

Subpial Demyelination in the Cerebral Cortex of Multiple Sclerosis Patients

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Abstract. The extent and pattern of demyelination in the cerebral cortex was determined in 78 tissue blocks from the brains of 20 multiple sclerosis (MS) patients and 28 tissue blocks from 7 patients without neurological disease. Tissue blocks from 4 predetermined areas (cingulate gyrus, frontal, parietal, and temporal lobe) were studied, irrespective of macroscopically evident MS plaques. All tissue blocks contained cerebral cortex and periventricular and/or subcortical white matter. One hundred and nine demyelinating lesions were detected in the cerebral cortex, of which 92 (84.4%) were purely intracortical and 17 (15.6%) were lesions extending through both white and gray matter areas. In 5 of the 20 MS brains, subpial demyelination was extensive in the 4 widely spaced cortical areas studied, thus considered to represent a general cortical subpial demyelination. The percentage of demyelinated area was significantly higher in the cerebral cortex (mean 26.5%, median 14.1%) than in white matter (mean 6.5%, median 0%) ($p = 0.001$). Both gray and white matter demyelination was more prominent in the cingulate gyrus than in the other areas examined ($p < 0.05$). These results indicate that the cerebral cortex is likely to be a predilection site for MS lesions and identify general cortical subpial demyelination as a distinct pattern occurring in a significant subpopulation of MS patients.

Key Words: Cerebral cortex; Demyelination; Gray matter; Multiple sclerosis; Myelin; Myelin basic protein.

INTRODUCTION

Multiple sclerosis (MS) is a disease of the human central nervous system (CNS) with multifocal demyelination, inflammation, and axonal loss. The etiology of MS is unknown, but environmental factors and multiple separate genetic loci contribute to disease susceptibility (1). MS lesions may occur in all CNS parenchymal areas, the predilection sites include optic nerve, brainstem, spinal cord, periventricular white matter, and subcortical areas (2). MS lesions are also present in the cerebral cortex, and cortical plaques have been found to constitute a significant proportion of MS lesions in the brain (3–6), even in a material that was originally selected for the presence of white matter lesions (7).

The clinical significance of cortical MS lesions has not been established. Cortical lesions may contribute to the sensory and motor deficits of MS, as well as to cognitive impairment, which is found in approximately 45%–65% of MS patients (8, 9). MRI studies have shown a significantly greater cortical/subcortical disease burden in cognitively impaired MS patients (10, 11). Cortical MS lesions may impair cortical function through neuronal damage or loss, as significant neuronal damage or loss

has been detected in cortical MS lesions (7, 12). The study of cortical lesions may be important for the understanding of the pathogenesis of MS, as the intensity of inflammation in cortical lesions is significantly reduced when compared with white matter lesions (7, 12).

The extent of cortical demyelination in MS may be studied *in vivo* using MRI or postmortem using histochemical or immunohistochemical techniques. The sensitivity of MRI for gray matter lesions is known to be low and is much lower than for white matter lesions. This may be due to technical reasons, such as partial volume effects with the cerebrospinal fluid (CSF) outside the cortex and to the nature of cortical MS lesions, such as differences in the degree of inflammation and blood-brain barrier damage (7, 12, 13). Thus, MRI may not be the best instrument to describe the extent and distribution of cortical demyelination in MS.

Autopsy studies have mainly used gross examination or histochemical methods for the detection of cortical MS lesions (3, 4, 12, 14, 15). Luxol fast blue (LFB), a standard histochemical method for the detection of myelin, has been found to have a lower sensitivity than immunohistochemistry with anti-myelin basic protein (MBP) antibody for the detection of cortical MS lesions (16).

A systematic immunohistochemical study of the percentage of demyelinated area in different cortical and subcortical regions in MS has, to our knowledge, not been reported previously. In this study, the extent and distribution of demyelination in the cerebral cortex and in white matter was studied in 4 predetermined areas in 20 MS patients and in controls using morphometric analysis of sections immunohistochemically stained for myelin basic protein (MBP).

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TABLE 1
Clinical Data and Mean Percentage of Demyelinated Area in the Cerebral Cortex and in White Matter in the MS Patients Studied

Patient no.	Age/Sex	Disease course	Disease duration (y)	Cognitive impairment	Epilepsy	% Demyelinated Area	
						White matter	Cortex
1	64/F	SP	34	+	0	0.0	6.1
2	45/F	SP	25	+	+	32.0	23.8
3	55/F	PP	22	0	0	0.5	61.4
4	54/M	PP	20	+	0	0.1	1.0
5	77/F	SP	31	+	0	2.3	16.7
6	79/F	SP	37	0	0	0.0	0.0
7	56/F	SP	26	+	+	3.1	23.4
8	35/M	SP	13	+	+	39.4	30.3
9	57/M	PP	16	+	0	1.3	72.3
10	53/F	SP	19	+	0	4.5	57.1
11	40/F	SP	10	+	+	0.0	0.0
12	52/M	PP	8	0	0	5.3	22.3
13	46/M	SP	15	0	0	0.0	18.0
14	62/F	PP	27	+	0	11.9	30.2
15	58/F	PP	14	+	0	2.8	42.8
16	51/M	SP	23	+	0	1.9	50.1
17	43/F	RR	4	+	0	6.6	28.6
18	42/F	RR	11	0	+	0.1	1.7
19	65/F	PP	10	0	0	0.8	25.9
20	43/M	RR	14	+	0	16.2	17.0

Patients MS3, MS9, MS10, MS15, and MS16 had a general cortical subpial demyelination (GSD), with extensive subpial demyelination in all 4 areas studied.

