Remyelinated Lesions in Multiple Sclerosis

Magnetic Resonance Image Appearance

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Background: Various types of pathologic mechanisms in multiple sclerosis (MS) can alter magnetic resonance imaging (MRI) signals, and the appearance of remyelinated lesions on MRI is largely unknown.

Objective: To describe the MRI appearance of remyelinated lesions in MS.

Design: Comparison of postmortem MRI findings with histopathologic findings.

Setting: Brain donations from a general community.

Patients: Magnetic resonance images from 36 rapid autopsies yielded 161 areas that could be matched with histologic characteristics, including 149 focal T2-weighted abnormalities, with a range of signal intensities on T1-weighted images. In a subset of 49 lesions, magnetization transfer ratio could be determined.

Main Outcome Measures: An observer blinded to the MRI findings assessed the presence of remyelination using light microscopic criteria; in 25 areas, in situ hybridization was used to assess the presence of oligodendrocytes expressing proteolipid protein messenger RNA.

Results: Remyelinated areas were found in 67 lesions (42%): partial remyelination was present in 30 lesions (19%), whereas 37 lesions (23%) were fully remyelinated. Remyelinated lesions contained enhanced numbers of oligodendrocytes containing proteolipid protein messenger RNA. All areas with remyelination shown histopathologically were hyperintense on T2-weighted images. Strong hypointensity on T1-weighted images was significantly associated ($\chi^2=29.8, P<.001$) with demyelinated and partially remyelinated lesions compared with fully remyelinated lesions. The magnetization transfer ratio of remyelinated lesions (mean [SD], 27.6% [41%]) differed (F=46.3, P<.001) from both normal-appearing white matter (35.2% [32%]) and demyelinated lesions (22.3% [48%]).

Conclusions: Remyelinated lesions return an abnormal signal on T2-weighted images. Both T1-weighted images and magnetization transfer ratio may have (limited) additional value in separating lesions with and without remyelination.

Arch Neurol. 2003;60:1073-1081

The central hallmark of multiple sclerosis (MS) is the presence of multifocal areas of demyelination. Magnetic resonance imaging (MRI) is very sensitive in the detection of MS lesions, and is commonly applied for diagnostic purposes. T2-weighted sequences depict almost any alteration in brain tissue composition and therefore lack histopathologic specificity; this may explain why measures of T2 lesion load correlate poorly with the degree of clinical disability. Several putative MRI markers (eg, T1 hypointensity and decreased magnetization transfer ratio [MTR]) have been suggested to be more specific for the degree of matrix destruction and axonal loss, the most likely correlate of persistent disability.

Axonal loss is a prominent feature of MS, but the degree of axonal loss varies considerably between lesions. The mechanisms leading to axonal loss are partially unknown, but it is likely that demyelination is a necessary (although not sufficient) condition. Acute axonal damage occurs in the earliest stages of the disease and correlates with the presence of inflammatory cells, especially macrophages and microglia and CD8+ T cells within the plaques; however, in MS and experimental allergic encephalitis, less axonal damage is found in remyelinated compared with demyelinated lesions. Denuded axons are more vulnerable to substances such as nitric oxide and tumor necrosis factor α, which can be produced by macrophages.

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To avoid axonal damage, denuded axons can be en-sheathed by processes of astrocytes. Alternatively, at tempts can be made to remyelinate the axons. Remyelina-
tion is a common feature of MS and is found in many active lesions, especially in the early or acute stages of the disease.15 In these early remyelinating stages, lymphocytes and macrophages are still present, whereas the typical shadow plaque in long-standing chronic MS is hy-
epcellular. Ultrastructurally, the myelin sheaths in re-
myelinated lesions are uniformly thin, have shorter in-
ternodes, and show a reduced staining intensity on myelin stains. Remyelination relies on the presence of oligoden-
drocytes that either survive the demyelinating attack or that are recruited from a progenitor pool, both condi-
tions have been observed in MS lesions.12-14 Although recent data suggest that remyelination of denuded axons may fail frequently,15

The MRI appearance of remyelinated lesions is largely unknown.16 Biopsy data and animal studies suggest that remyelinated lesions may return an abnormal MRI signal.17-19 The purpose of this study is to describe the MRI appearance of remyelinated MS lesions in a large sample of MS patients examined post mortem. We speculate that the altered physical properties of remyelination (com-
pared with normally myelinated lesions) lead to an ab-
normal T2 signal, but that the degree of hypointensity on T1-weighted images and loss of MTR is less marked in remyelinated lesions than in demyelinated lesions without signs of remyelination.

