IMAGING CORTICAL LESIONS IN MULTIPLE SCLEROSIS WITH ULTRA–HIGH-FIELD MAGNETIC RESONANCE IMAGING

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OBJECTIVE: To determine the sensitivity of T2*-weighted gradient-echo (T2*GRE) and inversion recovery turbo-field-echo (TFE) sequences for cortical multiple sclerosis lesions at 7 T.

DESIGN, SETTING, AND PARTICIPANTS: Autopsied brain tissue from individuals with multiple sclerosis was scanned with 3-dimensional T2*GRE and 3-dimensional inversion recovery white matter–attenuated TFE sequences at 7 T. Cortical lesions visible with either sequence were scored for each anatomical lesion type. Imaged brain tissue was then processed for immunohistochemical analysis, and cortical lesions were identified by labeling with antibody against myelin basic protein and CD68 for microglia. Magnetic resonance images were matched with corresponding histological sections and scored retrospectively to determine the sensitivity for each cortical lesion type.

MAIN OUTCOME MEASURE: Cortical lesion detection by 3-dimensional T2*GRE and white matter–attenuated TFE sequences.

RESULTS: The 3-dimensional T2*GRE and white matter–attenuated TFE sequences retrospectively detected 93% and 82% of all cortical lesions, respectively (with varying sensitivities for different lesion types). Lesion visibility was primarily determined by size as all undetected lesions were smaller than 1.1 mm at their smallest diameter. The T2*GRE images showed hypointense rings in some cortical lesions that corresponded with increased density of activated microglia.

CONCLUSIONS: Three-dimensional T2*GRE and white matter–attenuated TFE sequences at a 7-T field strength detect most cortical lesions in postmortem multiple sclerosis tissue. This study indicates the potential of T2*GRE and white matter–attenuated TFE sequences in ultra–high-field magnetic resonance imaging for cortical lesion detection in patients with multiple sclerosis.


Multiple sclerosis (MS) is an inflammatory disorder of the central nervous system and is regarded as a prototypical white matter (WM) disease.1 However, recent histopathological studies have shown extensive demyelination in the cortical gray matter (GM) of patients with chronic MS. On average, 15% to 25% of the total cortex is demyelinated in patients with progressive MS, and cases with more than 70% of demyelinated cortex have been reported.2-4 Four lesion types have been defined, namely lesions that extend across the WM and GM (type 1), subcortical lesions within the cerebral cortex that do not extend to the surface of the brain or to the subcortical WM (type 2), subpial lesions that typically affect entire and commonly multiple gyri (type 3), and lesions that extend throughout the full width of the cerebral cortex but do not reach into the subcortical WM (type 4).4

Because of their frequency and extent, cortical lesions are believed to contribute significantly to the disease process. It has therefore become a critical goal to study the effect of cortical lesions on clinical disability and on the disease course. Because of their small size, lack of inflammation, and low lesion contrast, cortical lesions are difficult to detect by conventional magnetic resonance imaging (MRI).3 Ultra–high-field MRI offers higher signal-noise ratio, spatial resolution, and contrast capabilities and has shown promise for improved lesion detection with T1- and T2-based sequences. In several studies, 2-dimensional (2-D) T2*-weighted gradient-echo (T2*GRE) and 3-dimensional (3-D) T1-weighted magnetization-prepared rapid-acquisition GRE sequences at 7 T provided high-resolution anatomical images of cortical lesions.5-9 These ultra–high-field studies were performed on patients with MS and demonstrate that cortical lesion imaging is feasible with this method. However, the sensitivity of these techniques for cortical lesion detection cannot be inferred from in vivo imaging alone without corresponding histopathological analysis.
Here, we provide a detailed assessment of the sensitivity of 3-D T2*GRE and 3-D inversion recovery WM-attenuated (WHAT) turbo-field-echo (TFE) sequences at 7 T in formalin-fixed MS brain for cortical demyelination. Our study shows a previously unachievable detection rate and serves as a guide for further studies in vivo.

