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MULTISEQUENCE-IMAGING PROTOCOLS TO DETECT CORTICAL LESIONS OF PATIENTS WITH MULTIPLE SCLEROSIS: OBSERVATIONS FROM A *POST-MORTEM* 3 TESLA IMAGING STUDY

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Abstract

Neocortical lesions (NLs) are an important component of multiple sclerosis (MS) pathology and may account for part of the physical and cognitive disability. Visualizing NLs of patients with MS using magnetic resonance imaging (MRI) poses several significant challenges. We optimized the inversion time (TI) of T₁-based magnetization-prepared rapid acquisition gradient-echo (MPRAGE) images by suppressing the signal of the lesions and enhancing their appearance as hypointensities, on the basis of the derived quantitative T₁ measurements. The latter were achieved by the means of 2D inversion recovery fast spin echo (IR-FSE), repeated using different inversion times (TI). Comparisons of detection of NLs by MPRAGE and dual echo T₂ and proton density-weighted (PDW) images were made. Four coronal brain slices from a deceased MS patient and two coronal brain slices from two formerly healthy donors were imaged using a 3 Tesla magnet (3T) equipped with a multi-channel coil. Based upon the averaged T₁ values computed from the MS specimen as well as visual inspection, an optimal TI of 380 msec was selected for the MPRAGE image. No NLs were seen in the specimens of the two healthy donors.

Of the 40 total NLs observed, 8 (20%) were visible in all three sequences employed. Three (7.5%) NLs were visible only in the PDW image and 5 (12.5%) were seen only in the T₂W image. Four NLs (10%) had clearly unique conspicuity in the MPRAGE image. Of those, 3 were retrospectively scored in the PDW image (1 NL) or in the T₂W image (2 NLs). We conclude that for the detection of MS-related NLs, high resolution T₁-based MPRAGE and T₂-W images offer complementary information and the combination of the two image sequences is crucial for increasing the sensitivity of detecting MS-induced NLs.

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Keywords

multiple sclerosis; post-mortem imaging; 3-Tesla MRI; cortical lesions; neurodegeneration; inflammation

INTRODUCTION

Magnetic resonance imaging (MRI) is currently regarded as the most sensitive non-invasive tool for detecting and quantifying multiple sclerosis (MS) pathology *in vivo* [for review see ref 1]. However, despite the sophisticated and advanced level of the imaging techniques presently available and employed, the amount of MS-induced pathology disclosed by MRI *in vivo* is less than that seen by histopathology *post-mortem*. This MRI limitation is especially pertinent to the detection and quantification of neocortical lesions (NLs) [2].

Visualizing NLs of patients with MS poses several significant challenges. First, as opposed to white matter (WM) lesions, NLs may be small in size relative to image resolution. This implies that, to be most suitable for detection of NLs, an MRI should have high signal to noise ratio (SNR), high contrast to noise ratio (CNR) between different tissues and high resolution. However, simply increasing resolution degrades SNR. As a result, the acquisition of images with high SNR acquired at high resolution often requires long scanning time. The second most significant challenge in visualizing NLs is provided by the low contrast between NLs and the surrounding healthy or seemingly normal GM tissue. The normal cortex and the NLs are both regions with low myelin content, and the integrity of myelin lipids is a major contributor of contrast in MRI images [3].

Identifying NLs as well as the ability to characterize and follow their evolution over time is crucially important for MS patients for two main reasons. First, NLs have the potential to substantially impact physical and cognitive abilities of MS patients. Additionally, monitoring the outcome of NLs during therapy may be an important indicator of drug effect beyond the resolution of the blood brain barrier breakdown, the decrease of chronic WM lesions and brain volume loss. Clearly, the demand for high-quality MRI able to demonstrate small MS-induced NLs is becoming increasingly urgent.