METHODS

Brain tissue was obtained through the rapid autopsy proce-
dure of the Netherlands Brain Bank (Amsterdam, the Nether-
lands), which has been approved by the ethics committee of the Vrije Universiteit Medical Center in Amsterdam. All pa-
tients (and their next of kin) had given written consent for au-
topsy during the course of their disease for the use of their brain and spinal cord tissue for scientific purposes. Because almost all patients had been staying in a nursing home, no formal expanded disability status scale assessments had been per-
formed during the last years of their life. Based on available medi-
cal documents, the MS disease type was determined retrospectively.

Immediately after death, the autopsy took place at the Vrije Universiteit Medical Center, and the brains were cut into 10-mm thick coronal sections. For each case, 1 or 2 slices were sub-
jected to MRI, which included T1-weighted (repetition time [TR], 500 milliseconds; echo time [TE], 28 milliseconds; 2 ex-
citation) and T2-weighted (TR, 2200 milliseconds; TE, 20 and 80 milliseconds; 1 excitation) spin-echo images. Gradient echo images (TR, 500 milliseconds; TE, 20 milliseconds; 2 excita-
tion) with and without an MT pulse were obtained in a subset of patients and were used to create MTR maps. Five-
millimeter thick MRI sections (1 mm in-plane resolution) were obtained at the center of the brain slice. Afterwards, the slices were halved using a 5-mm deep cutting device, ensuring that the cut surface of the halved slice corresponded with the im-
aging plane.3

Guided by the T2-weighted MRI, areas corresponding to hyperintense lesions visible on the scan were dissected (and 12 areas without focal T2 abnormalities). Care was taken to include lesions that were iso- and hypointense to T1-weighted images. Tissue blocks were fixed in

10% neutral-buffered formalin. All lesions were marked on the MRI scan to facilitate matching with the corresponding histochemically stained section afterwards. To confirm the clinical diagnosis, apart from the MRI-selected samples used in this study, many other brain and spinal cord sections were examined neuropathologically, confirming the diagnosis of MS in all cases.

For histopathologic evaluation, 5-µm-thick paraffin-
embedded sections were processed and stained with hema-
toxyl-cosin, Bodian silver impregnation to determine axo-
nal density, Luxol fast blue (LFB), and combined LFB and periodic acid–Schiff (PAS) staining to delineate areas of my-
elin breakdown and the presence of material positive for LFB and PAS in phagocytic macrophages.

To evaluate inflammatory activity, immunohistochemi-

The following primary antibodies were used: macrophage-specific marker KP1 (CD68), leukocyte common antigen (CD45), glial fibrillary acidic protein, and HLA-DR. The different lesional stages of the lesions in the collected material were determined on the basis of immunologic parameters, such as the presence of inflammatory cells (CD45-positive lymphocytes and macrophages and CD68-positive macrophages, con-
taining phagocytized myelin components); enhanced expres-
sion of major histocompatibility complex class II antigens (anti-
HLA-DR) and CD45 expression on leukocytes (including lymphocytes) and resident microglial cells; the intensity of ast-
rogliosis, using glial fibrillary acidic protein; as well as cer-
tain morphologic criteria, such as cellularity of the center and rim of the lesion as described previously.20,21

For each tissue block, the LFB-stained sections were used to verify whether the dissected samples matched correctly with the corresponding MRI. The appearance of the MRI was scored according to the degree of T2 hyperintensity as mildly or strongly hyperintense lesions or as normal-appearing white matter (NAWM); for focal lesions, the border was scored as sharp or fuzzy. On the T1-weighted MRIs, the lesions were classified as either isointense with white matter, mildly hypointense (sig-

nal intensity equal to or higher than gray matter), or severely hypointense (lower signal intensity than gray matter)20,21 by a rater (E.B.) blinded to the histologic classification. Magnetization transfer ratio was measured, in lesions where MT images were available, by another rater (J.G.), who was blinded to the histologic results.