**METHODS**

**MS TISSUE SAMPLES**

Human central nervous system tissue was obtained at autopsy according to an institutional review board–approved protocol. Postmortem intervals were between 4 and 8 hours. Brain was fixed with formalin for a minimum of 2 weeks. Ten coronal brain sections were used from 3 subjects with secondary progressive MS displaying predominantly chronic active and chronic silent WM lesions; patient 1 (6 coronal sections) was a 42-year-old man with a 12-year disease duration; patient 2 (2 sections) was a 69-year-old man with a 10-year disease duration; and patient 3 (2 sections) was a 60-year-old woman with a 15-year disease duration. Studies using samples of all 3 patients have been published previously.10,11

**CORTICAL LESION DETECTION IN POSTMORTEM TISSUE BY MRI**

Ten formalin-fixed brain slices from 3 individuals with secondary progressive MS were immersed in phosphate-buffered saline and imaged at 7 T (Achieva; Philips Medical Systems, Best, the Netherlands) using a transmit/receive knee coil with the following sequences: for the 3-D T2*GRE sequence: recovery time, 25 milliseconds; echo time, 12 milliseconds; flip angle, 5°; field of view, 150 × 150 × 25 mm; matrix size, 1000 × 1000 × 83; in-plane resolution, 150 × 150 µm; slice thickness, 300 µm; acquisition time, 3 hours 12 minutes; and 4 signal averages; for the 3-D inversion recovery WHAT-TFE sequence: inversion time, 91 milliseconds; shot interval between inversion pulses, 4000 milliseconds; TFE readout; recovery time, 5.7 milliseconds; echo time, 2.2 milliseconds; flip angle, 8°; turbo factor, 300; field of view, 150 × 150 × 25 mm; matrix size, 600 × 600 × 50; in-plane resolution, 250 × 250 µm; slice thickness, 500 µm; acquisition time, 1 hour 42 minutes; and 12 signal averages. The WHAT-TFE contrast is based on tissue T1-weighted differences with inversion time selected to null WM signal. Spatial resolution and number of signal averages for WHAT-TFE were adjusted to achieve a signal-noise ratio comparable to that of T2*GRE; the theoretically available signal with WHAT-TFE is one-eighth that of T2*GRE, necessitating a lower spatial resolution in WHAT-TFE images.

To calculate signal-noise ratios and contrast-noise ratios, image signal was determined in regions of interest. Signal-noise ratio was defined as the ratio of image signal and standard deviation of signal in air. Contrast-noise ratio was defined as the difference in signal-noise ratios between different image regions.

**DETECTION OF GM PATHOLOGY BY IMMUNOHISTOCHEMISTRY**

Following MRI, formalin-fixed MS blocks were transferred through sucrose, frozen in dry ice and N-methylbutane, and cut at 50-µm thicknesses on a large sliding microtome. Sections were mounted on large glass slides, incubated with primary antibody, and processed with the appropriate biotinylated secondary antibody and avidin-biotin staining kit with diaminobenzidine as the chromogen.

To detect myelin, we used rabbit antihuman myelin basic protein antibody (Dako North America, Inc, Carpentaria, California) at a concentration of 1:1000 and a 4-day incubation time. To immunolabel microglia or macrophages, we used mouse antihuman CD68 antibody (clone PG-M1) (Dako North America, Inc) at a concentration of 1:300 with an overnight incubation.

For each slice, 2 to 5 sections evenly spaced throughout the thickness of the entire block for MRI were used to create section maps outlining cortical demyelination and microglial activation (Figure 1).

Perls staining (Prussian blue reaction) for iron detection was performed by immersing slides in 4% ferrocyanide and 4% hydrochloric acid for 30 minutes in the dark. After rinsing in phosphate-buffered saline, iron was visualized using 3,3-diaminobenzidine (Vector Laboratories, Inc, Burlingame, California) for 30 minutes at room temperature. Sections were rinsed with distilled water, quickly dehydrated, and coverslipped with Permount (Vector Laboratories, Inc). Perls staining was performed on 5 blocks with a total of 15 CD68+ lesions.

Some sections were counterstained with cresyl violet to better delineate the GM-WM border.

Cortical lesions were classified according to their anatomical location as mixed GM-WM lesions (type 1) and cortical lesions that reside entirely within the cortex (types 2-4) as suggested by Bø et al.4 Type 2 lesions are small intracortical lesions often centered around a blood vessel, while type 3 lesions extend from the pial surface into cortical layer 3 or 4, often comprising entire or even multiple gyri. Type 4 lesions affect the entire width of the cortex from the pial surface to the WM.

