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RESEARCH ARTICLE

Long-term clinical, imaging and cognitive outcomes association with MS immunopathology

Alicja Kalinowska-Lyszczarz[1,2], Jan-Mendelt Tillema[1], William Oliver Tobin[1], Yong Guo[1], Stephen D. Weigand[3], Imke Metz[4], Wolfgang Brück[4], Hans Lassmann[5], Monica Giraldo-Chica[6], John D. Port[7] & Claudia F. Lucchinetti[1]

[1]Department of Neurology, Mayo Clinic, Rochester, Minnesota, USA
[2]Department of Neurology, Division of Neurochemistry and Neuropathology, Poznan University of Medical Sciences, Poznan, Poland
[3]Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota, USA
[4]Institute of Neuropathology, University Medical Center, Göttingen, Germany
[5]Center for Brain Research, Medical University of Vienna, Wien, Austria
[6]Department of Neurosurgery, Medical College of Wisconsin, Milwaukee, Wisconsin, USA
[7]Department of Radiology, Mayo Clinic, Rochester, Minnesota, USA

Correspondence
Claudia F. Lucchinetti, Department of Neurology, Mayo Clinic, 200 First Street SW, Rochester, MN 55901, USA. E-mail: clucchinetti@mayo.edu

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Abstract

Objective: In this observational study on a cohort of biopsy-proven central nervous system demyelinating disease consistent with MS, we examined the relationship between early-active demyelinating lesion immunopattern (IP) with subsequent clinical course, radiographic progression, and cognitive function. **Methods**: Seventy-five patients had at least one early-active lesion on biopsy and were pathologically classified into three immunopatterns based on published criteria. The median time from biopsy at follow-up was 11 years, median age at biopsy - 41, EDSS - 4.0. At last follow-up, the median age was 50, EDSS - 3.0. Clinical examination, cognitive assessment (CogState battery), and 3-Tesla-MRI (MPRAGE/FLAIR/T2/DIR/PSIR/DTI) were obtained. **Results**: IP-I was identified in 14/75 (19%), IP-II was identified in 41/75 (56%), and IP-III was identified in 18/75 (25%) patients. Patients did not differ significantly by immunopattern in clinical measures at onset or last follow-up. The proportions of disease courses after a median of 11 years were similar across immunopatterns, relapsing–remitting being most common (63%), followed by monophasic (32%). No differences in volumetric or DTI measures were found. CogState performance was similar for most tasks. A slight yet statistically significant difference was identified for episodic memory scores, with IP-III patients recalling one word less on average. **Interpretation**: In this study, immunopathological heterogeneity of early-active MS lesions identified at biopsy does not correlate with different long-term clinical, neuroimaging or cognitive outcomes. This could be explained by the fact that while active white matter lesions are pathological substrates for relapses, MS progression is driven by mechanisms converging across immunopatterns, regardless of pathogenic mechanisms driving the acute demyelinated plaque.

Introduction

The pathology and clinical manifestations of multiple sclerosis (MS) undergo changes over the lifetime of individuals affected with MS. They are influenced by lesion stage, severity, disease course, duration and therapy. Demyelinating activity is the hallmark of active MS lesions, which demonstrate patient-dependent immunopathological heterogeneity that persists over the time when lesions are active.[1–4] Four distinct immunopatterns (IPs) of early active MS lesions have been described,[1,5,6] reflecting differences in immune effector mechanisms of lesion formation. Patterns I and II are both associated with T lymphocyte and macrophage

infiltration, with additional antibody and complement deposition seen exclusively in pattern II lesions. Pattern III is defined as a distal oligodendrogliopathy, with preferential loss of myelin-associated glycoprotein (MAG) relative to other myelin proteins, and striking oligodendrocyte loss. Pattern IV, which has thus far only been described in three cases of progressive MS, is characterized by oligodendrocyte degeneration in the periplaque white matter.[1]

Immunopathological heterogeneity of lesions may harbor fundamental implications for individualizing MS patients' therapy and prognosis. While pathological data are rarely acquired for MS patients, typically through biopsy for a tumefactive lesion[7] or at autopsy, they are crucial for understanding mechanisms of the disease. Furthermore, there are only two large studies that have investigated MS lesion IPs with long-term clinical outcome measures. The only two studies come from our group. In the first one, we found no significant association between IP and clinical outcome measures in 91 patients after a median follow-up of 4.4 years.[8] In the other, which was just recently published, in a large cohort of 547 cases we found that pattern III was associated with a more fulminant initial attack than either pattern I or II, but long-term outcomes were not different across immunopatterns.[9]

There does seem to be a relationship between IP and therapeutic response in the acute relapse setting. Specifically, patients with pattern II lesions, which are characterized by marked humoral response, benefited most from apheresis treatment, while patients with pattern III lesions were unlikely to have a favorable response.[10,11]

In this detailed prospective long-term follow-up, we examined the relationship between MS immunopatterns (at disease onset) and (1) clinical course, (2) radiographic progression (lesion load, global and regional atrophy, cortical lesions, normal-appearing tissue integrity), and (3) cognitive performance. We hypothesized that pattern III lesions, given their association with oligodendrocyte pathology and limited remyelination, would be associated with more pronounced atrophy on MRI as well as more severe cognitive sequelae.

