

Postmortem verification of MS cortical lesion detection with 3D DIR

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ABSTRACT

Objective: To assess the sensitivity and specificity of 3D double inversion recovery (DIR) MRI for detecting multiple sclerosis (MS) cortical lesions (CLs) using a direct postmortem MRI to histopathology comparison.

Methods: Single-slab 3D DIR and 3D fluid-attenuated inversion recovery (FLAIR) images of 56 matched fresh brain samples from 14 patients with chronic MS were acquired at 1.5 T. The images of both sequences were prospectively scored for CLs in consensus by 3 experienced raters who were blinded to histopathology and clinical data. Next, CLs were identified histopathologically and were scored again on 3D DIR and 3D FLAIR (retrospective scoring). CLs were classified as intracortical or mixed gray matter (GM)-white matter lesions. Deep GM lesions were also scored. False-positive scores were noted and, from this, specificity was calculated.

Results: We found a sensitivity for 3D DIR to detect MS CLs of 18%, which is 1.6-fold higher than 3D FLAIR (improves to 37% with retrospective scoring; 2.0-fold higher than 3D FLAIR). We detected mixed GM-white matter lesions with a sensitivity of 83% using 3D DIR (65% sensitivity for 3D FLAIR), which improved to 96% upon retrospective scoring (91% for 3D FLAIR). For purely intracortical lesions, 3D DIR detected more than 2-fold more than 3D FLAIR (improved to >3-fold upon retrospective scoring). The specificity of 3D DIR to MS CLs was found to be 90%.

Conclusions: In this postmortem verification study, we have shown that 3D DIR is highly pathologically specific, and more sensitive to CLs than 3D FLAIR in MS. *Neurology*® 2012;78:302-308

GLOSSARY

BSA = bovine serum albumin; **CL** = cortical lesion; **DIR** = double inversion recovery; **FLAIR** = fluid-attenuated inversion recovery; **GM** = gray matter; **MS** = multiple sclerosis; **NEX** = number of excitations; **PBS** = phosphate-buffered saline; **PLP** = proteolipid protein; **TE** = echo time; **TI** = inversion time; **TR** = repetition time; **WM** = white matter.

Cortical lesions (CLs) are thought to contribute significantly to disease severity in multiple sclerosis (MS),¹⁻⁵ and dominate disease pathology in the progressive phase.⁶ Therefore, reliable in vivo detection of CLs is crucial.

Conventional MRI pulse sequences were found to largely miss cortical MS lesions^{7,8} and even with the use of newer MRI techniques such as fluid-attenuated inversion recovery (FLAIR),^{7,9-11} CL detection remained suboptimal. With the introduction of double inversion recovery (DIR) MRI, which simultaneously suppresses the signals from white matter (WM) and CSF,¹²⁻¹⁴ a substantial increase of MRI-detected CLs in patients with MS was found when compared to more conventional sequences.^{15,16} Subsequently, several cross-sectional and longitudinal DIR studies showed that CLs are associated with increased clinical, especially cognitive, impairment in MS.^{4,17-21}

A drawback of DIR as an imaging technique is its poor signal-to-noise ratio and the presence of flow and pulsation artifacts in 2D sequences.^{12-14,22} An improvement can be obtained with 3D single-slab methods, although the signal-to-noise ratio generally remains low.²³ Together

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Study funding: The VUmc MS Center Amsterdam receives a program grant from the Dutch MS Research Foundation (Voorschoten, the Netherlands), grant no. 09-358d.

Disclosure: Author disclosures are provided at the end of the article.

Table 1 Patient demographics

Patient/sex/ age, y	Postmortem delay, h:min	Disease duration, y	Disease type	Cause of death
1/F/47	4:25	16	SPMS	Rectum carcinoma
2/M/50	5:25	17	PPMS	Pulmonary carcinoma
3/F/66	6:00	23	SPMS	Unknown
4/M/55	6:20	32	SPMS	Respiratory insufficiency
5/M/61	9:15	19	SPMS	Euthanasia
6/F/40	9:00	9	RRMS	Hypovolemic shock
7/M/45	7:45	19	SPMS	Cardiac arrest
8/M/72	7:55	13	SPMS	Pneumonia
9/M/50	9:30	24	SPMS	Unknown
10/M/76	7:35	44	SPMS	Cardiovascular accident
11/M/44	12:00	14	PPMS	Pneumonia
12/M/66	7:30	26	PPMS	Unknown
13/M/57	7:55	25	SPMS	Euthanasia
14/F/88	7:55	25	SPMS	Cardiorespiratory insufficiency

