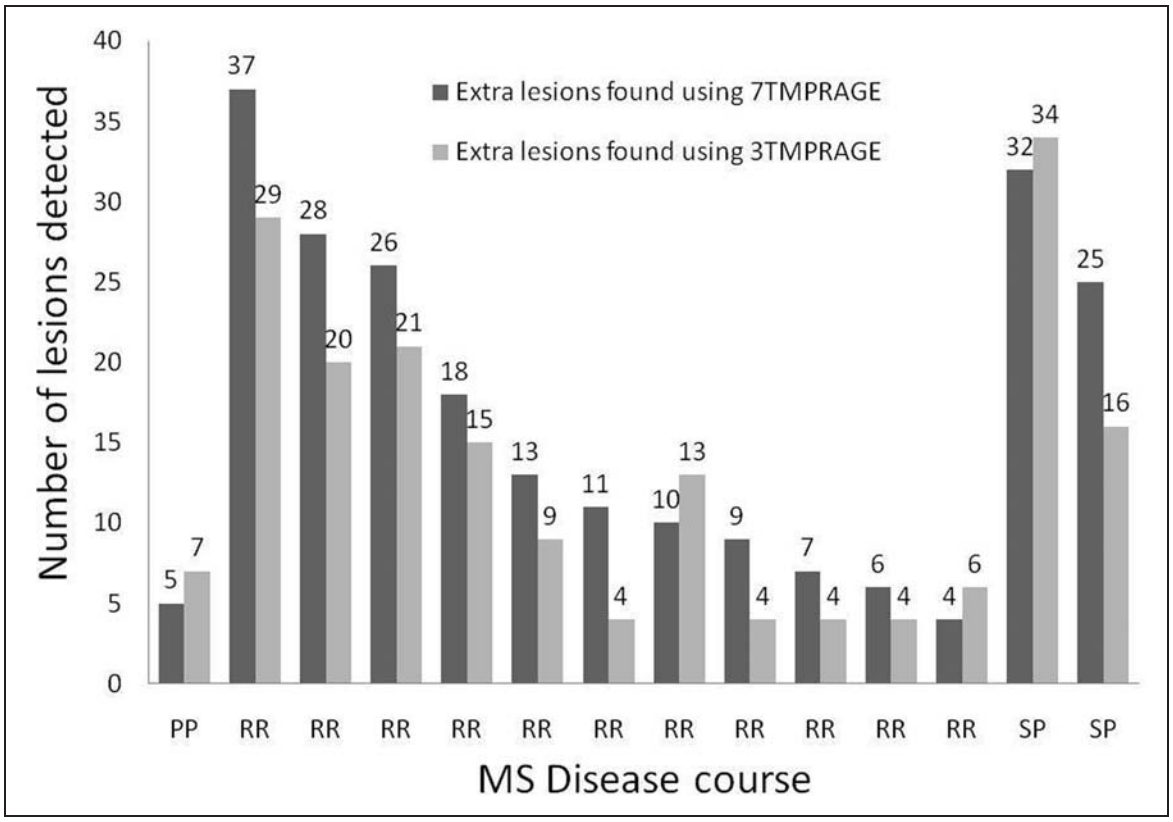

Figure 5. Significantly more extra lesions were found using 7T MPRAGE than using 3T MPRAGE. Number of additional (i.e. not detected using FLAIR) lesions detected using 7T and 3T MPRAGE for each patient. FLAIR: fluid attenuation inversion recovery, MPRAGE: magnetization prepared rapid acquisition gradient echo, PP: primary progressive, RR: relapsing remitting, SP: secondary progressive.

smaller than those that were ($p < 0.001$). Volume data are summarized in Table 2.

Review of digital images of post-mortem histopathological sections revealed the presence of subcortical white matter lesions which were wholly confined within the section, mimicking the small subcortical lesions detected by 7T MPRAGE imaging in vivo. On average, the area of a sectioned block was 281 mm^2, of which 64% was cortex and 36% subcortical white matter. Of the 124 blocks examined from patients with MS, five small subcortical white matter lesions were identified in total (mean area = 2.60 mm^2) in tissue blocks derived from two patients with secondary progressive and two with primary progressive MS. These lesions appeared completely demyelinated and appeared chronically inactive according to staining with lymphocyte and macrophage markers. No similar lesions were seen in tissue derived from non-neurological controls. Examples are shown in Figure 7.