Materials and Methods

Participants

Patients were recruited from the Multiple Sclerosis Lesion Project (MSLP), an ongoing prospective study on pathological, clinical and radiographic correlates in MS. Patients are identified as eligible for the MSLP when they have had a brain biopsy as part of their medical care for the diagnosis of MS. All available biopsies in this study were analyzed by a Mayo Clinic neuropathologist, confirming central nervous system inflammatory demyelinating disease (CNS-IDD) consistent with MS. All patients provided written informed consent according to the Declaration of Helsinki; the study was approved by Institutional Review Board (IRB).

Seventy-six participants were recruited for the current study during a face-to-face follow-up at Mayo Clinic, Rochester, MN between July 2014 and October 2018. They underwent detailed clinical, cognitive and neuroimaging evaluations. One patient was excluded based on clinical grounds (prior infliximab therapy),[12] leaving the final cohort of 75 patients with MS. All had at least one early active demyelinating lesion on histopathology, and no clinical or pathological evidence of another etiology.

Neuropathology review

Patients had undergone stereotactic or open brain biopsy as part of the diagnostic evaluation of an indeterminate brain lesion, which was pathologically confirmed to be consistent with CNS inflammatory demyelinating disease. Paraffin-embedded 5 μm sections were stained with hematoxylin–eosin, Luxol fast blue (LFB) myelin stain and periodic acid–Schiff (PAS) reaction. Immunocytochemistry was performed using an avidin-biotin technique,[13] with primary antibodies against myelin oligodendrocyte glycoprotein (MOG, 1:1000, Abcam, USA), myelin-associated glycoprotein (MAG, 1:1000, Abcam, USA), myelin proteolipid protein (PLP, 1:500, Serotec, USA), polyclonal rabbit anti-human glial fibrillary acidic protein (GFAP, 1:3000, Dako, Glostrup, Denmark), neurofilament (1:800, steam antigen retrieval with citric acid buffer pH 6.0, DAKO, Denmark), cyclic nucleotide phosphodiesterase (CNPase; 1:2000, Sternberger, USA), polyclonal rabbit anti-human AQP4 (1:250, Sigma-Aldrich, USA), polyclonal anti C9 neoantigen (C9neo, 1:2000, Dr. Paul Morgan, Cardiff, UK), and monoclonal mouse anti-human C9neo (1:400; Dr. Paul Morgan, Cardiff, UK), T cells (CD3, 1:200, DAKO; CD8, 1:50, DAKO), B cells (CD20, 1:100, DAKO) and macrophages (Kim1p; 1:5000, Dr. Wolfgang Bruck, Berlin, Germany).

Apoptotic oligodendrocytes were defined by nuclear condensation and fragmentation in cells stained by either MOG or CNPase antibodies. In situ hybridization was performed using digoxigenin-labeled riboprobes specific for PLP according to previously described techniques.[14] DNA fragmentation within cell nuclei was determined with the method of in situ tailing.[15]

Immunopattern classification

Demyelination pattern was described as perivascular (small focal demyelination around the vessel), coalescent (perivenous demyelination overlapped between adjacent vessels), or confluent (large demyelinating lesions beyond vascular regions). Stage of demyelinating activity was classified as: (1) no demyelination, (2) early active, (3) late active, (4) inactive, and (5) remyelination according to established criteria.[6] Early active plaques were defined by macrophages immunoreactive for minor myelin proteins (MOG, MAG) as well as major myelin proteins (PLP). They were further categorized into four previously described distinct immunopathological patterns.[1] Patients were grouped according to their early active lesion immunopattern.

Clinical assessment

All patients were evaluated by an MS subspecialty neurologist (CFL, WOT). Patient history, neurological examination, EDSS, clinical course, disease duration, treatment history, and standard medical information were obtained. At evaluation patients were classified as having radiologically isolated syndrome (RIS), clinically isolated demyelinating syndrome (CIDS) or MS by revised McDonald criteria.[16,17] Clinical course at the time of biopsy and at follow-up evaluation was categorized as monophasic (if the patient had not experienced another relapse following CIDS), relapsing–remitting, primary or secondary progressive according to Lublin and Reingold criteria.[18]

Cognitive assessment

All participants completed neuropsychometric assessment with the use of the CogState battery,[19] including six tasks: (1) Detection Task (processing speed); (2) Identification Task (attention); (3) One Card Learning Task (visual recognition/memory); (4) One Card Back Memory Task (working memory); (5) International Shopping List Task (verbal learning and memory); (6) The Groton Maze Learning Task (problem solving and reasoning). Patients were screened for depressive symptoms using the Patient Health Questionnaire-9 (PHQ-9), where the total score ranges from 0 to 27 (scores 1–4: minimal depression, 5–9: minor depression, 10–14: moderate depression, 15–19 moderately severe depression, 20–27: severe depression).[20]

MRI acquisition

MRI scans were acquired on 3 T Siemens Skyra using a 32-channel head coil, including T1-weighted magnetization-prepared rapid gradient echo (MPRAGE), Double-Inversion-Recovery (DIR), Phase-Sensitive Inversion-Recovery (PSIR), T2 and Fluid Attenuated Inversion Recovery (FLAIR), all obtained with 1.0 mm isotropic resolution. Diffusion tensor imaging (DTI) was acquired with 2.0 mm isotropic images (60 directions, axial spin-echo EPI, b-value 1000).