Motivated by this notion, over the past several years, numerous MRI sequences have been implemented with the specific aim of enhancing the identification of MS-related NLs. At first it was found that 2-dimensional (2D)-Fluid Attenuated Inversion Recovery (FLAIR) MRI slightly improved the detection of juxtacortical lesions [4–8], especially when image slice thickness was reduced to 1.5 mm [9]. Subsequently, on the basis of the research proposed by Geurts and coauthors [10], it was recognized that the double inversion recovery (DIR) MRI sequence has a superior sensitivity to 3D-FLAIR images in detecting NLs. DIR is mainly a T₂W sequence in which the signals from the cerebrospinal fluid (CSF) and the WM are suppressed at the same time. The sequence yields an image with GM enhancement and more precise cortex delineation with respect to conventional MRI T₂W sequences.

Concomitant to the use of T₂W image sequences, the role of T₁W imaging in detecting NLs has been investigated. High SNR T₁-spoiled gradient-recalled echo (SPGR) images were first shown to be sensitive in depicting NLs [11,12]. More insights into this finding were then provided with the demonstration that T₁-based MRI such as magnetization-prepared rapid acquisition gradient-echo (MPRAGE) could also unveil NLs [13] and, further, that this sequence was complementary to T₂W MRI, such as DIR, in disclosing the actual amount of MS-induced NLs [14]. The latter is an important observation. It highlights the concept that for the detection of NLs the use of a multisequence protocol is important.

To provide more insight into techniques to visualize NLs we compared visibility of NLs between T₁W and T₂W images by collecting high resolution *post-mortem* MRIs from samples of a brain of a patient with MS. In the present work we first obtained high resolution T₂W and proton density weighted (PDW) images on the basis of previously performed T₂ measurements in *post-mortem* tissue at 3Tesla (T) MRI [15]. On those T₂W and PDW images we identified the three tissues classes (i.e., normal-appearing WM [NAWM], normal-appearing gray matter [NAGM] and WM lesions). Next, quantitative brain T₁ maps were obtained. The latter were achieved by the means of 2D inversion recovery fast spin echo (IR-FSE), repeated using different inversion times (TI). Last, on the basis of the derived quantitative T₁ measurements of the three different tissue classes of the brain (i.e., NAWM, NAGM and WM lesions), we optimized T₁W MPRAGE images obtained by suppressing the signal of the lesions and enhancing their appearance as hypointensities. Comparisons of NLs detection between high resolution T₁W MPRAGE and dual echo T₂W and PDW images were made. The present study is part of a combined imaging-histopathological study which is underway in our group. We herein present only results obtained on the imaging optimization part.

MATERIALS AND METHODS

Study design and subjects

This study was performed in the Neuroimmunology Branch (NIB) of the NINDS-NIH with the approval of the Institutional Review Board. We imaged four *post-mortem* coronal slices from a sample of an patient deceased with MS and two coronal brain slices from two donors using a 3T magnet equipped with a multi-channel coil. *Post-mortem* specimens from healthy donors were obtained from a 69-year old male and 81-year old lady who died without neurological conditions and presented with a normal brain at the anatomopathological investigation. All subjects had previously signed informed written consents for autopsy and for the use of their organs for scientific research at NIH. While brain slices of the MS patient were used for all the quantitative and qualitative measurements reported in the present paper, the MRIs of the healthy donors were used only for comparisons with the patient's brain when NLs identification was performed.

MS patient's clinical history—The *post-mortem* MS specimen used in this study was obtained from a 70-year old male patient. The patient had not been seen in the NIB during his lifetime.

Retrospective evaluation of his clinical chart showed that the onset of MS occurred at age 40 with an episode of left optic neuritis. After recovery from the initial episode, he had a residual of a minor reduction of visual acuity and color vision which was often transiently worsened by heat or exercise. He had two months of “forgetfulness” with full recovery at age 42. Between ages 42 and 51 he had 11 relapses with varying amounts of recovery during remissions. These relapses involved the spinal cord five times with incomplete recovery, the brain stem twice and, possibly, the cerebellum also. The remaining episodes were consistent with primarily cerebral involvement and some additional spinal cord pathology. He developed bladder symptoms and erectile dysfunction which was treated with an implanted penile prosthesis. At age 48 he began using a cane. Gait progressively worsened thereafter. He needed automobile hand controls at age 51. He used a scooter, wheel chair and, infrequently, a walker beginning at age 52. He had progressively worse motor function, bladder control and constipation resulting in admission to a nursing home, and was bedridden with a permanent Foley catheter at age 65. By age 66 his cognitive impairment progressed to pronounced dementia. He was described as demented with euphoria at age 67

and non-verbal at age 70. He died with pneumonia at age 70. Spinal fluid exam at age 44 showed 2 oligoclonal bands.

