Can MS lesion stages be distinguished with MRI? A postmortem MRI and histopathology study

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Abstract In multiple sclerosis (MS), a histopathological distinction is made between different stages of white matter (WM) lesions. These lesions are characterized as preactive, active, chronic active or chronic inactive, depending on the degree of microglia activation and degree of demyelination. The different lesions are not distinguishable on conventional magnetic resonance imaging (MRI) scans at standard clinical field strengths, but might be distinguished using more advanced, quantitative MRI methods, such as T1 relaxation time (T1-RT) mapping. To investigate this, postmortem brain material from 20 MS patients was investigated, using both T1-RT MRI at 1.5T and histopathology. The brain material contained a total of 9 preactive, 18 active, 30 chronic active and 14 chronic inactive lesions, as well as 38 areas of normal appearing WM (NAWM). Our results show that, at 1.5T, T1-RT qMRI can only distinguish between categories NAWM/preactive, active and chronic WM lesions. Advanced imaging at standard field strengths, such as conventional imaging measures, is therefore insufficient to differentiate the WM lesions in MS, and higher field strengths may be required to achieve better pathological differentiation of these lesions.

Keywords Multiple sclerosis · MRI · Histopathology · Postmortem · Lesions · White matter

Introduction

Multiple sclerosis (MS) is an inflammatory and demyelinating disease of the central nervous system (CNS). Pathologically, a distinction can be made between different stages of white matter (WM) lesions that may be characterized as preactive, active, chronic active or chronic inactive, depending on their degree of microglia activation, adaptive immune response and demyelination [1]. Preactive lesions still have normal myelin density and morphology, but already show clusters of activated microglia. Active lesions show sharply bordered demyelination with myelin-laden macrophages. In chronic active lesions, macrophages have migrated to the rim of the lesion, leaving the center fully demyelinated and hypocellular. In chronic inactive lesions, there is complete demyelination; microglia and macrophages are no longer present. Conventional magnetic resonance imaging (MRI) techniques, such as T2-weighted MRI, are highly sensitive to MS WM lesions [2, 3] but do not provide information on the aforementioned pathological heterogeneity. However, for clinical purposes, it would be extremely useful if this pathological distinction could be made in vivo. This way, an inflammatory profile of MS patients could be more accurately described; the clinical impact of these different lesion stages, as well as their development over time, their occurrence in different patient and disease stages and their responsiveness to therapy could be monitored. Currently,
the technique of choice for visualizing active inflammatory WM lesions in vivo requires the administration of intravenous contrast agents. Quantitative MRI (qMRI) techniques, such as quantitative T1 relaxation time (RT) mapping, have shown to be both sensitive and more pathologically specific [4–7]. T1-RT correlates with myelin content and axonal count, which are both decreased in lesions compared to NAWM, and axonal count differs between different lesion stages [8, 9]. T1-RT mapping should therefore have the potential to detect inflammatory lesions at earlier stages and with more subtle pathology, which are now only evident postmortem. Use of such advanced MRI techniques could then improve clinical correlations of MRI-detectable abnormalities in vivo, as these correlations are generally low when using conventional techniques [10]. Therefore, the current study used advanced postmortem MRI and histopathology correlation to investigate whether T1-RT mapping can be used to distinguish the different stages of WM demyelination in MS.

Methods

Patients and autopsy

Coronal brain sections of 20 patients with MS (11 females, 9 males, mean age at autopsy 63.6 ± 11.5 years, mean disease duration 26.2 ± 15.3 years) were obtained after rapid autopsy (mean postmortem delay 8 h 21 min). Table 1 provides the demographic and neuropathological details of the donors. Autopsy procedure and tissue sampling followed the MS Center Amsterdam autopsy protocol which has been described previously [11]. Briefly, for each patient, five 10-mm-thick coronal hemispheric brain sections were cut and subjected to MR imaging. WM abnormalities visible on T2-weighted imaging were sampled.