MRI processing

All MRI scans were analyzed through identical processing steps, using FreeSurfer (MGH/Harvard, Boston, MA, USA, version 6.0)[21,22] and FSL (Oxford, UK, version 5.0.8).[23,24] Processing steps included: (1) registration of all sequences to the MPRAGE images via boundary based registration,[23] (2) identification & outlining of white matter lesions using MRIcron,[25] (3) obtaining quantitative lesion measures (lesion count), volume, mean fractional anisotropy (FA) and mean diffusivity (MD) and measure of T1-hypointense signal change (T1-ratio, calculating mean T1-intensity within lesion compared to surrounding normal appearing white matter), as previously described,[26] (4) volumetric and diffusion tensor imaging (DTI) metrics (FA and MD) were obtained from normal appearing white matter (NAWM), cortical and subcortical structures, (5) given anatomical defects related to either disease or surgery, a semi-quantitative measure of biopsy/index lesion destructiveness (biopsy lesion severity score) was assessed on a three-point scale (mild: at most small gliotic biopsy tract visible; moderate: defect limited to single gyrus; and severe: extensive tissue injury), (6) cortical GM segmentation maps and thickness measures were obtained with FreeSurfer.[21,22] Lesion maps were used to perform both T1 and WM segmentation lesion map filling to obtain accurate WM surface boundaries.[27] Tumefactive or large T2 hyperintense lesions were carefully outlined and reviewed again after white matter surface placement was obtained. Where surface errors were present due to large lesion or surgical cavity size, the entire region was outlined with a manually drawn binary mask and utilized as an exclusion mask for cortical thickness measures. All vertices were identified in this exclusion mask and their values of cortical thickness were not utilized in any of the statistical analyses. (7) Regional lobar analysis was performed as previously described.[27] Analyses were performed to assess thickness and cortical measures in both the "surgical" and "non-surgical" hemispheres. (8) Cortical lesions were scored conform to published guidelines,[28] with the following modification. We utilized PSIR to confirm presence of cortical lesions and to assist in localizing the cortical involvement (leukocortical, intracortical, transcortical and subpial). Lesions were scored and adjudicated by two raters (JMT, AKL).

Statistical analysis

We used R version 3.4.2. (http://www.R-project.org/) for all statistical analysis. We compared immunopattern groups on categorical variables using chi-squared tests. To compare immunopattern groups on numeric measures, we first performed a "global" test of group-wise differences using a Kruskal-Wallis test. If the test was significant at $P < 0.05$, we performed pairwise Wilcoxon rank-sum tests. These nonparametric tests were used because they were more robust to outliers yet had comparable power compared to their parametric counterparts. When analyzing volumetric measurements, we also performed linear regression models adjusted for age, disease duration, and total intracranial volume. CogState age-adjusted z-scores were used to account for potential confounding due to age.

Results

Clinical characteristics of the patient cohort

Demographic and clinical characteristics of the cohort are summarized in Table 1. Additional graphic representation of individual patient timelines between index attack, biopsy, last follow-up, and MRI/CogState evaluation is outlined in Fig. 1. The cohort included 45/75 women (60%). The median age at biopsy was 41 years (IQR 32–48, min 9, max 71), and median age at follow-up evaluation was 50 years (IQR 43–64, min 13, max 74). The median duration from the attack that prompted biopsy (index attack) to the last (face-to-face) follow-up evaluation, which coincided with MRI and CogState evaluation, was 11 years (IQR 4–15, min 0, max 40). Median EDSS at index attack was 3.5 (IQR 3–6, min 0, max 9.5), and at the follow-up it was 3 (IQR 2–3.5, min 0, max 8). As for immunotherapy exposure, 43 out of 75 patients received immunotherapy at some point until the last follow-up with at least one of the drugs: glatiramer acetate (n = 16), interferon-beta (n = 20), mitoxantrone (n = 2) cyclophosphamide (n = 4), azathioprine (n = 3), intravenous immunoglobulin pulses (n = 4), long-term steroids (intravenous methylprednisolone infusions or oral route, n = 8).

Immunopattern distribution

Among 75 biopsied individuals there were eight patients who had two sequential biopsies. The distribution of patterns was specific to the individual and stable across biopsies. Pattern I was identified in 14/75 (19%) patients, pattern II in 41/75 (56%), and pattern III in

Table 1. Comparison of clinical characteristics of different immunopathological patterns.

Characteristic	All (n = 73)	IP I (n = 14)	IP II (n = 41)	IP III (n = 18)
Female sex, n (%)	45 (60%)	12 (86%)	24 (59%)	7 (39%)
Age, years				
Index attack	41 (30–48) [9–71]	42 (41–46) [26–60]	41 (30–48) [18–71]	41 (27–46) [10–67]
Biopsy	41 (32–48) [9–71]	42 (41–49) [28–60]	41 (30–52) [18–71]	41 (29–47) [10–67]
Last follow-up	50 (43–64) [13–74]	57 (46–64) [44–72]	50 (41–65) [29–73]	48 (42–57) [13–74]
Disease duration, years				
Index attack–last follow-up	11 (4–15) [0–40]	13 (8–18) [2–27]	10 (4–15) [1–25]	10 (2–15) [0–29]
EDSS				
Index attack	3.5 (3–6) [0–9.5]	3.5 (3–5.5) [2–8]	3.5 (3–6) [1–9.5]	3.5 (3–6) [0–8]
Last follow-up	3 (2–3.5) [0–8]	2.5 (2–3.4) [0–8]	3 (1–3.5) [0–6]	3 (3–4) [0–6]
Diagnosis at last follow-up, n (%)				
MS	57 (76%)	10 (71%)	33 (80%)	12 (67%)
IDS	17 (23%)	3 (21%)	8 (20%)	6 (33%)
RIS	1 (1%)	1 (7%)	0	0
Course at last follow-up, n (%)				
RRMS	47 (63%)	9 (64%)	26 (63%)	12 (67%)
SPMS	3 (4%)	1 (7%)	1 (2%)	0
Monophasic	24 (32%)	3 (21%)	14 (34%)	6 (33%)
Asymptomatic	1 (1%)	1 (7%)	0	0

Values shown are number (%) or median (interquartile range) [range]. No statistically significant differences between different IPs were noted, except for sex (P = 0.03). For two cases where IP could not be identified the diagnosis and course were MS and monophasic, and MS and SPMS, respectively.