Microscopic analysis was performed by a rater (W.B.) blinded to the MRI appearance and to previous histopatho-
logic analysis of the same sections. According to their appear-
ance on the LFB stain, areas of interest were classified as showing normal myelin or demyelinated or remyelinated lesions. Remyelinated lesions were defined as focal abnormalities with decreased intensity of myelin staining, uniformly thin myelin sheaths (in relationship to axon diameter), and occurring ei-
ther at the edge of an otherwise demyelinated lesion (partial remyelination) or throughout the lesion (so-called shadow plaques).

A plasmid-derived digoxigenin-labeled complementary RNA probe encoding for the proteolipid protein (PLP) was ap-
plied in a nonradioactive in situ hybridization with slight modi-
fications to Breitschopf et al.18 Hybridization was performed at 65°C with a hybridization buffer containing 50% deionized for-
mamid, 2X silver sulfadiazine and chlorhexidine, 10% dex-
tran sulphate, 0.01% herring sperm DNA, and the probe (di-
luted 1:100) for 4 to 6 hours in a humidified chamber. The digoxigenin-labeled hybrids were visualized through the in-
cubation in an alkaline phosphatase buffer (100mM of Tris, pH 9.0, 50mM of magnesium chloride, 100mM of sodium chloro-
ride) containing 450 µg/mL of nitro blue tetrazolium (nitro-
blue tetrazolium reduction) and 175 µg/mL of 5-bromo-4-

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chloro-3-inodylphosphate for 4 to 16 hours at 4°C. After visualization of the hybridization signal, immunohistochemical analysis was performed to detect the PLP protein using an alkaline phosphatase/antialkaline phosphatase technique. Peroxidase reaction was visualized by alkaline phosphatase (resulting in a red reaction product).

The association between appearance on T2- and T1-weighted images and the presence of demyelination or remyelination was assessed using a Pearson $\chi^2$ test; post hoc comparisons were also performed using a $\chi^2$ analysis. The MTR data were compared between groups using a 1-way analysis of variance, with a post hoc Tukey for pairwise analysis. The threshold for significance was set at $P<.05$.

## RESULTS

Data were available from 36 patients (Table 1) with a mean age of 58.5 years (range, 34-83 years) and a mean disease duration of 22.8 years (range, 1.5-40 years). All patients were in the progressive phase of the disease, mostly secondary-progressive. A total of 161 areas could be matched with confidence and were used for analysis. In 36 areas, no signs of demyelination or remyelination were found. Demyelination without any evidence of remyelination was found in 36 lesions (36%) (Figure 1). Signs of remyelination were found in 67 lesions (42%): partial remyelination was present in 30 lesions (19%) and was usually confined to the border of a demyelinated lesion (Figure 2). Complete remyelination extending throughout the lesion was found in 37 lesions (23%) (Figure 3). When analyzed by type of disease, remyelinated lesions were at least as common in primary-progressive as in secondary-progressive patients. Within individual patients, some were found to primarily have fully remyelinated lesions, whereas others mainly had demyelination only or remyelination limited to the rim, suggesting that the type of lesion and its propensity for remyelination are determined individually.

Owing to the preservation of the tissue (fixed in 10% formalin and paraffin-embedded), in situ hybridization to assess the presence of PLP messenger RNA in oligodendrocytes was technically feasible in only 25 areas, derived from 10 patients with MS. Normal low-expression PLP messenger RNA expression was found in oligodendrocytes with normal myelin. In completely remyelinated lesions, high numbers of oligodendrocytes with strong PLP messenger RNA expression were found (Figure 4).
providing additional evidence that remyelination was taking place in the areas identified by the LFB staining.