Apart from MS, labile hypertension was diagnosed at age 63. He began having intermittent atrial fibrillation or tachycardia at age 69. Chest x-ray shortly before death showed pneumonia and borderline cardiac enlargement. *Post mortem* anatomic exam described cardiomegaly and atherosclerosis of the aorta and coronary arteries.

Tissue collection and preparation for the MRI procedure

Within a few hours of death, the cadavers were brought to the NIH for autopsy. From each subject the brain was removed and immediately fixed in 4% paraformaldehyde for 2 weeks. After 2 weeks, the whole brain was cut in 10-mm thick coronal slices. Each slice was then fixed in 4% paraformaldehyde until imaged. MR images were obtained at 2 months and 24 months after death for the 2 healthy donors, 36 months after death for the MS brain.

Each brain slice was placed into a cylindrical, specially-fabricated tissue container and imaged in 4% paraformaldehyde fluid in the container (see figure 1). The base ring of the container was manufactured from 140 mm inner diameter clear polyvinylchloride (PVC) sewer pipe of 22 or 28 mm height. PVC was chosen because it approximately matches the susceptibility of biological tissue and water and therefore does not cause extreme static magnetic field gradients at the container-tissue interface. In addition, PVC is chemically inert and mechanically stable. The detachable endplates were manufactured from polycarbonate and were bolted to the pipe using anodized aluminum screws. The container was sealed with a pair of rubber o-rings. A lateral opening in the ring was equipped with an adapter that allowed for evacuation, refilling and sealing.

MRI protocol

Each brain slice was scanned using a clinical 3T scanner (GE Signa Excite III, Twin Gradient, V 12.0) by employing a multichannel receiver-only array (8 channel coil). Table 1 indicates details of the pulse parameters used for acquiring the 2D dual echo spin echo (SE), PDW and T₂W images and the T1-based MPRAGE images.

A 2D IR Fast SE (IR-FSE) sequence with echo time (TE) 15 ms, repetition time (TR) 15 s, in-plane resolution = 0.86*0.86 mm², slice thickness = 0.8 mm and six different TIs (i.e., 50, 250, 500, 1000, 2000, 4000 ms, respectively) were acquired in successive scan series during a single MRI session. An accelerator factor of 4 was used to shorten the scan time. Total acquisition time was 6.5 hours.

Image Post processing

T1 map fitting—Once an inversion pulse is applied, the magnetic resonance signal, S_{IR} , is subject to T1 relaxation for an inversion time TI. Given $TR \gg T1$ of brain tissue, the signal

can be expressed as $S_{IR}(TI) = S_e \left[1 - (1 - k) \exp\left(\frac{-TI}{T_1}\right) \right]$, where S_e is the MR signal at a given TE and $k = \cos(\alpha_{eff})$ accounts for imperfect inversion by a pulse with effective flip angle α_{eff} . Quantitative T1 images were then calculated from the IR-FSE images by fitting the above exponential function to the data of the six different TIs.

T1 and k coefficient calculations and TIs optimizations for the MPRAGE image

—A single region of interest (ROI) of the same size as that previously obtained for the SNR measurement was laid over 15 WM lesions' areas in the T1 maps and the fitting maps. Lesions' areas were defined on the basis of the T₂W and PDW image. T1 and fitting

coefficient k values were averaged in each ROI. The optimal TI to suppress the lesion signal was given by the formula $TI_{\text{lesion}} = T1_{\text{lesion}} * \ln(1 - k_{\text{lesion}})$.