Abbreviations: RR: relapsing-remitting MS; SP: secondary progressive MS; PP: Primary progressive MS.

+ = present; 0 = not present.

MATERIALS AND METHODS

Tissue

The brains of 20 MS patients and 7 patients without neurological disease were obtained at autopsy and fixed in 10% buffered formalin. All available MS brains containing the areas of interest were obtained from the Brain Bank at the Department of Pathology, Haukeland Hospital (Bergen, Norway). Clinical data on the MS cases are provided in Table 1. The age of MS patients at the time of death ranged from 35 to 79 years (mean 54 years). The age-range of control patients was 27 to 90 years (mean 49 years). The causes of death in the control group were myocardial infarction, ruptured aortic aneurysm, hanging, drowning (2 patients), pneumonia, and decompression sickness. In the MS patients, the presence of cognitive impairment or epilepsy prior to the terminal relapse or disease was assessed using the available clinical documentation. Interviews with spouses, close relatives, and treating physicians were done when this was deemed necessary. No systematic effort to assess cognitive impairment had been made in any of the patients prior to the terminal episode. Cognitive deficit and epilepsy was recorded as either known to be present or not known to be present. Epilepsy was recorded as present if a clinical diagnosis of epilepsy had been made in the patient's clinical records.

Four predetermined areas—sections of the cingulate gyrus, superior frontal gyrus (frontal cortex), paracentral lobule (parietal cortex), and superior temporal gyrus (temporal cortex)—were selected in all brains, except in 1 MS brain, in which only 2 of these areas were available for selection. These areas were

selected because anatomical landmarks made sampling of nearly identical areas possible in the autopsy material (Fig. 1). The same areas were sampled in all brains, regardless of the presence of macroscopically evident pathology.

Tissue sections were stained using the hematoxylin and eosin, LFB, and Heidenhain methods for histological examination. For Heidenhain staining, a 3-day incubation in iron alum solution at room temperature was found to be optimal.

Immunohistochemistry

Paraffin sections were treated with microwave antigen retrieval (10 min at 750 W and 3 × 5 min at 500 W). Sections were then immunostained by the standard avidin-biotin complex procedure recommended by DAKO (Copenhagen, Denmark) using DAKO Tech-Mate 500 slide processing equipment. Diaminobenzidine from Dako was used as chromogen. We omitted the primary antibody and used irrelevant IgG antibodies as controls.

Antibodies

The primary antibodies used include mouse anti-myelin basic protein (Boehringer Mannheim Corp., Mannheim, Germany), rabbit anti-myelin basic protein (DAKO), and mouse anti-proteolipid protein (PLP) (Clone plpc 1; Instruchemie, Hilversum, The Netherlands).

Morphometry

An MBP-stained slide from each area was scanned in a slide scanner (Polaroid Sprint Scan 35, Polaroid Corp. Cambridge,

MA), thereby preparing a digital image. The areas of demyelination and the total area of white matter and gray matter in each slide was measured on the digital image using Leica Q500 MC morphometry equipment (Leica Camera, Solms, Germany). The calibration of the equipment was regularly controlled using a standard millimeter scale on a glass slide.

Statistical Methods

After analysis of the distribution of the data, nonparametric tests were chosen. The extent of demyelination in different regions and patient groups was compared using the Mann-Whitney test and correlations between groups were compared using Spearman's rho test. SPSS software (SPSS Inc., Chicago, IL) was used for statistical calculations.

RESULTS

Lesion Detection

In white matter, MS lesions were readily detectable by LFB staining, with a sensitivity comparable to that of MBP immunostaining (Fig. 2A, B), while cortical lesions were difficult to detect with a LFB staining, but easily detectable by MBP immunohistochemistry (Fig. 2C, D). In nondemyelinated cerebral cortex, myelin was well stained by MBP immunohistochemistry, but was difficult to discern from background staining using the LFB method (Fig. 2E, F). Intracortical lesions were particularly difficult to detect, because there were no associated changes in the subcortical white matter. Immunohistochemistry using anti-MBP antibodies was therefore used to detect and measure the areas of cortical demyelination. Similar patterns of staining were observed with both MBP-antibodies. Immunohistochemistry with anti-PLP antibody produced identical results as with the anti-MBP antibody (Fig. 3). Similar areas of cortical demyelination were also detected with Heidenhain's histochemical myelin staining, although this method appeared less sensitive compared with MBP- or PLP-immunostaining (Fig. 3). In the 78 tissue blocks from 20 MS patients studied, 109 demyelinating lesions were detected in the cerebral cortex (Table 2). In 18 of 20 MS patients, cortical lesions were detected in one or more of the 4 predetermined areas. In 2 MS patients, no MS lesion was detected in gray or white matter in the 4 areas studied and plaques were detected only in the brainstem and spinal cord at autopsy. These 2 patients had clinical signs and symptoms consistent with spinal cord/brainstem affection of MS and were included in the study (Table 1). No cortical or white matter demyelinating lesion was detected in the 28 tissue blocks from the 7 control patients studied. Intracortical myelinated fibers were organized in 3 patterns: radial fibers, oblique fibers, and horizontal fibers in normally myelinated cortices of both MS and control patients. As described previously, the highest density of myelin staining was found in cortical layers 4 to 6. Intermediate density of myelin staining was present in layer 3; in layers 1 and

2, the density of staining was low (17). Cortical lesions were classified according to a descriptive system based on lesion distribution similar to in previous studies (7, 12). This descriptive system should not be considered definitive, as classification may change with further knowledge of cortical lesion pathogenesis.