An abnormal signal on T2-weighted imaging was observed in 149 areas (12 areas had been included that were normal-appearing on MRI). All areas with either demyelination or remyelination histopathologically were hyperintense on T2-weighted images. Neither the degree of signal increase nor the appearance of the border (sharp/fuzzy) on T2-weighted images was associated with the histologic appearance of demyelinated and remyelinated lesions ($P>0.1$). T1-weighted appearance in relationship to histologic appearance of the lesions is shown in Figure 5. Most of the strongly hypointense lesions (58%) were demyelinated, and fewer were (partially) remyelinated. The association between appearance on T1-weighted images and histologic testing was statistically significant ($F=29.8$, $P<.001$). Post hoc analysis revealed that fully remyelinated lesions were mostly mildly hypointense, while 40% of partially remyelinated lesions were severely hypointense ($x^2=12.2$, $P=.002$); the degree of hypointensity did not differ between partially remyelinated and demyelinated lesions without any signs of remyelination ($x^2=1.1$, $P=.58$).

Magnetization transfer ratio data were available for 46 areas (Figure 6). In NAWM, the mean (SD) MTR was 35.2% (3.2%), while in demyelinated lesions, it was 22.3% (4.8%). Remyelinated lesions had a mean (SD) MTR in between the NAWM and demyelinated lesions (27.6% [4.1%]). The association between MTR and myelin score was highly significant ($F=46.3$, $P<.001$). Post hoc Tukey analysis showed that all groups differed significantly from each other; specifically, the mean MTR was lower in remyelinated lesions than in NAWM ($P<.001$) and higher than in demyelinated lesions ($P=.002$). The group of remyelinated lesions was too small to allow subgroup comparisons of partially and fully remyelinated lesions.

**COMMENT**

In this large sample of MS brain tissue, signs of remyelination were found in 42% of the areas that were investigated, confirming that remyelination is a frequent phenomenon in MS.2-10 Previous studies that have reported a lower frequency of remyelinated lesions in long-standing MS differ from our study in terms of tissue sampling; when only macroscopically visible and palpable...
lesions are harvested, a selection bias toward chronic, fully demyelinated lesions with gliosis may occur. Our strategy to use MR-guided sampling yields a wider range of lesion types, including all stages of lesional development and even those that do not appear to have myelin abnormalities on microscopy. This strategy could have been biased toward finding a rather high number of remyelinated lesions. In approximately half of the remyelinated lesions, a selection bias toward chronic, fully demyelinated lesions with gliosis may occur. Our strategy to use MR-guided sampling yields a wider range of lesion types, including all stages of lesional development and even those that do not appear to have myelin abnormalities on microscopy. This strategy could have been biased toward finding a rather high number of remyelinated lesions. In approximately half of the remyelinated lesions, a selection bias toward chronic, fully demyelinated lesions with gliosis may occur. Our strategy to use MR-guided sampling yields a wider range of lesion types, including all stages of lesional development and even those that do not appear to have myelin abnormalities on microscopy. This strategy could have been biased toward finding a rather high number of remyelinated lesions. In approximately half of the remyelinated lesions, a selection bias toward chronic, fully demyelinated lesions with gliosis may occur. Our strategy to use MR-guided sampling yields a wider range of lesion types, including all stages of lesional development and even those that do not appear to have myelin abnormalities on microscopy. This strategy could have been biased toward finding a rather high number of remyelinated lesions.