SNR and contrast-to-noise ratio (CNR) measurements—From each image sequence (i.e., T₂W, PDW and MPRAGE images) an SNR image was created in Analysis of Functional NeuroImages (AFNI) [15]. To calculate the SNR map, first a large ROI (i.e., 33.2 mm²) was placed in the background, far from noise artifacts, and the standard deviation (SD) of the signal intensity (SI) calculated. Thereafter the SNR image was calculated by $SNR = S/a * SD\text{-noise}$, where S is the signal, a is a correction factor equal to 1.4257312 for the 8-channel coil employed [16] and $SD\text{-noise}$ is the SD of the SI previously calculated in each image. On each of those three sequences 12 ROIs were placed over WM lesions, NAWM and NAGM using MEDx [17,18]. All the ROIs used had the same size, namely 8.3 mm² each, totaling 33.2 mm² for each tissue class. Care was taken to place each ROI away from the interface between different tissues to avoid partial voluming effect. SI of each ROI was calculated on these generated SNR images using MEDx and gave the SNR values. CNR

between two classes of tissue was defined as $CNR_{AB} = \frac{S_A - S_B}{SD_{\text{Noise}}}$, also equivalent to $SNR_A - SNR_B$ and was therefore given by the difference in their SNR calculated from each SNR-map.

Image Analysis

NLs identification—The number of NLs on the T₂W image, PD and MPRAGE were scored by two investigators (FB and EC) who interpreted the images by consensus. NLs were defined as areas of clearly discernible abnormal hyperintense (in T₂-W and PD-W images) or hypointense signal (in MPRAGE images) with respect to the surrounding tissue. Once identified, each NL was followed for numerous slices above and below its appearance to rule out the possibility that the abnormal signal seen corresponded to partial volume effect from CSF. NLs were classified as intracortical (namely type-I NLs hereafter) and mixed WM-GM (namely type-II NLs hereafter). The latter corresponded to lesions lying in the cortex but abutting the WM as well. Figures 2 and 3 show examples of type-I and type-II NLs.

RESULTS

T1 measurements and TI optimization

Average±SD (minimum and maximum values) of WM lesions T1 was 692.3±148.2 (460.4–884.4). Given the average and range of T1, optimal TI equal to 280, 330, 380 and 450 msec were computed. For each of the four TI an MPRAGE acquisitions was performed. Upon visual inspection, MPRAGEs with a TI equal to 380 msec were found to offer the highest lesions visibility and presented with higher NAWM/NAGM. The latter yielded a consequently better discrimination of NLs' from the cortex and it was preferred for the scope of this paper.

SNR and CNR measurements

In Table 2 SNR and CNRs of different brain regions reported for each sequence. In evaluating differences among sequences, it was observed that T₂W and MPRAGE images offered the best CNR between lesions and NAGM. However, higher SNR was detected in the NAGM in the PDW images when compared to the T₂W and MPRAGE image. This finding yielded to a substantial increase of NAWM/NAGM CNR in the PDW sequence.

NLs identification and comparisons in lesions' identification between sequences

No NLs were seen in the specimens of the two healthy donors.

A total number of 40 NLs was observed. Of those 40 NLs, 8 (20%) were visible in all three sequences employed. Three (7.5%) NLs were visible only in the PDW image and 5 (12.5%) were seen only in the T₂W image. Four (10%) NLs had clearly unique conspicuity in the MPRAGE image (see figure 2). Of those, 3 were retrospectively scored in the PDW image (1 NL) or in the T₂W image (2 NL).

Four (10%) NLs were equally visible only in both PDW and T₂W images (see figure 2 and 4), 12 (30%) NLs were seen only in both T₂W and MPRAGE images and 4 (10%) NLs were seen only in both PDW and MPRAGE images. Of the 14 NLs seen in both T₂W and MPRAGE images, 2 showed better contrast in the MPRAGE vs the T₂W image and were only retrospectively scored in the latter.

Of all the identified NLs, 11 (27.5%) were type-I NL (see figure 2) and 29 were found to be type-II NLs (see figures 2 and 3).