MRI protocol and qMRI maps

The postmortem brain slices were scanned according to our autopsy protocol [11, 12], using a whole body 1.5T magnetic resonance system (Sonata and Avanto, Siemens Medical Systems, Erlangen, Germany) with a standard circularly polarized head coil (Sonata) or a 12-channel phased-array head coil (Avanto). Conventional PD/T2-weighted images were acquired (TR/TE1/TE2: 2500/85/24 ms, in-plane resolution 0.5 mm × 0.5 mm, slice thickness 4 mm), centered in the middle of the slice and parallel to the coronal surface. For T1 mapping, six sets of images were acquired using a 3D gradient echo sequence (3D-FLASH; TR/TE: 20/4 ms; in-plane resolution 1 mm × 1 mm, slice thickness 4 mm), covering the same volume as the PD/T2-weighted images. Flip angles were 2°, 5°, 10°, 15°, 20° and 25°, respectively. For B1-mapping, five sets of images were acquired (TR/TE: 20/4 ms, in-plane resolution 2 mm × 2 mm, slice thickness 4 mm). Flip angles were 140°, 160°, 180°, 200° and 220°, respectively.

Image analysis

Pixel-by-pixel T1 calculations were performed with B1 correction as described by Venkatesan et al. [13]. Briefly, B1 maps were generated from the image volumes with nominal flip angles between 140° and 220° by determining the ratio between the true and nominal flip angle from the signal zero crossing that occurs at a true flip angle of 180°. Subsequently, T1 was determined for each pixel through a nonlinear least squares fit by using the image and the calculated B1 map [13].

Histopathology and immunohistochemistry

After MRI, the tissue blocks were fixed in 10% formalin, routinely processed and embedded in paraffin. Subsequently, 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% H2O2 for 30 min. After this, the sections were rinsed with 0.01 mol/L phosphate-buffered saline (PBS, pH 7.4). Staining and immunohistochemistry were performed on adjacent sections with antibodies against the following targets: microglia/macrophages (anti-HLA-DR, clone LN3) and proteolipid protein (PLP; Serotec, Oxford, UK). Bound primary antibodies were detected using EnVision method (DAKO Cytomation, Glostrup, Denmark) and 3,3′diaminobenzidine-tetrahydrochloride dihydrate (DAB) was used as a chromogen. Sections were counterstained with hematoxylin and mounted (Depex, BDH; Poole, UK).

Scoring, classification and matching

WM lesions were scored by an experienced pathological examiner (PvdV) and classified according to the van der Valk and De Groot criteria [11] into preactive, active, chronic active and chronic inactive lesions. Preactive lesions show myelin and clusters of activated microglia. Active lesions show sharply bordered demyelination with macrophages. Chronic active lesions show macrophages at the rim of the lesion, and in chronic inactive lesions microglia and macrophages are no longer present. In total, 71 WM lesions were selected: 9 preactive, 18 active, 30
Table 1 Demographic and neuropathology data of patients

| Patient | Sex | Age | DD (years) | PMD (h:min) | Scanner | MS type | Cause of death |
|---------|-----|-----|------------|-------------|---------|---------|----------------|
| 1       | F   | 44  | 8          | 10:15       | 1       | PPMS    | Heart failure  |
| 2       | M   | 63  | 24         | 7:05        | 1       | SPMS    | Cardiac arrest |
| 3       | F   | 69  | 53         | 7:30        | 1       | SPMS    | Heart failure  |
| 4       | F   | 70  | 40         | 6:55        | 1       | SPMS    | Urine tract infection |
| 5       | F   | 57  | 21         | 20:00       | 1       | SPMS    | Decubitus      |
| 6       | F   | 76  | 19         | 9:45        | 1       | SPMS    | Unknown       |
| 7       | F   | 81  | 64         | 4:00        | 1       | SPMS    | Unknown       |
| 8       | M   | 50  | 15         | 5:25        | 2       | PPMS    | Pulmonary carcinoma |
| 9       | F   | 66  | 22         | 6:00        | 2       | SPMS    | Unknown       |
| 10      | M   | 49  | 24         | 8:00        | 2       | SPMS    | Pneumonia     |
| 11      | F   | 77  | 24         | 10:00       | 2       | SPMS    | Euthanasia    |
| 12      | M   | 72  | 23         | 7:55        | 2       | SPMS    | Euthanasia    |
| 13      | M   | 56  | 14         | 5:00        | 2       | SPMS    | Cachexia      |
| 14      | F   | 60  | 7          | 8:50        | 2       | PPMS    | Euthanasia    |
| 15      | M   | 54  | 12         | 8:15        | 2       | PPMS    | Euthanasia    |
| 16      | M   | 75  | 50         | 10:10       | 2       | SPMS    | Pneumonia     |
| 17      | F   | 50  | 17         | 7:35        | 2       | SPMS    | Euthanasia    |
| 18      | M   | 67  | 37         | 11:00       | 2       | SPMS    | Heart failure  |
| 19      | M   | 54  | 29         | 7:00        | 2       | SPMS    | Euthanasia    |
| 20      | F   | 81  | 21         | 6:30        | 2       | PPMS    | Heart failure  |