IDS, isolated demyelinating syndrome; IP, immunopattern; RIS, radiologically isolated syndrome; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive MS (without relapses).

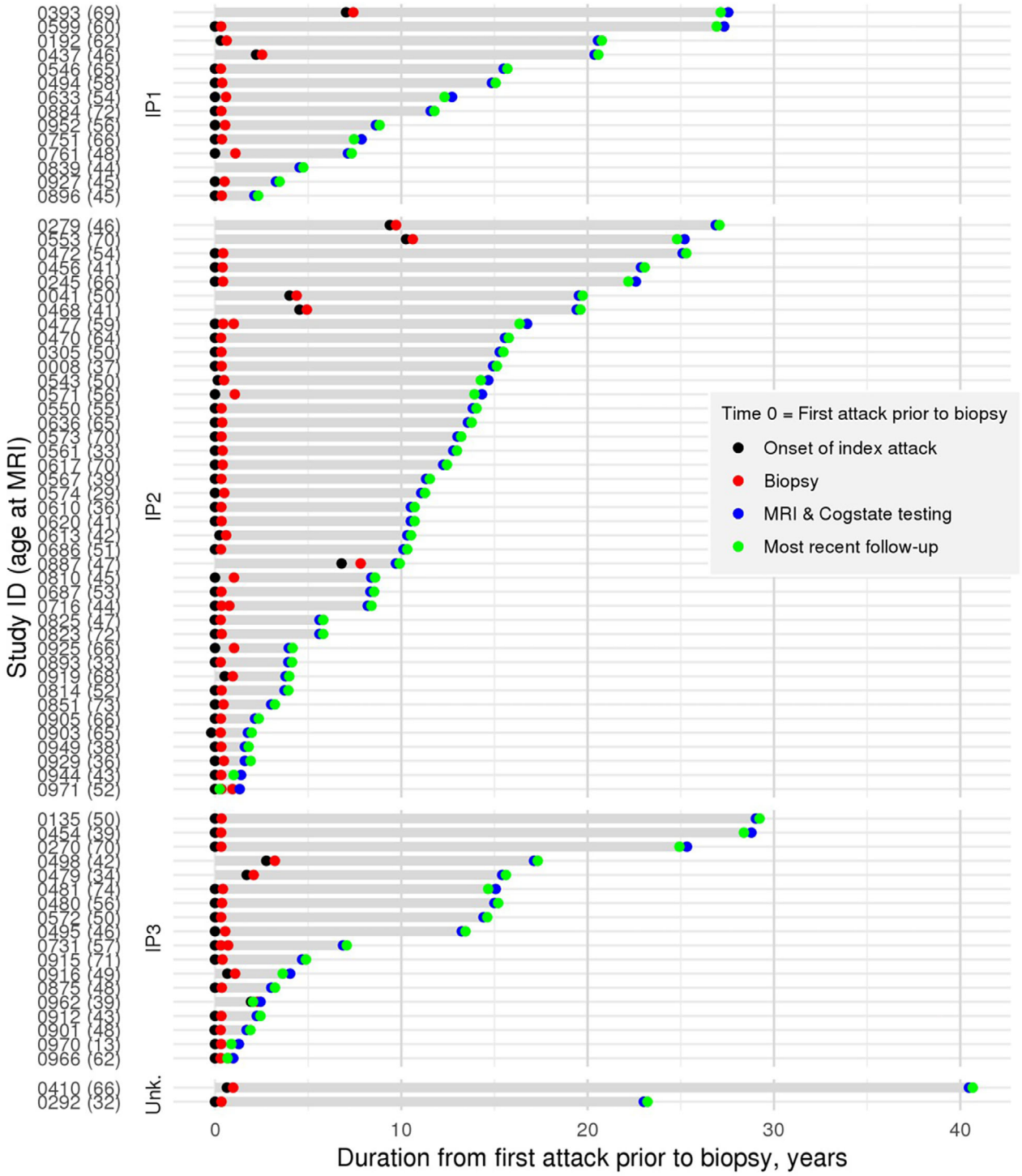

Figure 1. Patient timeline across immunopatterns. Time 0 is disease onset defined as the first attack prior to biopsy. Black dots indicate the time of the index attack. Red dots indicate the biopsy. Blue dots indicate CogState and MRI evaluations while green dots indicate most recent follow-up (which typically coincided with CogState and MRI evaluations).

18/75 (25%) patients. In 2 patients immunopattern could not be determined because of poor tissue and/or staining quality. No individual with immunopattern IV was identified in the present cohort. The typical pathological features of immunopatterns I, II and III are summarized in Fig. 2.