Figure 2. Partially remyelinated lesion. A and B, Coronal postmortem T1-weighted (A) and T2-weighted (B) magnetic resonance images (MRIs) of multiple sclerosis (MS) case 97-006. The frames contain a lesion that is mildly hypointense on T1. The lesion was dissected and analyzed histologically. C, Photograph of a Luxol fast blue (LFB)–stained, paraffin-embedded brain tissue section matching the frame on the MRIs contains a partially remyelinated lesion (arrow) confined to the border of an active demyelinated lesion in the white matter (asterisk) (details shown in F). D, Photomicrograph of the edge of the demyelinated MS lesion showing reduced LFB staining in the remyelinated (RM) area (scale bar=50 µm). E, High-power magnification shows that the axons are surrounded by thin myelin sheaths (LFB; scale bar=20 µm). F, CD68 immunostaining of the periventricular region shows an active demyelinated MS lesion containing CD68-positive phagocytes (arrows) (hematoxylin-eosin; scale bar=20 µm). NAWM indicates normal-appearing white matter.
eliminated lesions, remyelination was limited to the rim of the lesion, with the remainder of lesions being left without myelin (Figure 2). The other half of the remyelinated lesions showed remyelination throughout the lesion; these lesions can be designated as shadow plaques (Figure 3). A finding that may have been limited by the retrospective assessment of disease course and the small number of lesions per patient is that primary-progressive and secondary-progressive patients did not differ much in terms of remyelination and that this feature, rather, was bound to individual patients, suggesting that the factors determining the clinical phenotype may be different from those involved in the inhibition of remyelination.

The major finding of this study is the observation that all remyelinated lesions were hyperintense on T2-weighted images, even those labeled as shadow plaques. This is an intriguing finding, and is probably an indication that the myelin sheaths in those lesions are thinner (and the extracellular spaces wider), leading to slower relaxation rates compared with normally myelinated white matter, in which a higher number of myelin-water interfaces facilitate relaxation. The abnormal T2 signal in remyelinated plaques illustrates the unique sensitivity of MRI to detect alterations in the normal brain composition. On the other hand, it reinforces the notion that an increased signal on T2-weighted images is nonspecific. Assuming that at least part of the fully remyelinated plaques contained normally functioning axons, our findings help to explain the limited correlation between T2 lesion load and measures of persistent disability.\(^2\) Apparently, T2-weighted images show a cumulative history of events, regardless of lesion development, and reflect the duration of disease rather than the level of disability.

T1 hypointensity and reduced MTR have been proposed as putative markers of matrix destruction and axonal loss.\(^3\)\(^7\)\(^23\) Our data show that remyelinated lesions have signal characteristics in between normally myelinated and demyelinated tissue; this certainly applies to fully remyelinated lesions, or shadow plaques. The increased T1 relaxation rate in hypointense lesions probably reflects a slightly expanded extracellular space or glial prolifera-
tion, which may occupy the difference in space between tissue with normally thick myelin and the much thinner remyelinated sheaths found in remyelinated lesions. Similar to changes in the T2 relaxation rate, increased T1 relaxation rate and reduced MTR are not specific for remyelination; rather, it seems that the difference may be gradual, with the most severe changes occurring in fully demyelinated lesions with a large amount

Figure 4. In situ hybridization for proteolipid protein (PLP) (black) combined with immunohistochemical staining for the PLP protein (red) in the normal-appearing white matter (NAWM) (A and B), and a fully remyelinated (RM) lesion (C and D) in multiple sclerosis case 99-062. A, In the NAWM, moderate PLP messenger RNA (mRNA) expression was found in oligodendrocytes (arrows) (scale bar=20 µm). B, Higher-power magnification clearly shows the cytoplasmic staining for PLP mRNA (black) (arrows) in oligodendrocytes on a red background of dense myelin staining using PLP immunohistochemical staining (hematoxylin-eosin; scale bar=1 µm). C, In the RM lesion, a large number of oligodendrocytes with strong PLP mRNA expression (black) (arrows) are present (scale bar=20 µm). D, High-power magnification of the lesion in C shows the strong cytoplasmic staining for PLP mRNA (black) (arrows). Thin myelin sheaths are visible using PLP immunohistochemical staining (red) (hematoxylin-eosin; scale bar=1 µm).

Figure 5. Distribution of lesion appearance on T1-weighted spin-echo magnetic resonance imaging (MRI) according to myelin score. Note that most strongly hypointense lesions are demyelinated (DM) rather than partially remyelinated (pRM) or remyelinated (RM). The association between MRI appearance and histologic appearance is statistically significant ($\chi^2 = 29.8, P < .001$).