Abbreviations: SPMS = secondary progressive multiple sclerosis.

with regional variations in gray matter (GM) signal intensity²³ this may introduce difficulties when scoring cortical MS lesions. Recently, international consensus recommendations for CL scoring with 3D DIR were introduced,²⁴ but sensitivity and pathologic specificity of 3D DIR have never been formally assessed by comparison to the gold standard of histopathology.

In the current study we aimed to verify CL scoring on postmortem 3D DIR images by directly comparing them to histopathology. This way, sensitivity and specificity of 3D DIR as a technique could be determined.

METHODS Patients and autopsy. For this study, 40 brain slices of 14 patients with chronic MS were studied after rapid autopsy. Patients' characteristics are shown in table 1. As part of the MS Center Amsterdam autopsy protocol, areas of interest are generally sampled from a maximum of 5 coronally cut brain slices, under guidance of postmortem T2-weighted MRI.²⁵ As T2-weighted scans are usually not helpful in detecting GM lesions,⁷ GM areas of interest were selected randomly from the slices for the current study. A total of 60 cortical areas and 8 deep GM areas were selected and used for further histopathologic examination.

Standard protocol approvals, registrations, and patient consents. Ethics approval was obtained from the institutional ethics review board. Prior to death, all donors were registered at the Netherlands Brain Bank, Amsterdam, the Netherlands, and all donors gave written informed consent for the use of their tissue and medical records for research purposes.

Postmortem MRI. Single-slab 3D FLAIR images (repetition time [TR]/echo time [TE]/inversion time [TI]/number of excitations [NEX] 6,500/355/2,200/1; echo train length 191; measured voxel size $1.1 \times 1.1 \times 1.3 \text{ mm}^3$) and 3D DIR images (TR/TE/TI1/TI2/NEX 6,500/355/2,350/350/1; echo train

length 191; measured voxel size $1.1 \times 1.1 \times 1.3 \text{ mm}^3$) of selected brain slices were acquired using a whole body 1.5 T magnetic resonance system (Sonata and Avanto, Siemens Medical Systems, Erlangen, Germany) by using a standard circularly polarized head coil (Sonata) or a 12-channel phased-array head coil (Avanto).