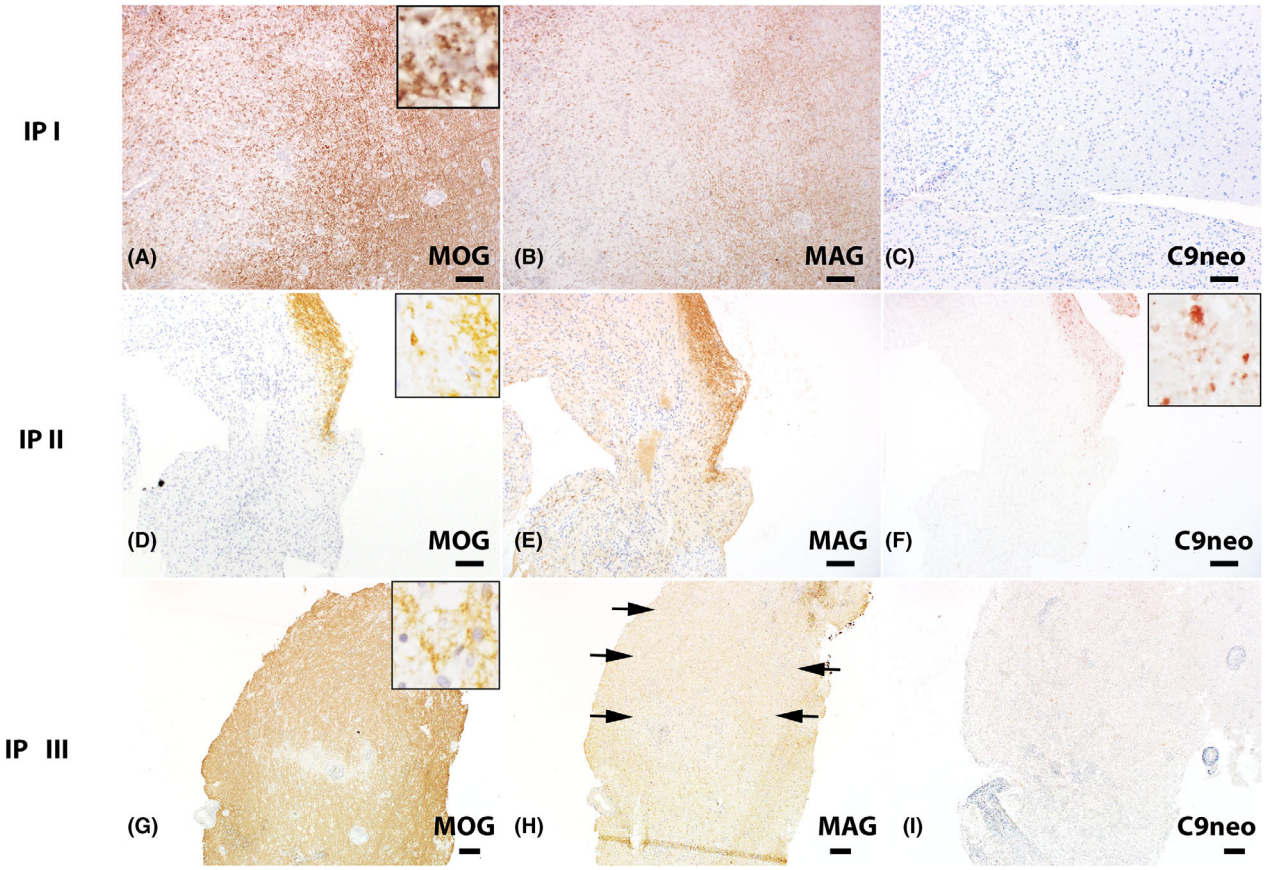

Figure 2. Pathologic characteristics of early active demyelination immunopatterns seen in the study cohort. Panels (A–C) depict immunohistochemistry features of immunopattern I (IP I); panels (D–F) depict immunopattern II (IP II), and panels (G–I) describe immunopattern III (IP III). Panels A, D and G show myelin oligodendrocyte glycoprotein (MOG) stain, with MOG-laden macrophages visualized in the enlarged view (40X) in the framed panel. This is a common feature of all early active lesions. Panels B, E and H show myelin associated glycoprotein (MAG) stain, with MAG loss corresponding to MOG loss area in immunopatterns I and II (panels B and E, respectively). In IP III (panel H) MAG loss (indicated with arrows) is more prominent and beyond MOG loss area. Panels C, F and I present complement C9 neo-antigen (C9neo) stain. IP I and IP III show no complement deposition. IP II (panel F) is accompanied by complement-laden macrophages in the early active demyelinating lesion (indicated in the enlarged view (40X) in the framed panel). Scale bar in A–F = 100 μm, scale bar in G–I = 200 μm.

Clinical characteristics across immunopatterns

Patients within specific immunopattern groups did not differ significantly with regards to age at index attack (P = 0.67), age at the last follow-up (P = 0.34), EDSS at the last follow-up (P = 0.38) or disease duration at the last follow-up (P = 0.45). The proportions of different disease courses were similar across immunopatterns (P = 0.39), as shown in Fig. 3. On the other hand, the proportion of women varied by immunopattern (P = 0.03) and ranged from 86% in pattern I to 39% in pattern III.

Neuroimaging characteristics across immunopatterns

Of the seventy-five patients enrolled in the study, three were excluded from detailed MRI analysis: one became ill and was not able to complete the scan, one had excessive motion artifact, and in one there was extensive lesion/post-operative tissue injury precluded detailed volumetric imaging analysis.

