ABSTRACT

BACKGROUND AND PURPOSE: At 7T MR imaging, T2*-weighted gradient echo has been shown to provide high-resolution anatomic images of gray matter lesions. However, few studies have verified T2*WI lesions histopathologically or compared them with more standard techniques at ultra-high-field strength. This study aimed to determine the sensitivity of T2WI and T2*WI sequences for detecting cortical GM lesions in MS.

MATERIALS AND METHODS: At 7T, 2D multiecho spin-echo T2WI and 3D gradient-echo T2*WI were acquired from 27 formalin-fixed coronal hemispheric brain sections of 15 patients and 4 healthy controls. Proteolipid-stained tissue sections (8 μm) were matched to the corresponding MR images, and lesions were manually scored on both MR imaging sequences (blinded to histopathology) and tissue sections (blinded to MR imaging). The sensitivity of MR imaging sequences for GM lesion types and white matter lesions was calculated. An unblinded retrospective scoring was also performed.

RESULTS: If all cortical GM lesions were taken into account, the T2WI sequence detected slightly more lesions than the T2*WI sequence: 28% and 16%, respectively (P = .054). This difference disappeared when only intracortical lesions were considered. When histopathologic information (type, location) was revealed to the reader, the sensitivity went up to 84% (T2WI) and 85% (T2*WI) (not significant). Furthermore, the false-positive rate was 8.6% for the T2WI and 10.5% for the T2*WI sequence.

CONCLUSIONS: There is no strong advantage of the T2*WI sequence compared with a conventional T2WI sequence in the detection of cortical lesions at 7T. Retrospectively, a high percentage of lesions could be detected with both sequences. However, many lesions are still missed prospectively. This could possibly be minimized with better a priori observer training.

ABBREVIATIONS: CNR = contrast-to-noise ratio; DIR = double inversion recovery; GML = gray matter lesion; WML = white matter lesion

Multiple sclerosis is traditionally regarded as a chronic inflammatory demyelinating disease of the white matter with a variable clinical course; primary-progressive or relapsing-remitting with possible conversion to secondary-progressive. Pathologic, immunologic, and imaging studies have confirmed that tissue damage in the gray matter is also a key component of the disease process.1-4 GM pathology occurs frequently, already early in the disease course, and explains cognitive and clinical disability better than white matter lesions.5,6 Nevertheless, visualizing these GM abnormalities has been challenging due to their small size, absence of inflammation, and partial volume effect from adjacent CSF and WM. The introduction of ultra-high-field MR imaging scanners and specific MR imaging pulse sequences has improved the detection of GM lesions due to a higher signal-to-noise ratio and better spatial resolution.7-9 7T T2*-weighted gradient-echo MR imaging has been shown to provide high-resolution anatomic images of GM lesions, and it has even been suggested that this sequence be used as the new criterion standard for GM lesion detection.10 It was reported to be 44% more sensitive than 1.5T MR imaging in detecting lesions with cortical involvement11 and up to 69% more sensitive than 3T double inversion recovery (DIR) imaging in detecting subpial lesions.10 Few groups have had the opportunity to study GM lesions that were visualized with 7T T2*WI in terms of histopathology. There-
Another study found that provides demonstration of tissue, and access to medical records was granted by the institutional ethics review board. However, the 2 sequences studied posed. Permission for performing postmortem examinations, use of their tissue and medical records for research purposes. All donors were registered at the Netherlands Brain Bank, Amsterdam, the Netherlands. All donors gave written informed consent for the use of their tissue and medical records for research purposes. Permission for performing postmortem examinations, use of tissue, and access to medical records was granted by the institutional ethics review board.

**Table 1: Demographic and neuropathologic data of subjects**

| Case No. | No* | Sex | Age (yr) | PMD (h:min)b | DD (yr) | MS Type | COD |
|----------|-----|-----|---------|-------------|--------|---------|-----|
| M5       |     |     |         |             |        |         |     |
| 1        | M   | 80  | 6:05    | 45          | SPMS   | Pneumonia |     |
| 2        | F   | 81  | 3:30    | 27          | PPMS   | Pneumonia |     |
| 3        | M   | 75  | 10:10   | 50          | NA     | Pneumonia |     |
| 4        | F   | 66  | 7:30    | 17          | NA     | Pulmonary hypertension |     |
| 5        | M   | 71  | 4:00    | 15          | SPMS   | Pulmonary carcinoma |     |
| 6        | F   | 54  | 6:00    | 16          | SPMS   | Liver cancer |     |
| 7        | M   | 63  | 4:30    | 25          | SPMS   | Pneumonia |     |
| 8        | M   | 78  | 3:00    | 33          | SPMS   | Euthanasia |     |
| 9        | M   | 59  | 5:00    | 21          | SPMS   | Euthanasia |     |
| 10       | M   | 56  | 10:10   | 13          | NA     | Suicide |     |
| 11       | F   | 56  | 8:25    | 32          | SPMS   | Pneumonia |     |
| 12       | F   | 54  | 3:30    | 31          | SPMS   | Heart failure |     |
| 13       | M   | 58  | 4:00    | 27          | SPMS   | Pneumonia |     |
| 14       | F   | 95  | 6:30    | 55          | SPMS   | Unknown |     |
| 15       | F   | 81  | 6:30    | 21          | SPMS   | Heart failure |     |