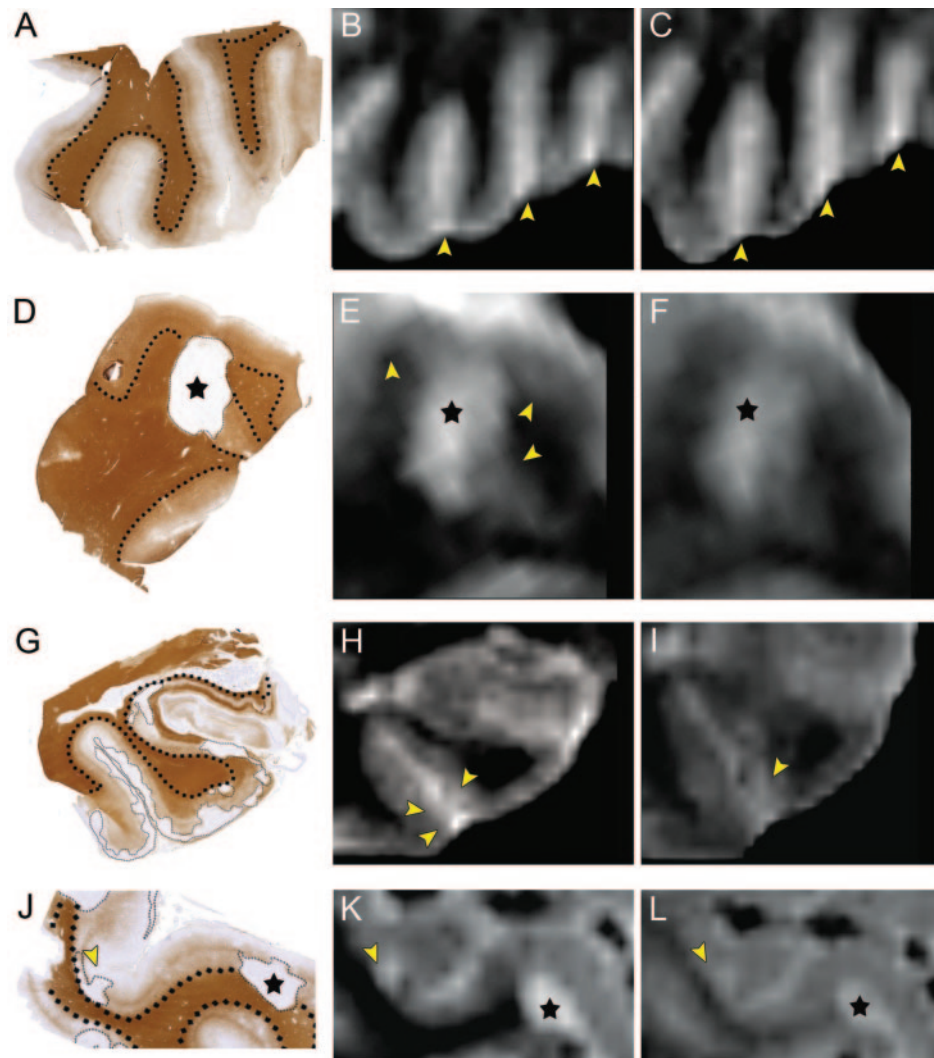
Histopathology and immunohistochemistry. After MRI, we selected a total of 68 tissue samples containing GM. The samples were fixed in 10% formalin and subsequently embedded in paraffin. Of these samples, 5- μm -thick sections were cut, mounted onto glass slides (Superfrost, VWR international, Leuven, Belgium), and dried overnight at 37°C. Sections were deparaffinized in a series of xylene, 100% ethanol, 96% ethanol, 70% ethanol, and water. Endogenous peroxidase activity was blocked by incubating the sections in methanol with 0.3% H_2O_2 for 30 minutes. After this, the sections were 3×10 minutes rinsed with 0.01 mol/L phosphate-buffered saline (PBS, pH 7.4). Then, sections were incubated with antiproteolipid protein (PLP; mouse IgG2a; 1:3,000; Serotec, Oxford, UK) diluted in PBS containing 1% bovine serum albumin (BSA; Roche Diagnostics, Mannheim, Germany) for 1 hour. Bound primary antibodies were detected using EnVision horseradish peroxidase complex (DAKO Cytomation, Glostrup, Denmark) and 3,3'-diaminobenzidine-tetrahydrochloridedihydrate (DAB) was used as a chromogen. Sections were counterstained with hematoxylin.

Matching and analysis. Cortical and deep GM lesions were scored (in consensus by A.S., S.D.R., and J.J.G.G.) on all 3D DIR and 3D FLAIR images which were viewed in a randomized fashion, and readers were blinded to histopathology and clinical information (i.e., prospective scoring). To avoid bias toward scoring in the sampled areas, CLs were assessed throughout the entire MRI of the brain slices, and thus before matching of selected tissue samples to the postmortem MRI. CLs were defined based on prior experience with scorings of cortical GM lesions in *in vivo* studies using different magnetic field strengths,^{4,16,26,27} and was consistent with the recently published consensus recommendations.²⁴ Among other points, these guidelines offer a scoring strategy to avoid mistaking CLs for cortical vasculature or artifacts caused by magnetic field inhomogeneities.

For the histopathologic scoring, we classified CLs as mixed WM-GM lesions (type I lesions) or purely intracortical lesions. Intracortical lesions included type II lesions (small, round intracortical lesions), type III or subpial lesions, and type IV lesions, which affect the entire width of the cortex.²⁸ The pathology reader (E.-J.K.) scored cortical lesion types and numbers in PLP-stained tissue sections and was blinded to MRI and clinical data. Deep GM lesions were also scored.