Semi-quantitative ratings

Distribution of semi-quantitative biopsy/index lesion severity score was without significant differences across immunopatterns (P = 0.73). The lesion damage in index lesion was distributed across immunopatterns as follows (pattern I, II and III respectively): mild (21, 14, 23%, P-value ***), moderate (36, 16, 28%, P-value = ***), and severe (43, 41, 50%, P-value = ***). Distribution of index lesion severity scores across immunopatterns, alongside neuroimaging examples, are presented in Fig. 4.

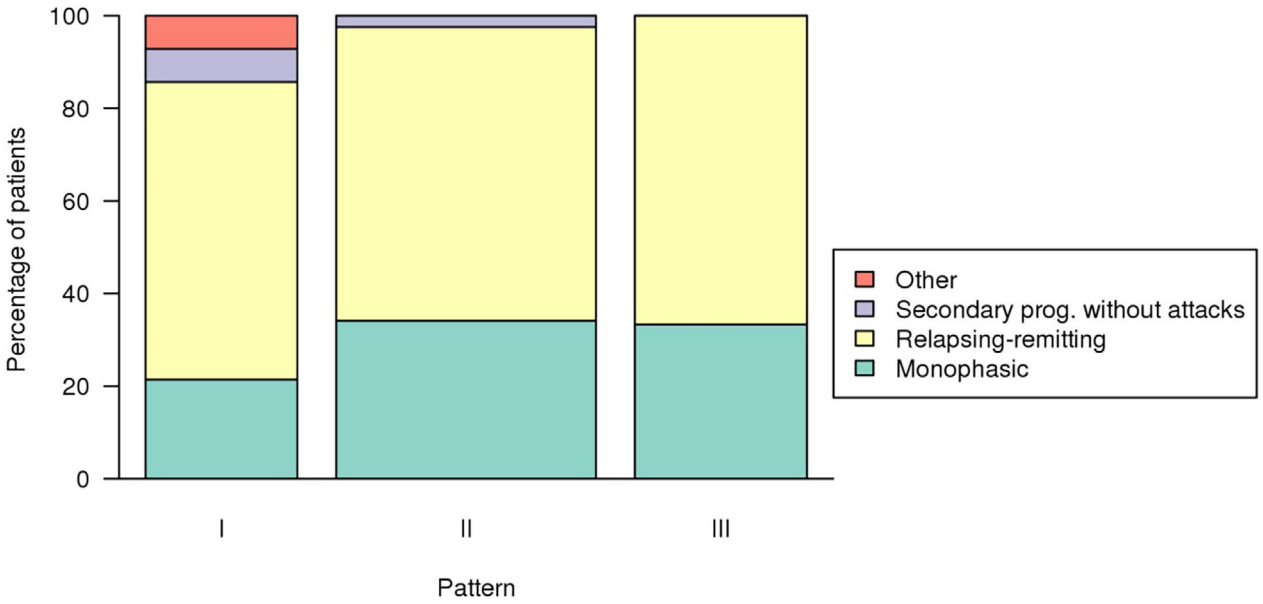

Figure 3. Clinical course at last follow-up by immunopattern showing percentage of patients. Bar widths indicate relative sample size. The proportions across immunopatterns are similar (P = 0.39).

Volumetric analysis

No significant differences were found between immunopatterns with regards to T2 white matter lesion number and volume at follow-up, except for immunopattern III vs II (higher WM lesion volume, lower lesion number, P = 0.05).

Although we found a slightly lower T1 ratio (darker T1 signal (black hole) within the lesions) in immunopattern I group, this was not significantly different between the groups (P = 0.27). We carefully reviewed whether there was any association with the surgical intervention and reviewed T1-ratios between surgical and non-surgical hemisphere, which did not result in any change of these findings.

Analyzing regional brain volumes, immunopattern subgroups did not differ significantly with respect to the following volumes: cortex, thalamus, cerebellum, midsection of corpus callosum, hippocampus, amygdala, caudate, and putamen (detailed measures are reported in Table 2). Subtle differences were detected in some of the deep gray matter structures (pallidum, caudate).

Cortical involvement on MRI

Gray matter thickness

Regional gray matter thickness did not differ between immunopatterns in general (P = 0.75), or in regional analysis (frontal (P = 0.29), precentral (P = 0.88), postcentral (P = 0.76), temporal (P = 0.39), parietal (P = 0.73), occipital (P = 0.46), cingulate (P = 0.39), and insula (P = 0.40)).

Cortical lesions

Out of 72 participants, 60 had one or more cortical lesions identified on MRI. Specifically, 58 had leukocortical, 19 had transcortical, 5 had intracortical lesions, and one patient had a single subpial lesion that could be detected. Across patients, the median number of cortical lesions identified was 4 (range 0 to 50). In 10 patients, there was a single large confluent WM lesion with vast cortical involvement (recorded as a single large cortical lesion), and in the additional 20 there were other cortical lesions identified alongside a large confluent WM-cortical lesion. No statistical difference was found between immunopatterns and cortical lesions.

Tissue integrity analysis

Fractional anisotropy and mean diffusivity measures of cerebral cortex, amygdala, hippocampus, thalamus, and normal-appearing white matter did not differ between immunopattern subgroups, as shown in Table 2. In order to assure tissue integrity measures were not affected by surgery, a separate analysis was performed by hemisphere (non-surgical vs surgical) between the groups, and no additional statistical significances were observed.