Control

|       |     |     |         |            |        |         |     |
|-------|-----|-----|---------|------------|--------|---------|-----|
| 20    | 4   | F   | 72      | >24:00     | –      | –       | Myocardial infarct |
| 21    | 3   | F   | 58      | <24:00     | –      | –       | Breast cancer |
| 22    | 3   | F   | 76      | <24:00     | –      | –       | Pneumonia |
| 23    | 2   | F   | 76      | <8:00      | –      | –       | Pneumonia |

Mean 70.5 ± 8.5

Note: PMD indicates postmortem delay; DD, disease duration since diagnosis; SPMS, secondary-progressive MS; PPMS, primary-progressive MS; COD, cause of death; NA, unavailable/unknown; –, not applicable.

The numbers indicate number of hemispheric sections included per case.

Control cases are not part of the rapid postmortem examination program and therefore have a longer PMD.

**MR Imaging**

Imaging was performed by using a 7T BioSpec USR70/30 imager (Bruker BioSpin MRI, Ettlingen, Germany), and a vendor-provided 8.6-cm-diameter radiofrequency transmit/receive coil (model 1P T12053V3). Each formalin-fixed brain section was placed into a rectangular plastic tissue container and immersed in 10% buffered formalin. Particular care was devoted to sequence optimization due to the effect of tissue fixation on sequence parameters. The MR imaging protocol included a 2D multiecho spin-echo T2*-weighted (TR/TE1/TE2/TE3 = 4000/19.1/38.2/57.3 ms; α = 90 and 180; averages = 6) and a 3D gradient-echo T2*-weighted (TR/TE = 25/12 ms; α = 5; averages = 16) sequence. All MR imaging sequences were acquired with an FOV of 100 × 80, matrix of 1000 × 800, and in-plane spatial resolution of 100 × 100 μm, with a section thickness of 1 mm.

Contrast-to-noise ratios (CNRs) were determined for the different MR image types in the MS samples on the basis of signal-intensity measurements in ROIs (ie, normal-appearing gray matter, GM lesions, normal-appearing white matter, WM lesions, formalin [noise]). The CNR between 2 tissue types was defined as |SI1 – SI2|/SD (noise). For T2WI, the TE of 19.1 ms was used for lesion detection and CNR calculation.

**Histology**

After MR imaging, the brain sections were cut in half to reveal the imaged plane and were embedded in paraffin. Eight-micrometer-thick sections were cut, mounted onto glass slides (Superfrost; VWR International, Leuven, Belgium), and dried overnight at 37°C. Sections were deparaffinized in a series of xylene, 100% alcohol (ethanol), 96% alcohol, and 70% alcohol and rinsed with 0.01-mol/L tris-buffered saline (pH, 7.8–8.0). Endogenous per-
oxidase activity was blocked by incubating the sections in tris-buffered saline with 0.3% H₂O₂ for 30 minutes. After this, the sections were rinsed with 0.01-mol/L phosphate-buffered saline (pH, 7.4). Staining was performed with antibodies against proteolipid protein (AbD Serotec, Oxford, UK) diluted in tris-buffered saline (1:500) containing 1% normal goat serum (Dako, Glostrup, Denmark) and stored overnight at 6°C. Immunolabeling was detected by incubating the sections in biotinylated goat antimouse (1:400; Vector Laboratories, Burlingame, California) and in Vectastain ABC (horseradish peroxidase, 1:200; Vector Laboratories) for 60 minutes at room temperature. Afterward, the sections were washed in 0.05-mol/L tris-hydrochloric acid (pH, 7.6). Peroxidase activity was demonstrated with 0.5-mg/mL 3,3′ diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, Missouri) in 0.01-mol/L tris-hydrochloric acid containing 0.03% H₂O₂ for 5 minutes, which led to a brown reaction product. Sections were counterstained with hematoxylin (Sigma) and mounted (dePex; BDH Chemicals, Poole, UK).