Cortical Lesion Types

Type 1: Lesions that extended across both white matter and gray matter were termed type 1 lesions (Fig. 4A) (7). Type 1 was the third most frequent lesion type (17 lesions), with the third largest mean lesion area (29.2 mm²), and accounted for 14.4% of the total cortical demyelinated area (Table 2). These lesions would usually not extend to the surface of the brain, and the center of the lesion would often be in the white matter (Fig. 4A). In general, subcortical white matter lesions crossed the white matter/gray matter border.

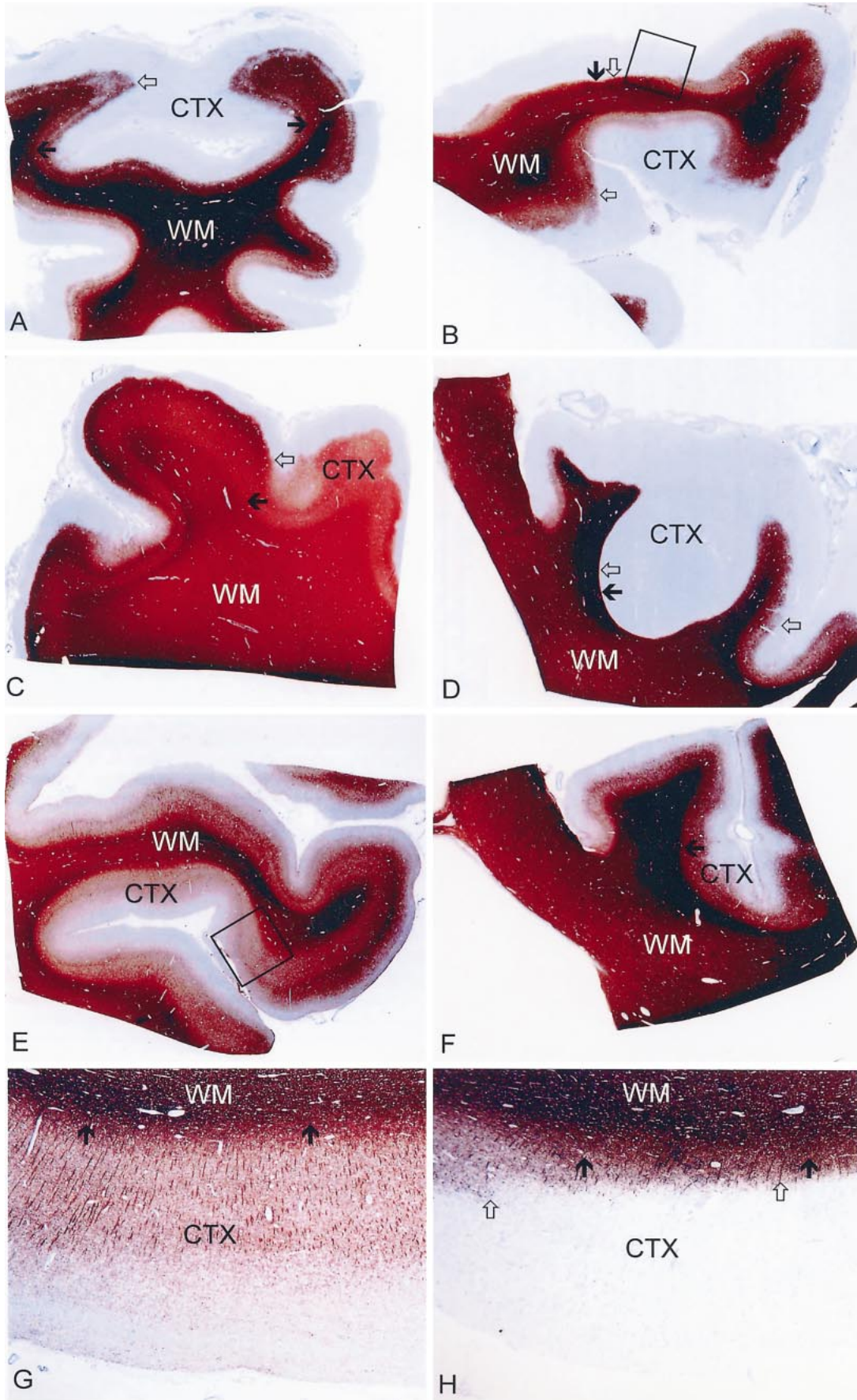
Type 2: Lesions within the cerebral cortex that did not extend to the surface of the brain or to the subcortical white matter were classified as Type 2 (Fig. 4B). These lesions had the lowest mean lesion area (2.4 mm²). Eighteen (17%) of the cortical lesions were Type 2, with only 1.2% of the total cortical lesion area (Table 2).

Type 3: The most common cortical lesion type was subpial, affecting the largest cortical area (Type 3) (Figs. 1A, C, 4C). Sixty-five (60%) of the cortical lesions were subpial, accounting for 67% of the total cortical demyelinated area (Table 2). A common appearance of type 3 lesions was that of long ribbons of subpial demyelination, often affecting several adjacent gyri (Fig. 1A, C). Other type 3 lesions were wedge-shaped, with the basis at the surface of the brain. Additionally, a combination of these patterns, with wedge-like lesion areas within bands of more superficial subpial demyelination, was often present (data not shown).