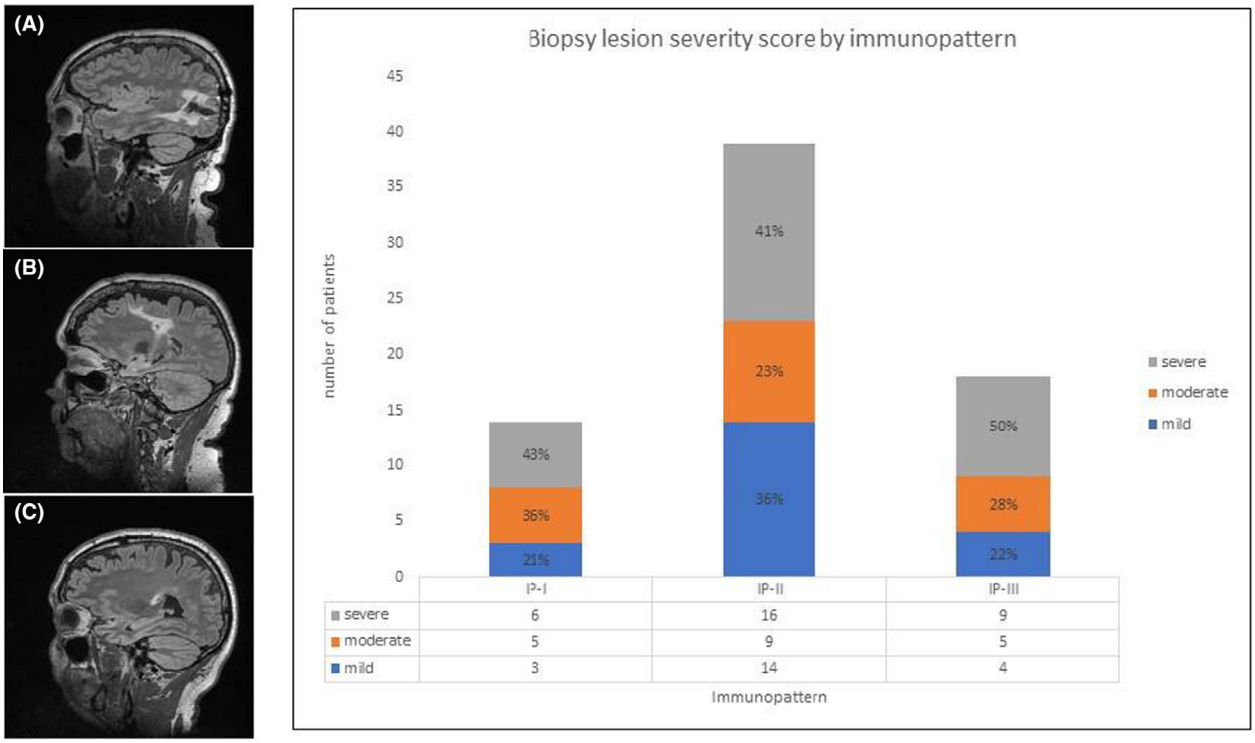

Figure 4. Neuroimaging examples and distribution of mild, moderate and severe biopsy/index lesion severity scores across immunopatterns. Panels (A–C) depict sagittal FLAIR MR imaging examples representative of severe (A), moderate (B), and mild (C) biopsy/index lesion severity score ratings. The right-hand panel presents the distribution of biopsy lesion severity scores by different immunopatterns, showing percentage of patients within colored bars (blue – mild destruction, as visualized in panel A; orange – moderate destruction, as visualized in panel B; and gray – severe destruction, as visualized in panel C) and number of patients in the bottom table. Distributions are similar (P = 0.73) across immunopatterns.

Cognitive characteristics across immunopatterns

CogState was completed in 72/75 participants: two did not complete one task, and one participant did not complete four tasks within the CogState battery. For most of the CogState tasks no differences in performance for both, raw data and age-adjusted z-scores, were found across the immunopatterns (Fig. 5). However, there was a difference between the immunopatterns for the episodic memory domain (international shopping list and delayed recall score), reaching significance based on a three-group Kruskal-Wallis test. On pairwise Wilcoxon rank sum testing, the difference between pattern I and pattern III was significant (P = 0.01 for total items and P = 0.01 for delayed recall). This difference between patterns I and III amounted to about one word on average and was significant after adjusting for disease duration and PHQ-9 total score. Also, the prevalence of cognitively impaired patients did not differ across immunopatterns.

PHQ-9 score was assessed in 65/75 patients. The median PHQ-9 score was 4 (IQR 1–8). PHQ-9 total score did not differ across the immunopatterns (P > 0.99). The distributions of patient-reported PHQ-9 related difficulties with work, including home maintenance and social interactions (P = 0.86).

Discussion

The immunopathological classification of the study cohort reflects previously reported distribution of early active lesion patterns.[1,9] We did not identify any pattern IV lesions in our cohort, which have so far only been reported in three cases with a primary progressive course.[1] Overall, we did not identify significant differences between long-term clinical, neuroimaging and cognitive correlates of different MS lesion immunopatterns, despite pattern III being pathologically characterized by limited remyelination and more pronounced oligodendrocyte damage.

Table 2. Summary statistics for radiographic measures across immunopatterns.