After separate prospective MRI and histopathology scorings, PLP-stained tissue sections were carefully matched to the corresponding plane of the 3D DIR and 3D FLAIR images. Matching was performed as described previously.²⁹ For an example of successfully matched tissue samples, see figure 1. After the blinded, prospective scoring of the postmortem MRI and the tissue-to-MRI matching, only those lesions that were present in brain areas sampled at autopsy were taken into account, and were used for further (retrospective) analysis. After histopathology scores had been made available to the MRI readers, a second, retrospective, unblinded scoring was performed, within the same areas from which tissue was sampled at autopsy. The sensitivity of the MRI sequences for detecting GM lesions was determined by dividing the number of lesions scored in the prospective or retrospective ratings by the number of lesions assessed on histopathology, times 100%. The specificity of the different MRI

Figure 1 Examples of postmortem MRI at 1.5 T, with corresponding histopathology



(A, D, G, J) Proteolipid protein (PLP) stained tissue sections; dotted lines indicate borders between white and gray matter; cortical lesions are circled by thin black lines. (B, E, H, K) Postmortem 3D double inversion recovery (DIR) images corresponding with the tissue sections. (C, F, I, L) Corresponding 3D fluid-attenuated inversion recovery (FLAIR) images. (A-C) Multiple sclerosis (MS) cortex with rather inhomogeneous signal intensity on MRI, but without any demyelinated lesions. The bright signal indicated by the arrowheads (B, C) is caused by blood and other fluid within the sulci, which should not be mistaken for subpial (type III) cortical pathology. (D-F) Mixed gray-white matter (type I) lesion (asterisk), which is seen on both 3D DIR and 3D FLAIR images. However, the gray matter border (arrowheads in E) is often easier identified on 3D DIR (E) as compared to 3D FLAIR (F). (G-I) Subpial (type III) cortical lesions (indicated by thin line in G and arrowheads in H and I) are slightly more conspicuous on 3D DIR (H) than on 3D FLAIR (I). (J-L) Mixed gray-white matter (type I) lesion (asterisks). Arrowhead in J-L: an intracortical lesion, which was prospectively scored on 3D DIR (K) and only retrospectively (i.e., with knowledge of histopathology) on 3D FLAIR (L).

sequences was determined as follows: $100\% - \left(\frac{\text{the number of false-positive scorings, i.e., hyperintensities on MRI without a corresponding lesion in histopathology}}{\text{false-positives} + \text{total number of true CLs detected with 3D DIR or 3D FLAIR}} \times 100\% \right)$. To assess relative gain or loss of lesions detected on 3D DIR vs 3D FLAIR, relative comparisons of lesion counts on these sequences were expressed as percentages, i.e., $\left(\frac{\text{lesions detected by 3D DIR} - \text{lesions detected by 3D FLAIR}}{\text{lesions detected by 3D FLAIR}} \times 100\% \right)$. Statistical analyses were performed using SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL).

RESULTS In total, we sampled 68 tissue samples containing GM under guidance of postmortem MRI for

further histopathologic analysis. Of these samples, we discarded 12 due to suboptimal matching with MRI (resulting from tissue processing, i.e., distortion and dehydration of the tissue or from a priori obvious partial volume artifacts), resulting in a final set of 56 samples of 14 patients with chronic MS. Of this final set, 8 samples were deep GM and 48 samples contained cortical GM.

Results of histopathology and MRI ratings are shown in table 2 and figure 1. In total, we identified 211 GM lesions on the PLP-stained tissue sections, consisting of 175 purely intracortical lesions (types

Table 2 Comparison of gray matter lesion scores between 3D DIR and 3D FLAIR, and PLP-stained tissue sections^a

Histopathology		Prospective rating MRI		Retrospective rating MRI	
Lesion type	Lesion count	3D FLAIR	3D DIR	3D FLAIR	3D DIR
I	23	15 (65.2)	19 (82.6)	21 (91.3)	22 (95.7)
II	61	5 (8.2)	5 (8.2)	6 (9.8)	10 (16.4)
III	103	0 (0)	7 (6.8)	6 (5.8)	34 (33.0)
IV	11	2 (18.2)	4 (36.4)	3 (27.3)	7 (63.6)
II-IV	175	7 (4)	16 (9.1)	15 (8.6)	51 (29.1)
DGM	13	1 (7.7)	1 (7.7)	5 (38.5)	4 (30.8)
Total	211	23 (10.9)	36 (17.1)	41 (19.4)	77 (36.5)

Abbreviations: DGM = deep gray matter; DIR = double inversion recovery; FLAIR = fluid-attenuated inversion recovery; PLP = proteolipid protein.