Type 4: Lesions that extended throughout the full width of the cerebral cortex, but did not reach into the subcortical white matter were classified as Type 4 (Figs. 1B, D, 4D). Nine (8%) of the cortical lesions were Type 4. These lesions had the largest mean area (66.2 mm²) and accounted for 17% of the total cortical demyelinated area (Table 2). Type 4 lesions had a striking selectivity for the cerebral cortex, with the border of the lesions (often for long stretches) corresponding to the white matter/gray matter border (Figs. 1D, 4D). Almost total demyelination of some gyri was observed without demyelination of the subpial white matter (Figs. 1D, 4D).

General Cortical Subpial Demyelination

In 5 of the 20 MS patients studied, subpial demyelination was extensive in all the 4 widely spaced tissue blocks selected, indicating a general cortical subpial demyelination (GSD) (Fig. 1A–D). The cortices of these patients harbored both type 3 and type 4 lesions (Fig.



1A–D). In patients with a GSD, cortical lesions affected a mean of 56.7% of the total cortical area examined, as compared to 16.3% in the other MS patients ($p < 0.001$). The mean percentage of demyelinated area in the white matter was 2.2% (median 1.9%) in the GSD patients, which was lower than in the other MS patients (mean 7.9%, median 2.3%). This difference was not statistically significant ($p = 0.20$).

Regional Differences in the Extent of Cortical Demyelination

A mean of 26.5% (median 14.1%) of the cortical area analyzed was demyelinated, while a mean of 6.5% (median 0%) of the white matter area was demyelinated ($p = 0.001$). When the material was subgrouped into each of the individual 4 areas, the percentage of demyelinated area was significantly higher in the cortex than in white matter in the frontal cortex ($p = 0.01$), temporal cortex ($p = 0.01$), parietal cortex ($p = 0.01$), and the cingulate gyrus ($p < 0.002$) (Table 3). The extent of cortical demyelination in the frontal, temporal, and parietal lobes and the cingulate gyrus significantly correlated with the extent of cortical demyelination in the each of the 3 other areas studied ($p < 0.05$). Demyelination in the cerebral cortex was mainly due to purely cortical lesions: Ninety-two (84%) of the cortical MS lesions with 85.6% of the cortical demyelinated area were purely intracortical, while 17 (15.6%) of the cortical MS lesions with 14.4% of the cortical demyelinated area extended through both gray and white matter (Table 2).

A mean of 43.8% (median 49.4%) of the cingulate gyrus cortical area was demyelinated in the MS patients. This was significantly higher than in the other areas examined (Table 3) ($p < 0.05$). The mean percentage of demyelination was 27.7% in the temporal cortex (median 19.4%), 24.1% in the frontal cortex (median 14.3%), and 11.5% in the parietal cortex (median 2.2%). These differences in percentages of cortical demyelinated area were not statistically significant.

Clinico-Pathological Correlation

In 4 of the 5 patients with GSD, symptoms and/or signs of cognitive impairment were noted in the available clinical documentation. Cognitive impairment was also

evident in 10 of the 15 other MS patients studied. Five patients had epilepsy; none of these had GSD. The age and duration of disease of the GSD patients was similar to that of the other MS patients. The mean percentage of cortical demyelinated area was higher in the primary progressive MS patients (36.6%) than in patients with secondary progressive (22.6%) or relapsing remitting MS (15.8%). In the white matter the percentage of demyelinated area was lower in the primary progressive MS patients (3.2%) than in patients with secondary progressive MS (8.3%) or relapsing remitting MS (7.6%). These differences were not statistically significant. The percentage of cortical demyelinated area did not correlate with the duration of disease ($p = 0.72$).

DISCUSSION

The present study determined the extent and pattern of myelin loss in 4 preselected cortical/white matter areas in MS patients. These data provide new information on the extent and distribution of cortical demyelination in MS and highlight the importance of cortical demyelination in the pathogenesis of chronic MS. Myelin loss in the cerebral cortex was extensive; a mean of 26.5% of the cerebral cortex was demyelinated in the 4 areas examined.

The extent of cortical or white matter demyelination in MS has to our knowledge not been determined in this way before. We have recently found cortical lesions in 62% of tissue blocks obtained at autopsy for the study of white matter lesions and in 44% of tissue blocks obtained at autopsy as control areas (7). Lumsden reported extensive involvement of the cerebral cortex, as 59% of lesions less than 10 mm in diameter were found to affect the cortex (4). In a study by Brownell and Hughes, 22% of MS lesions were found to affect the cerebral cortex (3).

We found a GSD in 5 of the 20 MS patients studied. Galaburda found extensive subpial demyelination in all levels of the brain of a patient with rapidly progressive disease (18). Neumann et al reported a case of atypical psychosis with disseminated subpial demyelination (19). In the study by Lumsden, cortical demyelination was found to be common, but an extensive subpial distribution of demyelination was not described (4). Lumsden described most cortical lesions to be small, only a few

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Fig. 1. Paraffin sections immunohistochemically stained with anti-MBP antibody and lightly counterstained with hematoxylin from an MS patient (A–D, H) and a control patient (E–G). The figure illustrates the 4 areas studied in each patient: the frontal lobe (A), the temporal lobe (B, E), the parietal lobe (C), and the cingulate gyrus (D, F). The MS patient has extensive subpial demyelination in the 4 tissue specimens (A–D). The MS patient is thus considered to have a general cortical subpial demyelination (GSD). In panels (A) and (C) the lesion is subpial, but does not span the full width of the cerebral cortex (Type 3 lesion). In panels (B) and (D) the lesion spans the full width of the cortex without reaching into subcortical white matter (Type 4 lesion). The cingulate gyrus specimens include periventricular white matter and white matter of the corpus callosum (D, F). Higher magnification images from panels (B) and (E) (boxes) show myelin staining throughout the width of control cerebral cortex (G), and subpial demyelination in the MS cortex (H). Open arrows indicate the lesion border, closed arrows delineate the white matter/cerebral cortex border.