DISCUSSION

Post-mortem investigations provide unique opportunities for understanding the mechanisms responsible for MRI contrast. In contrast to *in vivo* imaging, in *post-mortem* imaging, motionless scans with relatively long acquisition time can be performed. These yield images with increased SNR not achievable *in vivo* and excellent optimization of sequence parameters. The key purpose of the present work was to exploit the superb possibilities offered by *post-mortem* imaging for optimizing sequence parameters.

The T₂W and PDW sequence parameters herein used were optimized on the basis of previously reported findings in *post-mortem* imaging [19]. As already reported [20] and therefore expected, high resolution T₂W and PDW images greatly facilitated detection of small hyperintense NLs sustained by changes in T₂ relaxation times [20]. The boundaries between GM and WM were clearly discernible on both images and differentiation between purely intracortical and mixed WM/GM NL was possible.

No clear gain of one sequence over the other was observed, though the number of NLs seen in PDW images, either alone or in combination with other sequences, was slightly lower than that seen in T₂W images. On the contrary, PDW gave a better CNR between GM and WM than T₂W images. The latter is not surprising given the relatively higher GM/lesions CNR computed on the T₂W vs PDW of our study. The finding might be due to the long T₂ likely associated with the majority of the lesions. In general, increases in T₂ relaxation time are observed when moving from the healthy WM to the GM [21]. In MS brains, areas with longer T₂ relaxation times were found to correlate inversely with magnetization transfer ratios (MTRs) [22]. Decreased MTRs are known to be associated with myelin content and axonal density in lesions [23]. It is therefore likely that lesions more apparent in the T₂W than in the PDW image are those where more severe myelin loss has occurred.

The role of T1-MPRAGE images in disclosing cortical pathology

First, MPRAGE sequence parameters were optimized to obtain maximum contrast between GM or WM and NLs while maintaining a high resolution. Experiments performed in this work showed that T₁ measurements of WM lesions ranged from 500 to 850 ms, corresponding to TIs ranging from 280 to 450 ms for suppressing lesion signals and enhancing their appearance as hypointensities. On the basis of this computation as well as information deriving from images visual inspections, an intermediate averaged TI of 380

was used. We placed ROIs in visible WM lesions and T1 measurements were extrapolated from them. Certainly for NLs with a very different T1 than WM lesions, the optimization technique has some limitation. Nevertheless, this type of approach was used for two main reasons. First, SI of WM lesions on PDW images appears similar to SI of NLs. Second, and more importantly, since WM lesions are larger in size than NLs, larger and more representative ROIs could be traced while avoiding biases due to partial volume effects.

The usage of the T1 extrapolated from the calculate T1 allowed acquisition of images with a reasonable contrast between GM and WM tissue but with some inconsistency across the slice surface. Thus, PDW images were used as references for discerning those boundaries. The low visible contrast between NAWM and NAGM tissue on our MPRAGE sequence compared to the well known *in vivo* data is explained by three important factors. First, it is certainly due to the fact that the MPRAGE parameters employed in this work were tailored towards the detection of NLs and not the maximization of the contrast between the GM and the WM. Second, T1 changes are known to occur *post-mortem* with long-term formaldehyde fixation. Specifically, irrespective of the strength of the magnetic field employed, T1 declines as a function of time in fixed brains with greater decreases in GM than in WM [24,25]. This blurs the sharp WM/GM contrast observable *in vivo*. Last, T1 changes are known to occur in MS patients' cortex and WM [26], likely affecting the contrast normally present in MRI between the two tissue classes.

As has been shown *in vivo* [10–14] high-resolution T1-based sequences allowed the detection of small NLs. The lesion load seen in T₁-W images was lower than the one observed in the PDW ones. For few NLs, though, MPRAGE offered better or unique visibility than any other sequence. This finding parallels *in vivo* data [14]. We believe that the variable combination of myelin loss and water content may be responsible for the different appearance of lesions in T₁W and T₂W images. Lesions sharply demarcated in T₁-MPRAGE and less obvious or not visible in PDW or T₂W are those exhibiting pure T1 contrast. Those lesions correspond to areas of long T1 relaxation times which are known to be associated with the presence of a more destructive disease pattern *in vivo* [27]. We postulate that in those lesions massive demyelination and axonal loss associated with widening of the extracellular space and necrotic tissue infiltration [*for review* sees 28–30] are present. Conversely, for NLs or juxtacortical lesions remaining visible on a PDW image, it is likely that residual remnants of myelin sheets and reactive cells subtending scar formation might be present. Whether those residual components signify end-stage pathology or an attempted remyelination process deserves proper histopathological investigation.