^a Numbers in parentheses indicate the percentage of MRI-detected lesions as compared to histopathology (i.e., the pathologic sensitivity).

II–IV), 23 mixed WM-GM lesions (type I), and 13 deep GM lesions. Prospectively, we were able to detect 35 of the in total 198 CLs with 3D DIR MRI. As such, the sensitivity of 3D DIR for CL detection was 18%, which is 1.6-fold higher than the sensitivity of 3D FLAIR. Retrospective scoring improved the sensitivity of 3D DIR for CL detection to 37%, which is 2.0-fold higher than 3D FLAIR. The pathologic specificity for 3D DIR was 90% and for 3D FLAIR 81%. Those scored hyperintensities that were discarded as a GM lesion after comparison with histopathology (i.e., false-positives) later appeared to be explained by either sulci with blood and fluid that had been misinterpreted for superficial lesions in the prospective scoring or by juxtacortical lesions that had been mistaken for type I (mixed WM-GM) lesions.

The advantage of 3D DIR compared to 3D FLAIR was most evident for the purely intracortical lesions (type II–III–IV lesions; see panels H, I, K, and L of figure 1), showing a gain of 129% in the prospective scoring (i.e., 9 lesions more), which increased to 240% (i.e., 36 lesions more) in the retrospective scoring. Note that although the sensitivity for detecting intracortical lesions is enlarged with 3D DIR compared to 3D FLAIR, the majority of the CLs are still missed (figure 2). Mixed (type I) lesions were detected with a slightly higher sensitivity using 3D DIR (83% sensitivity) when compared to 3D FLAIR (65% sensitivity) in the prospective rating, and reached almost equal numbers for 3D DIR and 3D FLAIR in the retrospective scoring (96% sensitivity with 3D DIR vs 91% with 3D FLAIR). In terms of both prospective and retrospective detection of deep GM lesions, 3D DIR showed similar sensitivity compared to 3D FLAIR, confirming previous *in vivo* results.²⁷

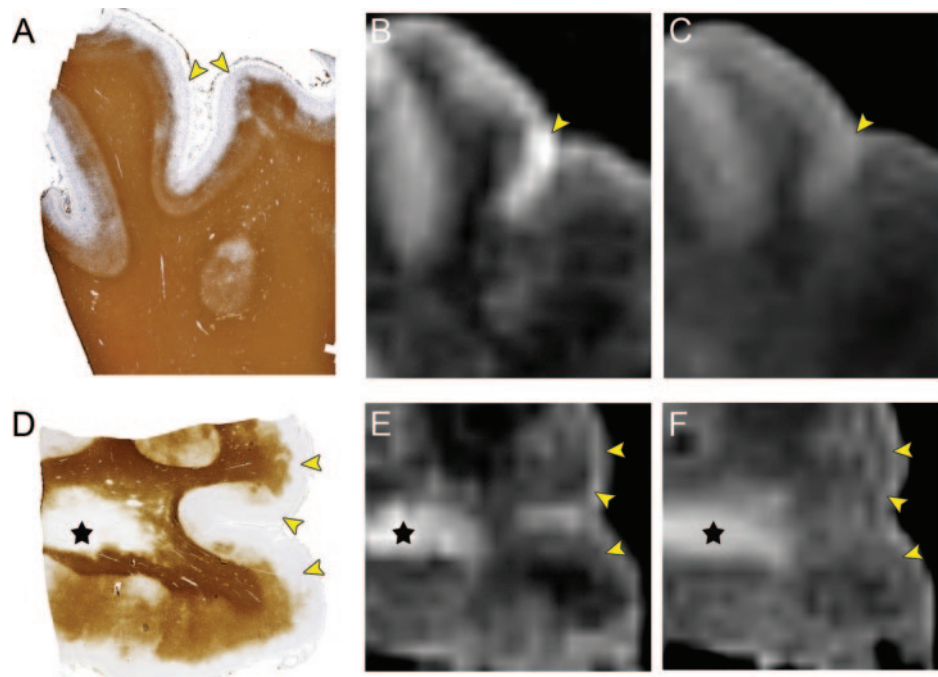
DISCUSSION Postmortem verification of 3D DIR hyperintensities in the GM of patients with MS has long been unavailable. In the current study, we demonstrated that although 3D DIR does not detect most GM lesions (especially not purely intracortical lesions), it has excellent pathologic specificity and higher sensitivity compared to 3D FLAIR (i.e., a relative gain of 3D DIR over 3D FLAIR in the detection of purely intracortical lesions of up to 240%).