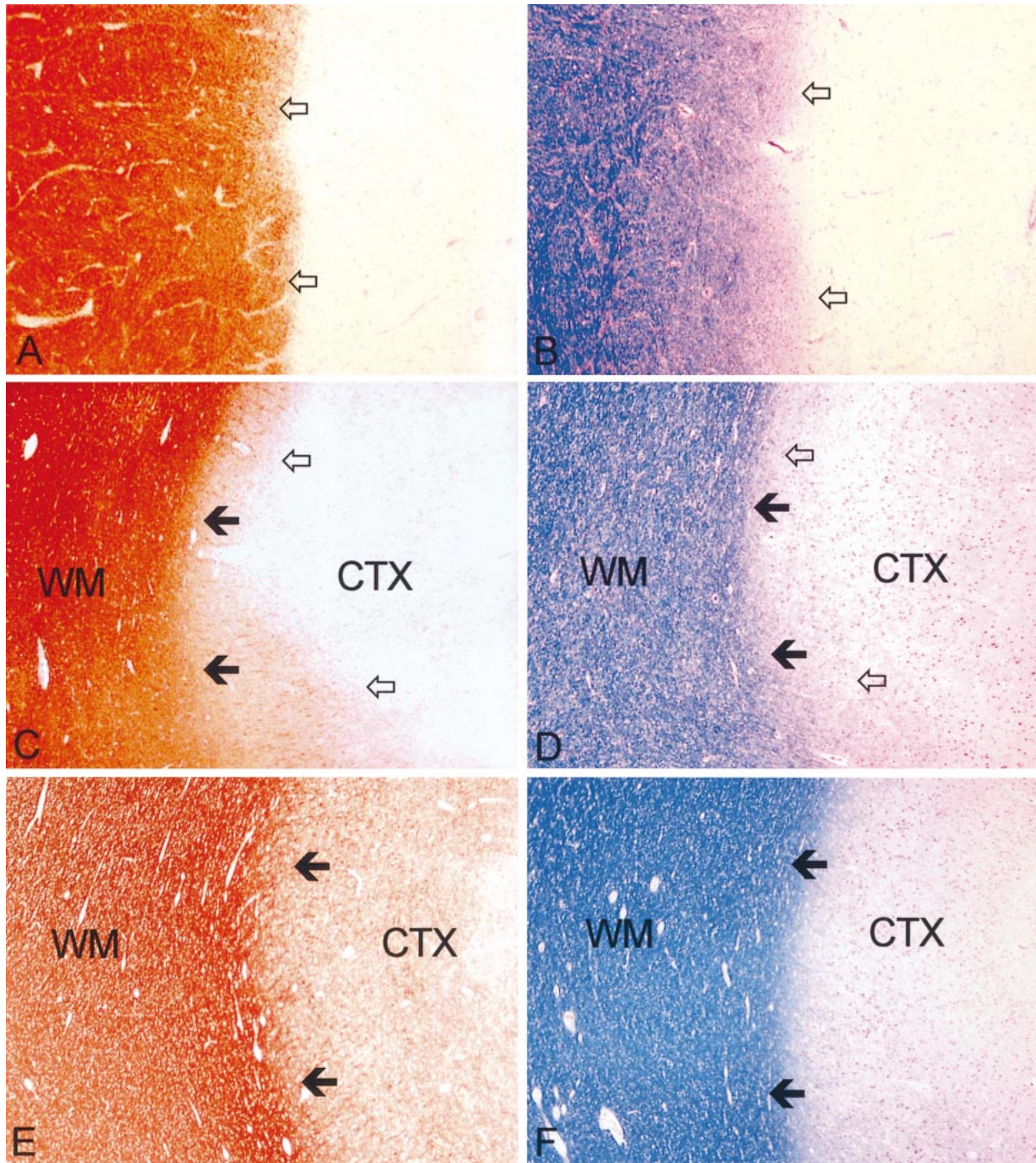


Fig. 2. Paraffin sections from MS brain (**A–D**) and control brain (**E, F**) stained with anti-myelin basic protein (MBP) antibody (**A, C, E**) and Luxol fast blue (LFB) (**B, D, F**). The border between white matter and cerebral cortex is indicated by closed arrows. The border of a white matter MS lesion is delineated by both LFB and MBP staining (**A, B**, open arrows). Cortical demyelination is clearly delineated by MBP immunohistochemistry (**C**), but not by LFB staining (**D**, open arrows). Abundant myelin staining is detected in the control brain cerebral cortex by MBP immunohistochemistry (**E**). In the same area in an adjacent section, myelin is heavily stained in the white matter, but difficult to discern from background staining in the cerebral cortex by LFB staining (**F**). Abbreviations: WM, white matter; CTX, cerebral cortex.

millimeters in diameter, in material from “early and severe” cases of MS. We have previously observed demyelinating lesions extending across several gyri in MS in material that was not systematically sampled (7). That

material could therefore not be used to measure the extent of cortical demyelination.