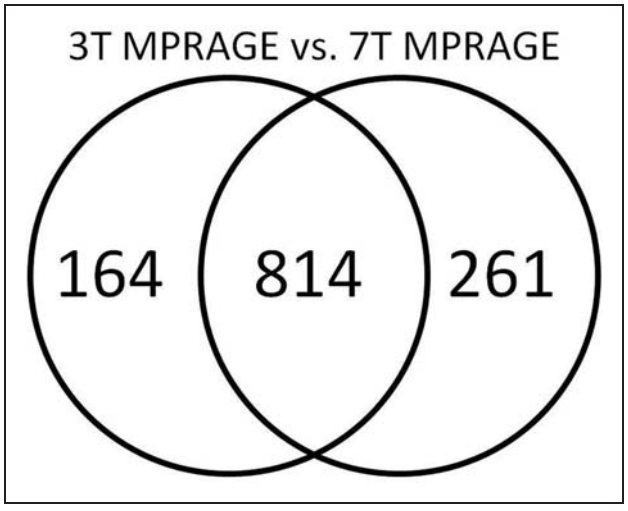

Figure 6. Comparison of lesion detection using 3T MPRAGE vs. 7T MPRAGE. The majority of lesions were common to both 3T and 7T MPRAGE. More lesions were seen on 7T MPRAGE only than on 3T MPRAGE only. MPRAGE: magnetization prepared rapid acquisition gradient echo.

Discussion

There is a growing body of evidence that the radiologically observed changes in NAWM are clinically relevant in MS.[19–21] Studies using MR techniques, including magnetization transfer, T_1 relaxation time mapping, spectroscopy and diffusion tensor imaging, continue to uncover abnormalities in NAWM in patients with MS.[1,22–28]

In this study, we used 3T and 7T MPRAGE MRI to identify lesions in areas of white matter that were defined as NAWM using 3T FLAIR. It follows that discrete lesions such as these are likely to contribute to the radiological changes that have been reported in NAWM. The total volume of the additional lesions found using MPRAGE was relatively small, but their contribution to NAWM abnormality should not be assumed to be proportionately small. There are two reasons for this: first, it is known that pathological effects of focal MS lesions project beyond the lesion and into surrounding NAWM;[2,3] second, it is probable that there are yet more focal lesions (perhaps even smaller) which were undetected in our study (and if they do represent completely demyelinated lesions, as the post-mortem data suggest, then despite their small size they could significantly impact on the average tissue properties).

FLAIR MRI is used routinely in clinical practice (alongside other T_2-weighted spin echo sequences) to detect MS lesions on account of its high sensitivity,[8] aided by its combination of strong T_2-weighting and CSF signal suppression. Currently, acquiring high-quality FLAIR images at 7T is challenging, preventing us from performing a direct comparison of 7T FLAIR vs. 3T FLAIR during the period over which this study was performed. Three-dimensional MPRAGE was

Table 1. Counts of lesions (per patient) detected on 3T FLAIR that were missed on 7T MPRAGE

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	total
7T missed FLAIR lesions bottom half of supratentorial volume	4	3	15	13	20	9	10	7	2	12	7	7	3	3	115
7T missed FLAIR lesions top half of supratentorial volume	6	1	4	16	11	5	10	4	4	7	3	0	1	1	73

Table 1 shows counts of lesions (per patient) detected on 3T FLAIR that were missed on 7T MPRAGE, according to whether they were located in the bottom or the top half of the supratentorial volume. A paired *t*-test was applied to the differences in numbers of lesions detected in the bottom (Group One) vs. top (Group Two) halves of the supratentorial volume.

Significantly more lesions were missed on 7T MPRAGE in the bottom half of the supratentorial volume compared with the top half ($p = 0.0172$). The mean of Group One minus the mean of Group Two equals 3.00 (95% confidence interval of this difference = 0.62 to 5.38). FLAIR: fluid attenuation inversion recovery, MPRAGE: magnetization prepared rapid acquisition gradient echo.

Table 2. MPRAGE lesion volume comparison

7T MPRAGE lesion volumes (mm^3)			3T MPRAGE lesion volumes (mm^3)		
Lesion type	Mean	SD	Lesion type	Mean	SD
Not seen on FLAIR	11.2	15.2	Not seen on FLAIR	13.5	29.6
Also seen on FLAIR	96.6	334.3	Also seen on FLAIR	103.5	518.0

FLAIR: fluid attenuation inversion recovery, MPRAGE: magnetization prepared rapid acquisition gradient echo, SD: standard deviation.