Naturally, the scoring criteria and the images used to define lesions in the GM influence the eventual number of scored lesions. Hence, the results of the present study are especially true for the 3D DIR protocol and the scoring criteria used here. However, for CL identification in the current study, we followed recently proposed CL scoring guidelines of an international panel of experts.²⁴ Hyperintensities were not excessively scored as CLs, as is proven, e.g., by the low number of false-positive scorings (reflected in high specificity) in our study. The apparent distinction in CL numbers scored prospectively and retrospectively also shows that our scoring was conservative. Prospectively, especially type II intracortical and type III subpial lesions were missed (92% and 93%, respectively), and a considerable portion (more than 2 thirds) of all intracortical lesions were still missed when scored retrospectively.

To explain why some CLs are better visible than others, it is important to understand which specific properties of CLs determine their contrast and therefore their relative (in)visibility on MRI. Several factors are known to be responsible for the low contrast between CLs and surrounding GM, including the generally noninflammatory characteristics of cortical lesions (i.e., no complement deposition, gliosis, or blood–brain barrier disruption), the intrinsically low myelin density in the upper cortical layers, and the small size of CLs.^{30–33} The 3D DIR and 3D FLAIR sequences that were used for the current postmortem study are also used *in vivo*, and image contrasts are comparable. However, a difficulty in the postmortem setting is that additional artifacts caused by blood and water in the sulci may hamper CL detection. As such, it might well be that the sensitivity of 3D DIR and 3D FLAIR for detecting CLs is slightly higher in the *in vivo* setting, where these artifactually high signal intensities are adequately suppressed by the inversion pulses. However, the current results cannot lend sufficient force to this expectation.

Unfortunately, as a result of the consensus approach for scoring of CLs that was adopted in this study, an interrater consistency score could not be provided. Furthermore, as sequential imaging on the 2 scanner systems used in this study was not possible due to time constraints and for reasons of decaying

Figure 2 Two examples of cortical lesions (CLs) that were not scored on 3D double inversion recovery (DIR) and 3D fluid-attenuated inversion recovery (FLAIR) in the prospective scorings and were also not discriminated in the retrospective phase for different reasons



(A, D) Proteolipid protein (PLP) stained tissue sections. (B, E) postmortem 3D DIR images corresponding with the tissue sections. (C, F) Corresponding 3D FLAIR images. (A) PLP staining indicates an extensive subpial (type III) CL involving the superficial layers of the cerebral cortex (arrowheads); (B) corresponding 3D DIR image indicates a hyperintense area in the cortex (arrowhead) that was not scored as a lesion due to nuisance of the high (artifactual) signal produced by fluid in the sulcus (see also figure 1, A through C, and text). (D) PLP-stained tissue section showing an extensive subpial CL, in some areas affecting all layers of the cerebral cortex; despite this extensive demyelination, the lesion was not scored on the MRI (E, F) during prospective and retrospective scorings, because the subtly increased signal intensity on both 3D DIR and 3D FLAIR throughout the entire cortex (indicated by arrowheads) made the distinction between the signal of the lesion and that of normal cortex nearly impossible. White matter lesions were always readily visible on both MRI sequences (asterisks).

tissue quality, putative effects of the different scanner types on the numbers of detected CLs were not explored. These issues should be regarded as limitations of the current work, and will need to be investigated in future studies. Ongoing and future imaging of patients with MS at higher magnetic field strengths may further increase the sensitivity for the detection of GM lesions.³⁴ This has already been shown by in vivo studies using 3D DIR MRI at 3 T²⁷ and T2*-weighted imaging at 7 T.^{35,36} However, whether imaging techniques at higher magnetic field strengths also show high(er) pathologic specificity remains to be determined. With 3D DIR being increasingly used for CL detection in MS, and consensus recommendations²⁴ and postmortem verification of this technique now being available, the need for a standardized acquisition protocol also becomes more pressing, as comparison of CL scores between centers will otherwise remain difficult.