In the present material, intracortical lesions accounted for 84% of cortical lesions and 86% of the cortical

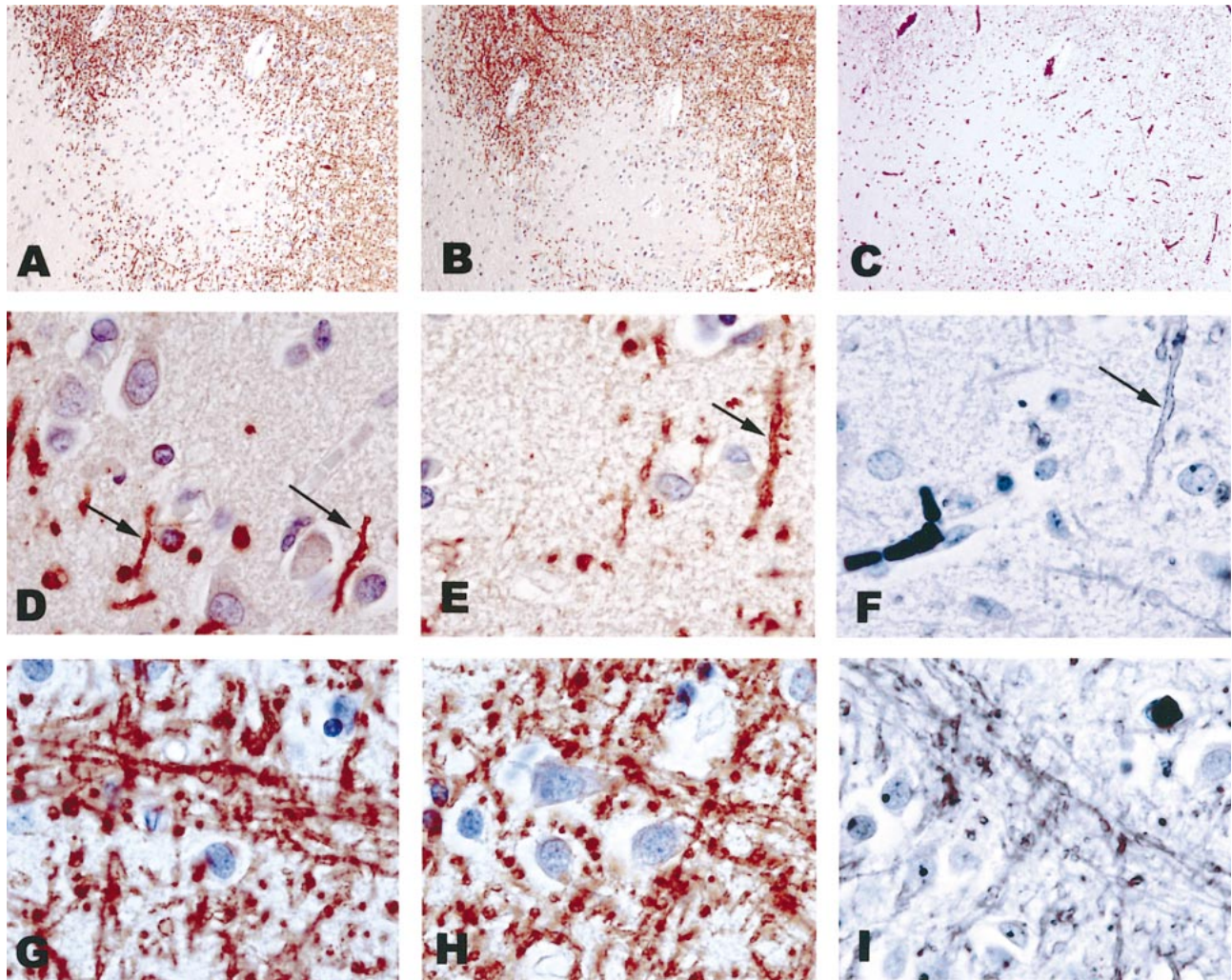


Fig. 3. Adjacent paraffin sections from an MS patient, immunohistochemically stained with monoclonal anti-MBP antibody (**A, D, G**), monoclonal anti-PLP antibody (**B, E, H**), and Heidenhain's myelin staining (**C, F, I**). With all 3 myelin markers a cortical lesion with a similar area of myelin loss is detectable at low magnification (**A–C**), although the contrast level of Heidenhain's staining (**C**) is lower than for the immunohistochemical stains (**A, B**). At the edge of the lesion, short strands of myelin are observed (**D–F**, arrows). In a neighboring nondemyelinated area, normal-staining cortical myelin is detected with all 3 markers (**G–I**).

demyelinated area. Studies using histochemical techniques have detected a much lower proportion of purely cortical (intracortical) lesions. Brownell and Hughes found that 5% of MS lesions were intracortical and 17% were at the junction of cortex and white matter (3). In the study by Kidd et al, only 24% of cortical lesions were intracortical (5). The extent of subpial demyelination may have been underestimated previously because of technical factors. We found the standard histochemical procedure Luxol fast blue to have a good sensitivity for lesions at the leucocortical junction, but a low sensitivity for subpial demyelination (Fig. 2). This is consistent with the results of Itoyama et al, who found MBP immunohistochemistry to be more sensitive than LFB histochemistry, particularly for the detection of cortical MS lesions (16).

We attempted to improve the sensitivity of LFB staining for the detection of intracortical lesions by reducing the destaining time, although this was not successful due to higher levels of (nonspecific) background staining (data not shown). With Heidenhain's myelin staining modified to optimize sensitivity for cortical myelin, similar areas of cortical demyelination were detected as with immunohistochemical staining, but the sensitivity for cortical myelin still appeared lower than with immunohistochemistry.