Limitations of the present study and conclusion

The first limitation of our study stems from the fact that all the MS specimens analyzed were derived from a single case. The patient died relatively old and with an advanced stage of MS. This implies that only chronic lesions were likely to have been studied in the present investigation. While this does not interfere with the conclusions of the findings, it does weaken the power of the generalization of our data. In this regard we recognize that studies performed in larger samples are warranted to establish the patient-dependency of our observations.

Additionally, the lack of information regarding the pathological significance of the present findings, leave the results, in part, inconclusive. As stated earlier in the introduction, histopathological analyses are underway to understand the pathological substrate of our findings.

Finally, one should bear in mind that the values of T1 generated from our sample are certainly influenced by the very long time period (i.e., 36 months) the sample was kept in

formalin. Thus care needs to be taken before generalizing from the numerical values obtained here, and applying them to other *in vivo* and *post-mortem* studies.

With the present data we conclude that for the detection of MS-related NLs, high resolution T₁-based MPAGE and T₂W and PDW images offer complementary information, although the interface GM/WM can be better identified on PDW images. It is likely that both sequences, when obtained with a 3T magnet, still do not capture the entire amount of disease pathology present in the cortex of MS patients. However, the combination of the three image sequences is crucial for increasing the sensitivity in detecting MS-induced NLs.

Our evidence, in combination with previously reported work, renders the histopathological validation of the findings mandatory in order to optimize their transition to the daily clinical practice.

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Figure 1. Cylindrical home-made tissue containers
The base ring of the container was manufactured from 140 mm inner diameter clear PVC sewer pipe of 28 (*figure 1a*) or 22 mm (*figure 1b*) height.

Figure 2A **Figure 2B** **Figure 2C** **Figure 2. MPRAGE (figure 2a) PDW (figure 2b) and T₂W (figure 2c) Images**

The white dashed arrow points to a type-I NL visible only in the MPRAGE image, not visible in the PDW image and barely visible in the T₂W images.

Figure 3A **Figure 3B** **Figure 3. PDW (figure 3a) and T₂W (figure 3b) Images**

The white dashed arrow points to a type-I NL visible in both PD and T₂W images. The white solid arrow points to a type-II NL. As one can see, PDW images offered better visibility of both lesions. Additionally, the intracortical portion of the type-II NLs was not visible on the T₂W image. None of the NLs was identified on the MPRAGE image.

TABLE 1**PULSE SEQUENCE PARAMETERS**

	DUAL ECHO T₂W/PD	MPRAGE
Field of view (FOV)	22	22
In plane resolution	1024*1024	1024*1024
Repetition time (TR), msec	4,000	7.3
Echo time (TE), msec	76/8.4	3.2
Inversion time (TI), msec	N/A	380/450
Flip Angle (FA)	N/A	10
Echo train length	16	N/A
Bandwidth	31.25	31.25
Thickness (mm)	1.0	1.0
Inter-slice gap	0	0
Number of slices	30	30
Slice coverage	Whole	
Imaging plane	Oblique	
Acquisition time, mints	26	25
Number of excitations	6	3

N/A= not applicable; mm=millimeters; msec=milliseconds; mints=minutes.

TABLE 2**SNR AND CNR MEASUREMENT OF THE MS BRAIN**

Sequence	SNR			CNR		
	NAWM	NAGM	LESIONS	NAWM NAGM	NAWM LESIONS	NAGM LESIONS
T ₂ W	37.6±5.1	82.0±16.6	129.7±21.2	44.4	122.2	47.7
PDW	132.5±14.6	187.9±28.7	217.8±18.8	55.4	82.3	29.9
MPRAGE	74.6±8.2	59.5±13.3	14.4±8.1	15.1	60.2	45.1