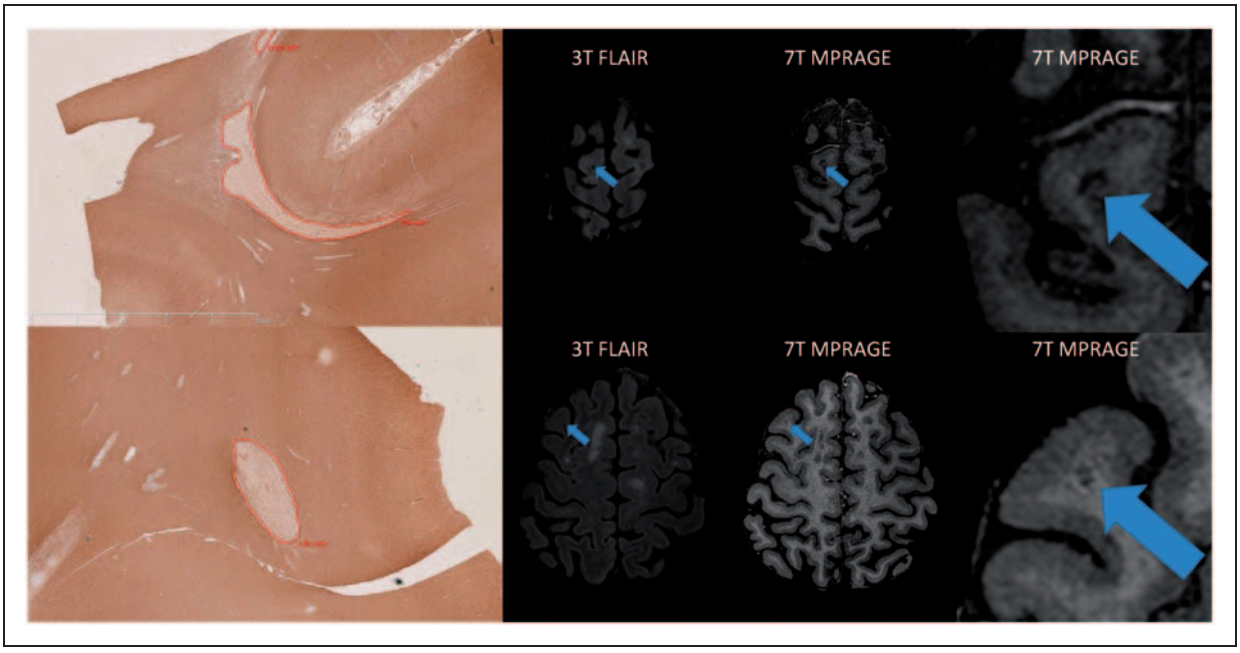

Figure 7. Immunohistochemically stained post-mortem tissue demonstrating small white matter lesions that mimic typical lesions found in vivo on 7T MPRAGE but not on 3T FLAIR images. Left panels: post-mortem brain tissue from patients with MS, immunostained for myelin basic protein. Middle panels: MRI slices containing typical lesions (arrowed) that were identified in vivo using 7T MPRAGE but were not identified using 3T FLAIR. Right panels: enlarged views of lesions as seen on 7T MPRAGE. Top left panel shows a juxtacortical white matter lesion (lesion area 2.28 mm^2 within this section) involving 'U-fibres', without adjacent cortical demyelination. Top right shows a U-fibre involving hypointensity on 7T MPRAGE which appears to be an analogous white matter confined lesion (lesion area 3.50 mm^2 in slice shown), rather than just the white matter part of a mixed leukocortical lesion. Bottom left shows a small subcortical lesion (lesion area 1.56 mm^2 within this section) and an analogous small subcortical lesion seen on 7T MPRAGE is shown bottom right (lesion area 3.00 mm^2 in slice shown). FLAIR: fluid attenuation inversion recovery, MPRAGE: magnetization prepared rapid acquisition gradient echo, MS: multiple sclerosis.

more practical to implement at both field strengths whilst also maintaining good contrast between lesions and adjacent white matter, relatively short acquisition times, and (especially at ultra high-field) high spatial resolution.