**Scoring, Classification, and Matching**

MR imaging lesions were manually marked on all T2*WI, and all T2WI with TE = 19.1 which had contrast similar to that of clinically used T2WI sequences. The MIPAV (National Institutes of Health, Bethesda, Maryland) application was used for manual prospective and retrospective lesion scoring. The MR imaging reader scoring was blinded to clinical information and histopathologic results. Lesions were scored throughout all of the MR imaging slices to avoid bias toward scoring within the sampled areas. A subset of images (n = 5 for each sequence) was rated by a second independent reader to ascertain the quality of scoring and calculate an intraclass correlation coefficient for each sequence.

Histopathologically, lesions were defined as areas of complete demyelination (lack of proteolipid protein) and were scored by a histopathologic reader blinded to the clinical and MR imaging data. GM lesions were scored and classified according to criteria described in Bø et al, 14 in which a distinction among 4 cortical lesion types is made. Type I lesions involve the deeper layers of the GM and the adjacent WM; type II lesions are small demyelinated lesions, often centered around blood vessels and confined within the cortex; type III lesions extend from the pial surface into the cortex, most often reaching to cortical layers 3 or 4. When these lesions involve the entire span of the cortex without entering the subcortical white matter, they are defined as type IV lesions. After MR imaging and histopathologic scoring, hemispheric tissue sections were matched to the corresponding MR imaging planes by using WM lesions and as many cortical anatomic landmarks as possible. After the blinded prospective scoring of the postmortem MR imaging and the tissue-to-MR imaging matching, histopathology scores were made available to the MR imaging readers and a second, retrospective, unblinded scoring was performed in consensus between the raters.

**Analysis of Data**

Histopathologic lesion count was considered the criterion standard. Therefore, prospective and retrospective sensitivity of MR imaging sequences for detecting lesions was determined by divid-
cause they did not fulfill the criterion of a “fully demyelinated lesion” (see “Materials and Methods”).

Contrast-to-noise ratios for the various tissue types are shown in Table 3. Although only descriptive, the T2WI sequence showed higher WM to white matter lesion (WML) CNR, which could account for the higher prospective sensitivity than T2*WI sequence (Table 2). Regarding the T2WI and T2*WI sequences, a paired samples t test showed no significant difference between the comparably low GM-GML CNRs.

**DISCUSSION**

In the current study, we have demonstrated that prospectively (ie, without knowledge of histopathology [location and type of lesions]), the standard T2WI sequence detected more cortical lesions than the T2*WI sequence (ie, 28% versus 16%, respectively), though this difference was not statistically significant. When only intracortical lesions were taken into account, this difference between sequences vanished completely. It also vanished when lesion location was revealed to the reader (retrospective scoring). An explanation for this slight prospective difference could be that the T2*WI sequence is more susceptible to global inhomogeneities such as tissue-to-formalin boundaries, leading to local T2* signal decay, which could hinder prospective lesion detection.

Retrospective detection of cortical lesions increased to 83% for the T2WI and 84% for the T2*WI sequence. When we focused on intracortical lesions, retrospective detection increased to 80% and 82%, respectively. This retrospective detection sensitivity is much higher than that in previous postmortem MR imaging studies at lower field strengths. Previous studies at 1.5T detected only 31% or 56% of intracortical lesions with a T2WI sequence and 29% with a DIR sequence. With a FLAIR sequence, 9%, 21%, or 71% of intracortical lesions were detected retrospectively. Compared with previous postmortem MR imaging studies at ultra-high-field (7T) strength, our T2WI and T2*WI retrospective detection rates are higher than the 67% found with R2* maps, comparable with the 82% found with WM-attenuated turbo field echo, and slightly lower than the 93% detected with T2*WL. However, prospective...
lesion detection in these studies varied between 42% and 48%, which is higher than our cortical detection rate of up to 28%. One explanation for this higher detection rate in other studies could be the type of lesions identified; most lesions found by Yao et al were the more easily detectable type I lesions, while most lesions in our sample were the more difficult detect type III lesions. Another explanation could be the higher CNR as found in the study by Pitt et al. Their T2*WI sequence had a GML-GM CNR of 3.4, while our study only had an average CNR of 1.7, making distinction between GML and surrounding GM more difficult. These differences could have led to a more optimal sequence for lesion detection by Pitt et al. Future studies should be performed to see how lesion heterogeneity and within-sequence differences influence lesion detection. As shown by the high retrospective lesion count, 7T MR imaging has greatly improved the possibility of detecting cortical or intracortical lesions. However, the challenge remains to actually detect them prospectively and in vivo.