Mean ± SD 63.6 ± 11.5 26.2 ± 15.3 8:21

PMD postmortem delay (h:min), DD disease duration in years since diagnosis

a Scanner; 1, Avanto; 2, Sonata

Table 1 shows the demographic and neuropathology data of patients. Chronic active and 14 chronic inactive lesions. Furthermore, 38 areas of normal appearing WM (NAWM) were selected after histopathological inspection. Sections containing WM lesions were matched to corresponding post-mortem T2-weighted MR images as described previously [12]; see Fig. 1 for an example. Lesions were outlined (ROIs) on the T2 images using MIPAV software (Medical Image Processing, Analysis and Visualization, National Institutes of Health; mipav.cit.nih.gov). Subsequently, ROIs were copied onto the T1 qMRI maps and T1-RT values were obtained. ROIs were also placed in the NAWM, so as to act as a control measurement. An average T1-RT value was obtained over all voxels within a ROI (lesion or NAWM). Then each lesional/NAWM T1-RT value was assigned to their corresponding histopathological group (preactive, active, chronic active, chronic inactive or NAWM) and statistical analysis was performed. A flow-chart of the autopsy, histological, matching and analyzing procedure can be found in Fig. 2.

Statistical analysis

Descriptive and statistical analysis was performed using SPSS 20.0 for windows (SPSS, Inc., Chicago, IL). For the analysis between lesion types, we used the general estimated equation (GEE) for related data (71 lesions from 20 patients; some lesions from the same patient and therefore not independent). Scanner (Sonata or Avanto) and lesion area (mm²) were added as covariates. Holm–Bonferroni correction was used for multiple testing; significance level was set at p < 0.05.

Results

T1-RT increased consistently when moving from NAWM through preactive and active lesions to chronic lesions. Table 2 provides an overview of the mean (±SD) T1-RT values of the different lesion types. Statistical analysis of T1-RT revealed that NAWM differed significantly from active, chronic active and chronic inactive lesion types (all p < 0.001). Preactive lesions also differed significantly from active, chronic active and chronic inactive lesion types (all p < 0.001). However, NAWM and preactive lesions did not differ significantly from each other (p = 0.742). Furthermore, active lesions differed significantly from chronic inactive lesions (p < 0.05), but not from chronic active lesions (p = 0.286). Chronic active and chronic inactive lesions did not differ significantly from each other (p = 0.316). When lesion types were
grouped into three distinct groups, i.e., NAWM/preactive (mean T1-RT = 798.32 ± 67.72 ms), active (mean T1-RT = 1140.89 ± 204.82 ms) and chronic (mean T1-RT = 1271.36 ± 231.19 ms, including chronic active and chronic inactive lesions), all three groups differed significantly from each other. In other words, NAWM/
preactive lesions differed significantly from active \( (p < 0.05) \) and chronic lesions \( (p < 0.001) \), and active lesions differed significantly from chronic lesions \( (p < 0.05) \).

**Discussion**

In the current study, we set out to investigate whether the different stages of WM demyelination that are defined in MS histopathology can be differentiated by quantitative T1-RT mapping. Upon analysis of the data, histopathologically defined WM lesion types could be distinguished from NAWM, and a distinction could also be made between the overarching categories NAWM/preactive, active and chronic lesions (including chronic active and chronic inactive lesions). However, further subclassification into pathological lesion types based on T1-RT measures was not possible.