The focus of this study was not to examine 'which sequence is best for overall lesion detection'; indeed, many lesions were detected using 3T FLAIR that were not identified on MPRAGE images. Instead, we have focused on trying to determine whether additional small demyelinating lesions exist which could account for some of the NAWM abnormality. In our study, image slice thickness was greatest for 3T FLAIR, intermediate for 3T MPRAGE and smallest for 7T MPRAGE. It is known that reduction in slice thickness enables visualization of greater numbers of smaller lesions.[29] However, it is unlikely that slice thickness alone accounts for all the additional lesions detected using MPRAGE images. As can be seen in Table 2, there was some variance in the size of additional lesions detected using both 3T and 7T MPRAGE (i.e. though smaller on average, lesions undetected on FLAIR were not uniformly small). We postulate that a combination of slice thickness/resolution, increased SNR (at least near the cranial vertex, using the existing 7T receive coil) afforded by ultra high-field strength, and differing

contrast mechanisms of MPRAGE vs. FLAIR offered complementary information contributing to the detection of additional lesions on MPRAGE images.

A disadvantage of MPRAGE is that MS lesions, veins and CSF are all hypointense, necessitating the additional steps of cross-checking MPRAGE findings with T_2*-weighted and FLAIR images. On MPRAGE images, veins in cross-section can be similar in appearance to small lesions, both appearing as small circular hypointensities, if viewed on a single slice in isolation. Veins can usually be distinguished from small spherical or ovoid lesions, because veins are comparatively longitudinally extensive through adjacent slices. Another limitation of this study is that the MRI sample size was small. Larger studies would be required to characterize differences in lesion counts between different MS clinical course types and levels of disability, for example to measure correlation between subcortical lesion load and cognitive impairment.

A strength of this study was that we avoided drawing conclusions based on simple lesion counts. Though time-consuming, ascertaining whether each individual lesion was detected on each sequence is more representative of lesion detection performance.

In our study, more than half the additional lesions detected using 3T and 7T MPRAGE were subcortical or juxtacortical. This is despite the fact that detection of subcortical lesions has been regarded as a specific strength of FLAIR imaging.[30–33] MS subcortical white matter lesions are more likely to cause significant cognitive impairment than comparable lesion loads in other locations.[34] A 1.5 T MRI study found fast-FLAIR to be superior to conventional spin echo in identifying juxtacortical MS lesions, and presence of these lesions correlated with impaired performance in verbal memory tasks.[35] Lesion load in U-fibres has been associated with impaired memory and executive function, using conventional T_2-weighted MRI.[36] Our histopathological finding of small subcortical white matter lesions substantiates the similar subcortical lesions found using 7T MPRAGE imaging in vivo. Only five subcortical lesions were detected in 124 blocks, which seems incongruous with the high-field MRI results, where such lesions represent over half the total number. The incongruity is attributable to sampling differences inherent in the two techniques: the block area (grey and white matter) was on average only 281 mm^2, i.e. just over 16 mm × 16 mm. While a relatively large number of patients were sampled, the tissue area examined histopathologically was small in comparison to the MRI images. Whilst the histopathological technique cannot count the total number of small subcortical lesions in the whole brain, our aim was simply to look for subcortical lesions that bore resemblance to the MRI lesions.

In conclusion, we have demonstrated that MRI with 3T and 7T MPRAGE enables detection of MS lesions in areas defined as NAWM using standard clinical 3T FLAIR. Focal MS lesions contribute to the abnormalities known to exist in the NAWM. In future, combinations of 7T MPRAGE and 3T FLAIR-visible lesion maps may refine segmentation of NAWM. Thus, future studies using (e.g. diffusion or MT) maps of NAWM could be better placed to improve our knowledge about the other possible mechanisms leading to damage beyond discrete lesions.

Funding

This study was funded by research grants from the UK Multiple Sclerosis Society (grant number 919) and Medical Research Council (grant number G0700584).

Conflict of interest statement

Dr Mistry's research fellowship is funded by a research grant from the UK Multiple Sclerosis Society (grant number 919), and he has received funding for conference attendance from Merck-Serono and Bayer-Schering. Dr Tallantyre received funding for conference attendance from Bayer-Schering and Novartis. Dr Evangelou has received honoraria for participating in advisory boards for Bayer- Schering, Merck-Serono and Novartis and has received funding for attending conferences from Novartis, Bayer-Schering and Merck-Serono. The other authors report no disclosures.

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