Lesions are staged according to inflammatory activity into active, chronic active, and chronic inactive. Degree of inflammation is determined by the density and distribution of macrophages or microglia. Active cortical lesions have a distinct border of macrophages or microglia at the lesion edge and cores with increased macrophage or microglia density compared with normal cortex. Chronic active cortical lesions have a border of macrophages or microglia (usually thinner than active lesions) but decreased or normal densities in their centers compared with normal cortex. Chronic inactive cortical lesions show no increase in macrophage or microglia density at the lesion border or the center.

Slides were scanned with a ScanScope XT scanner (Aperio Technologies, Inc, Vista, California) at ×20 magnification. The GM-WM borders and demyelinated cortical lesions were outlined on digitized images of myelin basic protein–labeled sections using the ImageScope viewer (Aperio Technologies, Inc), creating a map of the histological section. Sections were carefully matched to corresponding MRI planes using cortical anatomy, ependymal lining, and WM lesions as landmarks.

**ANALYSIS**

In a first-pass, prospective analysis, the 2 MRI readers (E.W. and C.R.Z.) scored total lesion count and lesion count by anatomical type (types 1-4), blinded to histopathological findings. On subsequent comparison, a consensus was reached in cases of divergent lesion assessment.

Per tissue block, 2 to 5 histological sections were scored and cortical lesions were classified into anatomical subtypes. Finally, the subset of MRI sections corresponding to histological sections underwent a second retrospective review and lesions were scored against lesions defined by immunohistochemical labeling.
RESULTS

T2*GRE AND WHAT-TFE IMAGES IDENTIFY CORTICAL DEMYELINATION IN POSTMORTEM MS BRAIN TISSUE

We investigated the utility of very high-resolution 3-D T2*GRE and 3-D WHAT-TFE MRI sequences at 7 T for cortical lesion detection in postmortem brain slices from 3 patients with MS. Using the T2*GRE sequence, a total of 148 cortical lesions were detected in 10 brain slices, while the WHAT-TFE sequence showed 118 cortical lesions (Table 1).

Contrast-noise ratio measurements for both sequences are tabulated in Table 2. Contrast-noise ratios are higher with the WHAT-TFE sequence than with the T2*GRE sequence; however, adjustment for differences in spatial resolution reduces contrast-noise ratios in the WHAT-TFE sequence substantially. Thus, more lesions are visible on T2*GRE images owing to higher resolution, but they have lower contrast than on WHAT-TFE images. In particular, the high resolution on T2*GRE imaging allows one to discriminate the external band of Baillarger (line of Gennari) (Figure 2A). By tracing lesions throughout the whole thickness of brain slices, we found that cortical lesions could not always be assigned to 1 single lesion category according to the classification of types 1 through 4 by Bø et al. A number of lesions combined characteristics of type 1 and 3 or type 1 and 4 lesions, presumably because of expansion and eventual fusion of different lesion types (Figure 2B).

A total of 129 lesions (types 1-4) were identified in all histological sections. This is less than the total lesion count on MRI because the sections represent only a part of the entire block. Tabulating the prospective lesion count in the subset of MRIs that correspond to the histological sections, we found that 46% (T2*GRE) and 42% (WHAT-TFE) of histologically confirmed lesions were seen on prospective scoring. These scores improved to 93% and 82%, respectively, on retrospective scoring (Table 3). Lesion visibility was partially determined by size as all undetected lesions had a diameter of 1.1 mm or less (Figure 3). Several thinly myelinated cortical lesions were

Table 1. Cortical Lesions Detected on T2*GRE and WHAT-TFE Imaging, With Prospective Scoring Blinded to Histopathological Findings

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>T2*GRE</th>
<th>WHAT-TFE</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>40 (27)</td>
<td>27 (23)</td>
</tr>
<tr>
<td>2</td>
<td>25 (17)</td>
<td>13 (11)</td>
</tr>
<tr>
<td>1-3</td>
<td>14 (10)</td>
<td>21 (18)</td>
</tr>
<tr>
<td>3</td>
<td>42 (28)</td>
<td>19 (16)</td>
</tr>
<tr>
<td>1-4</td>
<td>15 (10)</td>
<td>26 (22)</td>
</tr>
<tr>
<td>4</td>
<td>12 (8)</td>
<td>12 (10)</td>
</tr>
<tr>
<td>Total</td>
<td>148 (100)</td>
<td>118 (100)</td>
</tr>
</tbody>
</table>

Abbreviations: T2*GRE, T2*-weighted gradient-echo; WHAT-TFE, white matter–attenuated turbo-field-echo.