This means that, at a standard clinical field strength of 1.5T, there is additional value in distinguishing active from chronic lesions, but determining which exact lesion types are predominant in which patients or how specific lesional stages correlate with clinical disability is still limited and requires more research, possibly at higher field strength with better signal-to-noise ratio and spatial resolution. T1-RT measurement would still be an interesting MRI candidate at higher field strength, as this technique is highly sensitive to pathology and may detect tissue abnormalities where other advanced MRI techniques cannot \[4\]. This remains important when attempting to visualize subtle tissue pathology such as microglial clustering or incipient demyelination. However, future studies would probably benefit from combining T1-RT with other advanced MRI measures, such as MTR or DTI, as a combination of qMRI metrics with different pathological substrates may increase pathological specificity and hence improve MRI characterization of lesional heterogeneity in MS.

A limitation of this study is that although from in vivo measurements it is known that the accuracy and reproducibility of the T1-RT mapping method used are acceptable given the field strength \[4, 13\], we cannot fully exclude that there may be T1-RT errors related specifically to its application in fresh brain slices. Due to the rapid autopsy setting, imaging time had to be minimized to preserve tissue. Therefore, there was no time to perform a direct quantification of any potential errors related specifically to the application in fresh brain slices by, e.g., comparing to a trusted other technique such as inversion recovery spin echo imaging with an array of inversion times, because of the long acquisition times for such techniques. Furthermore, our sample size was limited when looking at the numbers of lesions within some of the lesion categories. As a result, the power to detect differences between, e.g., preactive and other types of lesions or NAWM was a priori low. However, if lesion differentiation can be improved by future (q)MRI efforts, a translation to the in vivo setting would be highly interesting. Initially, sensitivity and specificity of classifying lesions by qMRI, or T1-RT in particular, would have to be determined. Subsequently, an in vivo study with parameters similar to those used in this postmortem study is recommended, to see how T1-RT values change between the postmortem and in vivo setting and how this affects classification. Lesional changes may then be studied in vivo and in direct relation...
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**Conflicts of interest**

Laura E Jonkman has nothing to disclose. Alexandra Lopez Soriano has nothing to disclose. Sandra Amor serves on the editorial board of CNS and Neurological Disorders, Multiple Sclerosis and Related Disorders, Italian MS society and Finnish Academy. Frederik Barkhof serves on the editorial boards of Brain, European Radiology, Neuroradiology, Multiple Sclerosis journal and Radiology and serves as a consultant for Bayer-Schering Pharma, Sanofi-Aventis, Biogen-Idec, Teva, Novartis, Roche, Synthon BV and Jansen Research. Paul van der Valk has nothing to disclose. Hugo Vrenken has received funding for collaborative research projects from Pfizer, Novartis and Merck-Serono and a speaker honorarium from Novartis; all funds were paid directly to his institution. Jeroen JG Geurts serves on the editorial boards of Neurology, Multiple Sclerosis Journal, MS international, BMC Neurology and the Scientific Advisory Board of the Dutch MS Research Foundation. He has served as a consultant for MerckSerono, Novartis, Biogen Idec, Genzyme and Teva Pharmaceuticals.

**Ethical Standard**

Prior to death, all donors gave written informed consent for the use of their tissue and medical records for research purposes (The Netherlands Brain Bank, Amsterdam, The Netherlands). Ethics approval was obtained from the institutional ethics review board.

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**Table 2** Mean, standard deviation and minimum and maximum of T1-RT (in ms) for lesion types

| Lesion type/tissue       | Mean     | Standard deviation | Minimum | Maximum |
|--------------------------|----------|--------------------|---------|---------|
| NAWMc                    | 785.15   | 78.69              | 704.20  | 981.22  |
| Preactive                | 846.15   | 66.11              | 744.50  | 939.00  |
| Active<sup>a,b</sup>     | 1140.89  | 204.82             | 839.50  | 1432.45 |
| Chronic active<sup>a,b</sup> | 1225.58  | 197.77             | 1003.03 | 1561.04 |
| Chronic inactive<sup>a,b,d</sup> | 1320.32  | 370.05             | 942.97  | 2171.69 |

<sup>a</sup> Significant difference (p < 0.001) with normal appearing white matter (NAWM)
<sup>b</sup> Significant difference (p < 0.001) with active lesions
<sup>c</sup> Significant difference (p < 0.001) with normal appearing white matter (NAWM)
<sup>d</sup> Significant difference (p < 0.05) with active lesions
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