The current postmortem study is the first to verify CLs as scored on 3D DIR in the postmortem setting, by direct comparison to histopathology. It was

shown that single-slab 3D DIR has excellent pathologic specificity and a higher sensitivity than 3D FLAIR in detecting CLs in patients with MS. The latter confirms previous in vivo data.¹⁶ These findings now further establish the usefulness of 3D DIR for the MS clinical and research setting, and may be used to further fine-tune the discussion revolving around the imaging of CLs. Specifically, other MRI techniques (e.g., T1-based, phase-sensitive inversion recovery techniques) may now be further investigated to determine their sensitivity for CL detection in MS relative to 3D DIR, and future protocols might also explore the added value of combining sequences to optimally visualize lesions in the GM of patients with MS.

AUTHOR CONTRIBUTIONS

A. Seewann: data collection, study design, and writing the paper. E.-J. Kooi: data collection, study design, and writing the paper. S.D. Roosendaal: data collection and study design. P.J.W. Pouwels: study design, intellectual contributions, and writing the paper. M.P. Wattjes: study design, intellectual contributions, and writing the paper. P. van der Valk: study design, intellectual contributions, and writing the paper. F. Barkhof:

study design, intellectual contributions, and writing the paper. C.H. Polman: study design, intellectual contributions, and writing the paper. J.J.G. Geurts: data collection, study design, and writing the paper, guarantor of study.

ACKNOWLEDGMENT

The authors thank the Netherlands Brain Bank (Dr. I. Huitinga) and the autopsy team of the MS Center Amsterdam for providing the high-quality brain material with ultrashort postmortem delay, as well as the postmortem MRI data used for this study; and Dr. Jeroen Beliën (Department of Pathology, VU University Medical Center), who expertly digitized tissue sections for image analysis.

DISCLOSURE

Dr. Seewann reports no disclosures. E.-J. Kooi receives research support from the Dutch MS Research Foundation. Dr. Roosendaal receives research support from the MS Research Foundation. Dr. Pouwels reports no disclosures. Dr. Wattjes serves on the speakers' bureaus of and has received speaker honoraria from Bayer Schering Pharma, Janssen, and Biogen Idec. Dr. van der Valk reports no disclosures. Dr. Barkhof serves on scientific advisory boards for Lundbeck Inc., Bayer Schering Pharma, sanofi-aventis, UCB, Novartis, Biogen Idec, BioMS Medical, Merck Serono, and GE Healthcare; serves on the editorial boards of *Brain*, the *Journal of Neurology*, *Neurosurgery and Psychiatry*, *European Radiology*, the *Journal of Neurology*, and *Neuroradiology*; has received speaker honoraria from Novartis, Merck Serono, and BioClinica; serves as a consultant for sanofi-aventis, UCB, Novartis, Biogen Idec, BioMS Medical, MediciNova, Inc., and GE Healthcare; and receives research support from the Dutch MS Research Foundation. Dr. Polman serves on scientific advisory boards for and has received funding for travel and speaker honoraria from Actelion Pharmaceuticals Ltd, Biogen Idec, Bayer Schering Pharma, GlaxoSmithKline, Teva Pharmaceutical Industries Ltd., Merck Serono, Novartis, and UCB, Roche; serves on the editorial boards of *Lancet Neurology* and *Multiple Sclerosis*; and receives research support from Biogen Idec, Merck Serono, Novartis, UCB, European Community Brussels, and MS Research Foundation Netherlands. Dr. Geurts serves on scientific advisory boards for the Dutch MS Research Foundation and Merck Serono, and serves on the editorial board of *MS International*. He has received speaker honoraria from Merck Serono, Biogen Idec, and Teva Pharmaceuticals.

Received April 12, 2011. Accepted in final form August 3, 2011.

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