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seen with both imaging modalities (Figure 4). These lesions could represent remyelinated lesions, although this has not been verified by electron microscopy.

**T2*GRE MAGNITUDE IMAGING IDENTIFIES IRON-LADEN MICROGLIA IN CORTICAL LESIONS**

Most cortical plaques as imaged by the T2*GRE sequence showed homogeneous, hyperintense signals on magnitude images (Figure 5A). Among the lesions, 23% displayed heterogeneous signal patterns, ie, mixed hyperintense and hyperintense signals, or more commonly, peripheral hypointense halos around a hyperintense lesion center (Figure 5C, G, and H). Hypointense rings occurred predominantly in leukocortical lesions but also in type 3 (subpial) and type 4 lesions. Correlating these images with tissue sections that were labeled with anti-CD68 antibody or stained with Perls (iron) stain, we demonstrated a close association of hypointense MRI signal with the presence of iron-rich microglia within or at the edge of active and chronic active lesions (Figure 5C-L).

Phase imaging of our tissue specimens produced signals of variable intensity and diminished GM-WM contrast. The example in Figure 5F shows a hyperintense rim.

**COMMENT**

This study shows that a high rate of cortical lesion detection can be achieved in postmortem tissue with ultra-high-field MRI using 3-D T2*GRE and 3-D WHAT-TFE sequences. Prospectively, 46% (T2*GRE) and 42% (WHAT-TFE) of cortical lesions were detected. These counts improved to 93% and 82%, respectively, with retrospective scoring, ie, after comparison with histological sections. The relatively low numbers on prospective counting reflect the moderate contrast-noise ratios in cortical lesions, which require observer training.

The contrast-noise ratios are comparable to those found by Geurts et al using proton density sequences at 4.7 T. However, because the spatial resolution at 7 T is higher than that in the study by Geurts et al, the cortical lesion contrast is significantly higher, which is reflected in the vastly improved lesion count (93% and 82% vs approximately 30% on retrospective scoring).

Another determinant for cortical lesion detection was lesion size as all undetected lesions had a smallest diameter of less than 1.1 mm. These factors might explain the relatively low detection rates of type 2 lesions (86% T2*GRE, 71% WHAT-TFE) and type 3 lesions (93% T2*GRE, 77% WHAT-TFE). Type 2 lesions are small and round, while type 3 lesions are thin and located in the upper cortical layers, which contain little myelin and thus produce less lesion contrast. Differences in sensitivity between WHAT-TFE and T2*GRE sequences presumably reflect differences in resolution (150 × 150 × 300 µm by the T2*GRE sequence vs 250 × 250 × 500 µm by the WHAT-TFE sequence).

A corollary finding is that by immunohistochemistry, approximately 20% of cortical lesions in our samples had features of more than 1 lesion type, presumably because of merging of 2 or more lesions. Detection of WM lesions was not addressed in this study, although hyperintense WM plaques were clearly evident with both sequences.

The second finding of this study is that hypointense rings on T2*GRE magnitude imaging correlate with iron-laden microglia present at the edge of chronic active lesions. Similar rings have been reported in WM lesions in patients with MS using T2*GRE magnitude imaging and, more prominently, phase imaging. Our study therefore suggests that hypointense rings in patients with MS represent activated microglia or macrophages and can thus be used to stage MS lesions by inflammatory activity in vivo.

Phase imaging in vivo is presumably better suited to detect iron than T2*GRE magnitude imaging. Phase images from our specimens showed diminished GM-WM contrast and lesion boundaries with variable appearance. Similar findings have been reported in WM of formalin-fixed tissue by Yao et al. This suggests that factors other than iron content contribute to the phase effect, warranting further studies into contrast mechanisms in phase images in formalin-fixed tissue.