The patients with GSD had very extensive demyelination in the cerebral cortex, with a mean of 56.7% of the total cortical area examined being demyelinated. In the white matter of these patients, the extent of demyelination was lower than in the other MS patients (mean percentage

TABLE 2
Cortical Lesion Subtypes in MS Cases: Number and Percentage of Total Area of Demyelination

Lesion type	Number	Mean lesion size (mm ²)	Percentage of total demyelinated Area
1 (mixed WML/GML)	17	29.2	14.4
2	18	2.4	1.2
3	65	35.5	67.0
4	9	66.2	17.3
Intracortical lesions (2–4)	92	32.1	85.6

Type 1 lesion: lesion with a continuous area of demyelination extending through both gray and white matter.

Type 2 lesion: intracortical lesion without contact with white matter or pia mater.

Type 3 lesion: subpial lesion.

Type 4 lesion: lesion extending throughout the full width of the cerebral cortex, but not extending into white matter.

Intracortical lesion: type 2–4.

Abbreviations: GML, gray matter lesion; WML: white matter lesion.

of demyelinated area 2.2 vs 7.9). Although this difference in the extent of white matter demyelination was not significant, these data may indicate a heterogeneity in the pathogenesis of MS in different patients, with selective

TABLE 3
Percentage of Demyelinated Area in the 4 Regions Studied

	White Matter		Cerebral Cortex	
	Mean	Median	Mean	Median
Frontal cortex	4.0 ± 1.6	0.0	24.1 ± 5.5	14.3
Parietal cortex	3.5 ± 3.1	0.0	11.5 ± 3.8	2.2
Temporal cortex	7.7 ± 4.1	0.0	27.7 ± 7.1	19.4
Cingulate gyrus	11.0 ± 4.5	2.0	43.8 ± 7.9	49.4

mechanisms mediating proportionally more extensive cortical demyelination in some patients. This is supported by the observation that extensive cortical demyelination in one cortical area was correlated with extensive cortical demyelination in other remote areas of the brain, and by the selectivity of type 4 lesions for the cerebral cortex, as the full width of the cerebral cortex can be demyelinated without demyelination of the subcortical white matter (Figs. 1B, D, 3D).

As is commonly found in autopsy studies, the MS patients included in this study had a relatively long mean duration of disease (19 years). Therefore, it is important to measure the extent of cortical demyelination in different patient materials with a more acute disease course

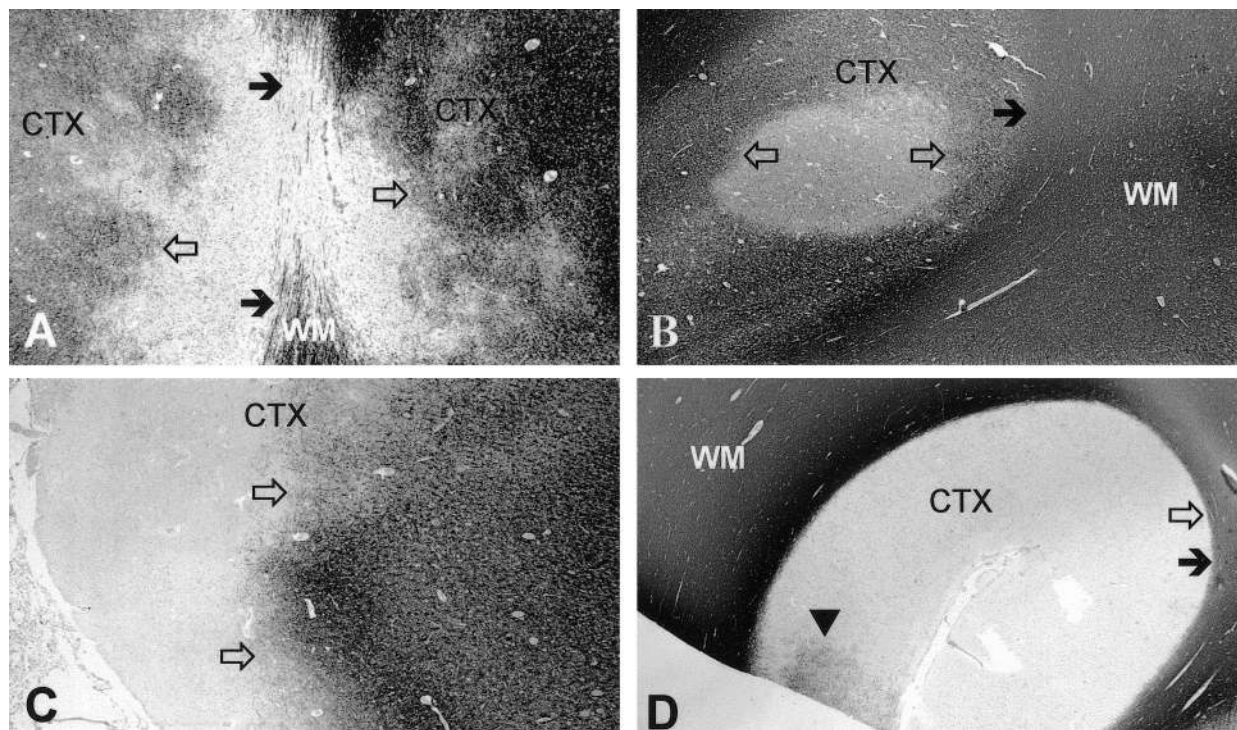


Fig. 4. The classification of cortical MS lesions. Paraffin sections from MS brain immunostained with anti-MBP antibody. The cerebral cortex/white matter borders are delineated by closed arrows. The lesion borders are delineated by open arrows. Type 1 lesions (**A**) extend through both white and gray matter. Type 2 lesions (**B**) are intracortical, having no contact with white matter or with the surface of the brain. Type 3 lesions (**C**) extend inward from the surface of the brain. Type 4 lesions (**D**) extend through the whole width of the cortex without reaching into white matter. A small area of probable remyelination is observed (arrowhead). Abbreviations: WM = white matter; CTX = cerebral cortex.