Regardless of 7T MR imaging with increased signal-to-noise ratio (SNR) and spatial resolution, the number of prospectively detected cortical lesions on MR images remains low; in our study, up to 84% of cortical or intracortical lesions remained undetected. In another study at 7T, up to 57% were still missed. Lesion size has been found to affect the visibility of cortical lesions at both 1.5T and 7T. Furthermore, extensive cortical demyelination could hinder visibility of type IV lesions; when most of the cortex is affected, there is no normal-appearing gray matter present, making it difficult to differentiate areas of demyelination and normal-appearing gray matter (Fig 2). Perhaps quantitative MR imaging could provide additional information in these areas. Automated segmentation could be another option to aid cortical lesion detection, though this could be extremely challenging due to a lack of contrast in the cortex, especially in the upper layers where most cortical lesions are located. Nevertheless, retrospective lesion detection shows that it is possible to find cortical lesions on MR imaging when lesion location is (histopathologically) known, indicating that observer training is important and could dramatically increase future prospective sensitivity.

There were only 7 WMLs observed during histopathologic analysis. This seems low, but the coronal sections were sampled from more frontal regions of the brain, a preferential area for cortical pathology, but not far for WMLs, which are more frequently located periventricular.

Sequence parameters were optimized for the effect of fixation, SNR, and spatial resolution. Fixed tissue has a decrease in T2 signal, which leads to lower contrast and requires an increase in averages to achieve a reasonable SNR. This results in an increase in acquisition time, a methodologic limitation when trying to compare the results from this study with the in vivo setting. The sequences used can be optimized for the 7T in vivo setting, but identifying cortical lesions will remain especially challenging for smaller sized lesions. Another limitation in correlative studies between histopathology and MR imaging is matching tissue sections to MR images. Tissue sections were 8 μm, while MR images had a section thickness of 1 mm. However, accurate matching was made possible due to the many anatomic landmarks in the full-hemispheric sections used in this study.

Looking at the in vivo setting, DIR is reported to improve cortical lesion detection at 3T compared with 1.5T, while T2WI or FLAIR is not. In turn, a 75% increase in cortical lesion detection was found with 7T T2WI versus 3T T2WI. For T1 and FLAIR, this was even 91% and 238%, respectively. Another study found a 65% increase in cortical lesion detection with 7T T2*WI versus 3T DIR. The same research group also investigated how various lesion types contributed to physical and cognitive performance. They found that type III–IV lesions had the strongest relationship to physical disability. In turn, type I lesions and, to a lesser extent, type III–IV lesions had a relationship with cognitive performance. At 3T, DIR detected 538% more intracortical lesions than T2WI and 152% more intracortical lesions than FLAIR. This finding was supported by another study in which DIR detected 43% more cortical lesions than FLAIR. However, a study from Kilsdonk et al at ultra-high-field strength (7T) showed that FLAIR detected 89% more cortical lesions than DIR, and that DIR and T2WI obtained nearly identical mean cortical lesion counts (115 versus 116). This finding indicates that a sequence that may be optimal at a lower field strength (DIR at 3T) may lose its benefit at a higher field strength (7T), and vice versa: A sequence suboptimal at a lower field strength may have an advan-

### Table 3: Contrast-to-noise ratio (±SD)

|              | T2WI     | T2*WI   |
|--------------|----------|---------|
| WM-WML       | 12.07 (0.90) | 5.03 (1.68) |
| GM-GML       | 2.01 (0.74)  | 17 (1.37)   |
| GM-WM        | 7.5 (1.58)   | 4.96 (3.79) |

**Note:** WM-WML indicates white matter-to-white matter lesion CNR; GM-GML, gray matter-to-gray matter lesion CNR; GM-WM, gray matter-to-white matter CNR.
tage over other sequences at higher field strengths (FLAIR or T2* at 7T). It would be useful if future studies could elucidate which sequences have optimal lesion detection sensitivities at which field strength. Phase-sensitive inversion recovery looks promising at 3T with a 307% increase over DIR,29 but how does it perform at 7T? How do FLAIR, T2WI, and T2*WI compare at 7T in 1 comparative study?

**CONCLUSIONS**

Our findings suggest that at 7T, T2WI and T2*WI sequences are equally capable of detecting up to 83%–84% of cortical lesions in postmortem MS samples. However, many lesions are still missed prospectively. With observer training, the expectation is that not only the “tip” but a large part of the proverbial “iceberg” of GM lesions may be uncovered.

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