Figure 6. Mean magnetization transfer ratio (MTR) according to lesion type. The difference between groups is statistically significant (F=46.3, $P < .001$). Not enough remyelinated (RM) lesions were available to analyze partially and fully remyelinated lesions separately. DM indicates demyelinated; NAWM, normal-appearing white matter.
of extracellular water. Such changes are likely to be most apparent in lesions with severe tissue destruction and axonal loss. In any case, the MRI appearances of the subgroups overlap, implying that a single snapshot will not suffice to assess the absence or presence of remyelination reliably in vivo.

Sequential analysis of T1-weighted images and MTR may be more promising in this respect. Several studies have reported that MS lesions frequently are hypointense initially, with around a third of initially hypointense lesions reverting back to isointensity (with normalization of MTR) at follow-up. In rats with chemically induced demyelination and spontaneous remyelination, this pattern of evolution parallels remyelination; our data, although unable to provide longitudinal evidence, certainly could support this hypothesis in humans. A recent pathologic-radiologic study performed with patients who underwent brain biopsy for diagnostic reasons found that lesions in the stage of early remyelination showed a tendency to develop from initially hypointense to more isointense lesions over time.

An intriguing question in MS is why some lesions fail to remyelinate, even though surviving oligodendrocytes and their progenitors have been shown to survive in at least a subset of lesions. Proposed explanations include the presence of antibodies against oligodendrocyte progenitor cell surface antigens, specific mutations in the myelin basic protein gene, or a deficit in oligodendrocyte-axon signaling. The lack of appropriate growth factor signals may also play a role in this context. Recently, beneficial effects of the inflammatory component of the MS plaque have been suggested. This may be in accordance with an earlier observation in a small number of MS biopsy cases in which early remyelinating lesions were found to be gadolinium-enhancing, in the early stage of remyelination, high numbers of inflammatory cells were found.

Several strategies have been devised to promote remyelination in MS. Apart from interventional procedures, such as transplantation of progenitor cells, several pharmaceutical approaches have been attempted. Intravenous immunoglobulins have been shown to stimulate remyelination in animal models and showed promising results in patients with longstanding treatment of refractory optic neuritis. Larger studies with intravenous immunoglobulins had a favorable impact on relapses and development of new MRI lesions, but a large study using intravenous immunoglobulins in patients with a fixed deficit failed to induce significant improvement. The latter may have been due to the selection of patients, who had little residual capacity for remyelination, and may indicate that such treatments should be instituted earlier in the course of the disease.

In conclusion, we show that remyelination is associated with an abnormal signal on T2-weighted images. Both T1-weighted images and MTR may have (limited) additional value in separating lesions with and without remyelination.

Accepted for publication March 27, 2003.

Author contributions: Study concept and design (Drs Barkhof and van der Valk); acquisition of data (Drs De Groot, Bergers, Hulshof, Geurts, Polman, and van der Valk); analysis and interpretation of data (Drs Barkhof, Bruck, Bergers, Hulshof, and van der Valk); drafting of the manuscript (Drs Barkhof); critical revision of the manuscript for important intellectual content (Drs Barkhof, De Groot, Bergers, Hulshof, Geurts, Polman, and van der Valk); obtained funding (Drs Barkhof); administrative, technical, and material support (Drs De Groot, Bergers, Hulshof, and Geurts); study supervision (Drs Barkhof, Bruck, Polman, and van der Valk).

The MS-MRI center is supported by the Dutch Foundation for Multiple Sclerosis Research, the Netherlands, and the Vrije Universiteit Medical Center.

We thank Brain Bank for supplying the human multiple sclerosis tissue (coordinator, Rivka Ravid, MD), Wouter Kamphorst, MD, for the neuropathologic evaluation, and Lisette Montagne and Marieke Pijmans for performing the histochemical and immunohistochemical stainings.

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