and/or a shorter mean duration of disease. This will determine whether cortical demyelination is an early or late event in the clinical course of the disease. A correlation of the extent of cortical demyelination with duration of disease was not found in this study. Primary progressive MS patients had a lower percentage of demyelinated area in the white matter than secondary progressive MS patients and relapsing remitting MS patients. This finding is consistent with the results of MRI studies comparing the extent of demyelination in primary progressive MS, secondary progressive MS, and relapsing remitting MS patients (20, 21). In the cerebral cortex, however, the percentage of demyelinated area was higher in the primary progressive MS patients than in the other MS patients. Although this difference was not statistically significant, it may imply a role of cortical demyelination in the chronic progression of MS.

The differences in the lesion distribution in MS patients may reflect various autoantigens, as differences in the pattern of demyelination depend on the antigen that has been demonstrated in EAE (22). Biochemical differences between white matter and cortical myelin and/or oligodendrocytes could explain a selectivity of the demyelinating process for cortical myelin, but to our knowledge this has not been demonstrated.

There were significant differences in the extent of demyelination in the 4 regions studied. The most extensive cortical myelin loss was detected in the cingulate gyrus, with less demyelination in temporal, frontal, and parietal cortex. Also, in white matter the cingulate gyrus was the area with the most extensive demyelination, but regional differences were much smaller. These regional differences should be cautiously evaluated, due to the small sample size.

Regional differences in the extent of cortical demyelination have also been reported by Lumsden, who found the highest number of cortical lesions to be in the superior frontal gyrus (4). The number of lesions detected was not adjusted for the gyral area, however, the percentage of demyelinated area was thus not studied.

MRI and Sensitivity for Cortical Demyelination

Our results differ from the results of MRI studies, where intracortical demyelination is found to be minor compared to subcortical demyelination (5). However, MRI may not be sensitive for detecting intracortical lesions. In a comparison of postmortem MRI and histological examination, only 2 of 14 histologically detectable intracortical lesions were detected by MRI (5). In another similar study, none of the 39 cortical plaques identified histologically were detected by MRI, while in periventricular areas the maximum possible number of lesions detected by MRI often exceeded the number of histological plaques (23). The reason for this low sensitivity of

T₂-weighted images for intracortical lesions may be longer relaxation times of gray matter lesions because of a high cellularity of the cerebral cortex, partial volume effects due to the proximity to the CSF (5), and the absence of significant inflammation in cortical lesions (7, 12, 13). The relative insensitivity of MRI in the detection of cortical lesions may contribute to the relatively weak correlation of brain MRI lesion load with disability in MS (5, 23). The fluid-attenuated inversion recovery (FLAIR) MRI technique has been shown to give a better contrast between demyelinated and "normal" gray matter and to improve the sensitivity of MRI for cortical lesions. Using FLAIR sequences, the sensitivity for cortical lesions has been improved by approximately 20%–60% (24–26). In a study of 7 MS patients with relapsing remitting or secondary progressive disease using FLAIR, 45% of MS lesions were found to be cortical/subcortical (25).

Clinical Significance of Cortical Demyelination

Due to the poor sensitivity of MRI for cortical lesions, the clinical significance of cortical MS lesions is difficult to determine. Cortical MS lesions could contribute to motor and sensory deficits, cognitive impairment, and epilepsy through demyelination and axonal and neuronal loss or damage. Neuronal injury in cortical lesions was noted by Dawson (12). In a recent paper, we have described significant neurite transection and neuronal loss in cortical MS lesions (7).

The prevalence of epilepsy is 2% to 5% in MS patients, which is significantly higher than in the general population (27, 28). The frequency of partial seizures is increased in MS patients with epilepsy (29), and lesions implicated in the causation of seizures has been detected in cortical/subcortical areas (30). No association of cortical demyelination with epilepsy was found in this study. This may be due to heterogeneity in cortical lesion pathogenesis, since neuronal affliction could conceivably vary in different lesions, and lesions in some areas may be more epileptogenic. This may be more important than the general extent of cortical demyelination in generating epilepsy.

Cognitive impairment affects 45% to 65% of MS patients (8, 9) and has been correlated with juxtacortical lesion load in MS patients in an MRI study (31). In the present study, 4 of 5 patients with general cortical subpial demyelination were known to have had symptoms and/or signs of cognitive impairment. This may indicate that intracortical lesions underlie dementia in MS.

In conclusion, we have demonstrated that purely cortical demyelination in MS is extensive, and that general cortical subpial demyelination occurs in a significant subpopulation of chronic MS patients. Although cortical lesions reaching across several gyri have been described in previous studies (7, 12), the occurrence and extent of this

as a general pattern has not, to our knowledge, been previously demonstrated. Demyelination and neuronal/axonal injury in these extensive cortical lesions may mediate clinically significant cognitive, motor, and sensory deficit in MS.

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