The extended scan times feasible with postmortem tissue and the use of a knee coil both allow one to obtain a signal-noise ratio and resolution that cannot be reached in patient imaging. Thus, tissue imaging cannot be translated directly to in vivo imaging. Moreover, MRI tissue characteristics differ between formalin-fixed and live tissue; in particular, T1-weighted relaxation times are significantly shorter in formalin-fixed tissue, while proton density and T2*-weighted values are only slightly reduced. Nevertheless, T1-, T2-, and T2*-weighted and proton density measurements both in formalin-fixed tissue and in live tissue can be used to compute and optimize the expected contrasts in different MRI sequences. This will allow size threshold estimation for cortical lesion imaging in vivo. Similar contrast-resolution estimates for 1.5 T and 3 T may help to determine feasibility of cortical lesion imaging with optimized MRI pulse sequences at lower field strength. Because of the small size and low lesion contrast, high spatial resolution and image contrast as provided by ultra-high-field imaging may be a prerequisite for cortical lesion imaging. Specific se-
quences such as T2*-GRE and T1-weighted magnetization-prepared rapid-acquisition GRE have shown success with cortical lesion depiction in patients with MS. Our WHAT-TFE sequence is a variant of magnetization-prepared rapid-acquisition GRE with an inversion time that nulls WM signal to improve lesion detectability.

One advantage of inversion recovery TFE sequences is their lower radiofrequency power requirement at 7 T compared with standard spin-echo methods. Magnitude and phase T2*-GRE images show exquisite details in vivo of normal cortical anatomy as well as subtle lesion microstructure such as central, penetrating vessels within lesions and hypointense rings surrounding WM lesions.

A different approach for improving visualization of cortical lesions is 3-D double inversion recovery imaging. Double inversion recovery sequences can be acquired with a 1.5-T field strength and are thus easily applicable to patient studies. Cross-sectional and longitudinal studies have indeed shown that symptoms such as cognitive impairment and epilepsy are associated with increased cortical lesion load as detected by double inversion recovery. However, the sensitivity of double inversion re-

Table 3. Cortical Lesion Counts on T2*GRE and WHAT-TFE Imaging Stratified by Cortical Lesion Subtypes

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>Histological Lesions, No. (%)</th>
<th>Prospective Count, No. (% of Histological Lesions)</th>
<th>Retrospective Count, No. (% of Histological Lesions)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T2*GRE</td>
<td>WHAT-TFE</td>
<td>T2*GRE</td>
</tr>
<tr>
<td>1</td>
<td>36 (28)</td>
<td>17 (47)</td>
<td>19 (53)</td>
</tr>
<tr>
<td>2</td>
<td>28 (22)</td>
<td>9 (32)</td>
<td>8 (29)</td>
</tr>
<tr>
<td>3</td>
<td>57 (44)</td>
<td>29 (51)</td>
<td>20 (35)</td>
</tr>
<tr>
<td>4</td>
<td>8 (6)</td>
<td>4 (50)</td>
<td>7 (88)</td>
</tr>
<tr>
<td>Total</td>
<td>129 (100)</td>
<td>59 (46)</td>
<td>54 (42)</td>
</tr>
</tbody>
</table>

Abbreviations: T2*GRE, T2*-weighted gradient-echo; WHAT-TFE, white matter–attenuated turbo-field-echo.

a Prospective counting, ie, blinded to histopathological findings, was performed on the magnetic resonance images that match the histological sections.
b Retrospective counting compares lesions found on magnetic resonance imaging with those found by immunohistochemistry.
Discovery for cortical lesions has not been determined in a postmortem study and the number of cortical lesions in these in vivo studies is far lower than those reported in the histological literature. This is not necessarily indicative of poor sensitivity because the respective patient populations differ significantly with regard to disease duration. However, 3-D double inversion recovery sequences may be susceptible to flow-related artifacts as well as regional variations in GM signal intensity and may thus lead to false-positive lesion detection.24

The effect of cortical lesions on MS remains unclear. It is suspected that cortical lesions contribute to clinical symptoms that are seemingly related to cortical pathology such as cognitive impairment, depression, and seizures. Moreover, recent studies have highlighted that GM damage is a dominant pathological finding in progressive MS.25,26 The predominance in progressive MS as well as the absence of inflammatory markers suggest a mechanism for cortical lesion formation different from that in WM. The true impact of cortical lesions can be determined only through accurate and clinically feasible detection of cortical demyelination. Ultra–high-field MRI combined with 3-D T2*GRE and WHAT-TFE sequences shows high accuracy for cortical lesion detection in vitro. These findings bode well for future application of this technology for cortical lesion imaging in patients with MS.

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