	All	IP I	IP 2	IP 3	*P*
Lesions					
WM lesion number	14 (6, 32) [1 to 172]	8 (6, 22) [1 to 74]	17 (6, 36) [1 to 172]	10 (6, 18) [1 to 55]	0.31
WM lesion volume	10 (3, 23) [0 to 66]	11 (3, 15) [0 to 46]	7 (3, 26) [0 to 48]	13 (6, 21) [1 to 66]	0.54
Mean T1 ratio	0.74 (0.70, 0.79) [0.00 to 0.85]	0.72 (0.66, 0.76) [0.34 to 0.79]	0.74 (0.72, 0.80) [0.00 to 0.85]	0.74 (0.70, 0.80) [0.35 to 0.84]	0.27
Biopsy lesion severity (mild/moderate/severe)	31%/26%/43%	21%/36%/43%	36%/23%/41%	22%/28%/50%	0.73
Volumes					
Cortex, total	494 (459, 537) [384 to 705]	477 (452, 500) [384 to 524]	512 (461, 549) [408 to 617]	492 (466, 511) [417 to 705]	0.18
Hippocampus	7.9 (7.4, 8.2) [6.0 to 10.8]	7.7 (7.3, 7.9) [6.8 to 8.7]	7.9 (7.1, 8.3) [6.4 to 10.8]	7.9 (7.8, 8.3) [6.0 to 10.8]	0.21
Amygdala	3.2 (2.9, 3.5) [2.1 to 5.4]	3.0 (2.7, 3.3) [2.5 to 3.8]	3.1 (2.8, 3.6) [2.1 to 4.1]	3.2 (3.1, 3.4) [2.2 to 5.4]	0.26
Thalamus	12.8 (11.6, 14.4) [7.8 to 18.9]	12.3 (10.7, 12.7) [10.0 to 15.0]	13.1 (11.8, 14.8) [7.8 to 18.8]	12.9 (12.3, 14.1) [10.3 to 18.9]	0.12
Pallidum	3.5 (2.9, 3.8) [1.5 to 5.6]	2.8 (2.1, 3.0) [1.8 to 3.8]	3.5 (2.9, 3.9) [1.5 to 5.6]	3.5 (3.5, 4.2) [2.5 to 4.8]	0.004**
Putamen	8.5 (7.2, 9.3) [4.7 to 11.8]	7.8 (7.2, 8.4) [5.8 to 9.0]	8.5 (7.0, 9.8) [4.7 to 11.5]	8.9 (8.1, 9.1) [5.9 to 11.8]	0.20
Caudate	6.5 (5.7, 7.1) [4.4 to 9.1]	5.9 (5.4, 6.5) [4.5 to 6.8]	6.6 (6.2, 7.4) [4.4 to 9.1]	6.9 (5.4, 7.1) [5.0 to 8.6]	0.03*
Cerebellum	110 (110,118) [80 to 143]	108 (99,114) [93 to 122]	110 (102,119) [90 to 142]	104 (100,118) [89 to 143]	0.75
Corpus callosum	2.9 (2.5, 3.4) [0.8 to 4.0]	2.8 (2.4, 3.1) [1.6 to 3.5]	3.0 (2.7, 3.4) [1.4 to 3.9]	2.8 (2.5, 3.4) [0.8 to 4.0]	0.30
Fractional anisotropy					
Cortex	0.13 (0.12, 0.13) [0.09 to 0.15]	0.12 (0.11, 0.13) [0.10 to 0.14]	0.13 (0.12, 0.14) [0.09 to 0.15]	0.13 (0.12, 0.13) [0.10 to 0.15]	0.10
Hippocampus	0.14 (0.13, 0.15) [0.11 to 0.17]	0.13 (0.12, 0.14) [0.12 to 0.17]	0.14 (0.13, 0.15) [0.11 to 0.17]	0.14 (0.13, 0.15) [0.11 to 0.17]	0.55
Amygdala	0.17 (0.16, 0.19) [0.13 to 0.21]	0.16 (0.16, 0.17) [0.15 to 0.19]	0.18 (0.16, 0.19) [0.13 to 0.21]	0.17 (0.15, 0.19) [0.13 to 0.21]	0.44
Thalamus	0.29 (0.28, 0.31) [0.26 to 0.36]	0.29 (0.28, 0.31) [0.26 to 0.33]	0.30 (0.28, 0.32) [0.26 to 0.36]	0.29 (0.28, 0.31) [0.26 to 0.34]	0.72
Corpus callosum	0.52 (0.43, 0.56) [0.20 to 0.64]	0.55 (0.46, 0.56) [0.20 to 0.64]	0.53 (0.41, 0.56) [0.22 to 0.61]	0.49 (0.46, 0.55) [0.31 to 0.59]	0.72
NAWM	0.32 (0.31, 0.34) [0.29 to 0.38]	0.32 (0.31, 0.33) [0.31 to 0.37]	0.32 (0.31, 0.33) [0.29 to 0.36]	0.33 (0.31, 0.34) [0.29 to 0.38]	0.52
Cortical thickness	2.6 (2.6, 2.7) [2.5 to 3.0]	2.6 (2.5,2.7) [2.5 to 2.8]	2.6 (2.6, 2.7) [2.5 to 3.0]	2.6 (2.6–2.7) [2.5 to 3.0]	0.84

Unless otherwise indicated, values shown are median (interquartile range) [range]. Volumes are in cm^3, fractional anisotropy is unitless, and cortical thickness is expressed in mm.
*$P < 0.05$
**$P < 0.01$
***$P < 0.001$.

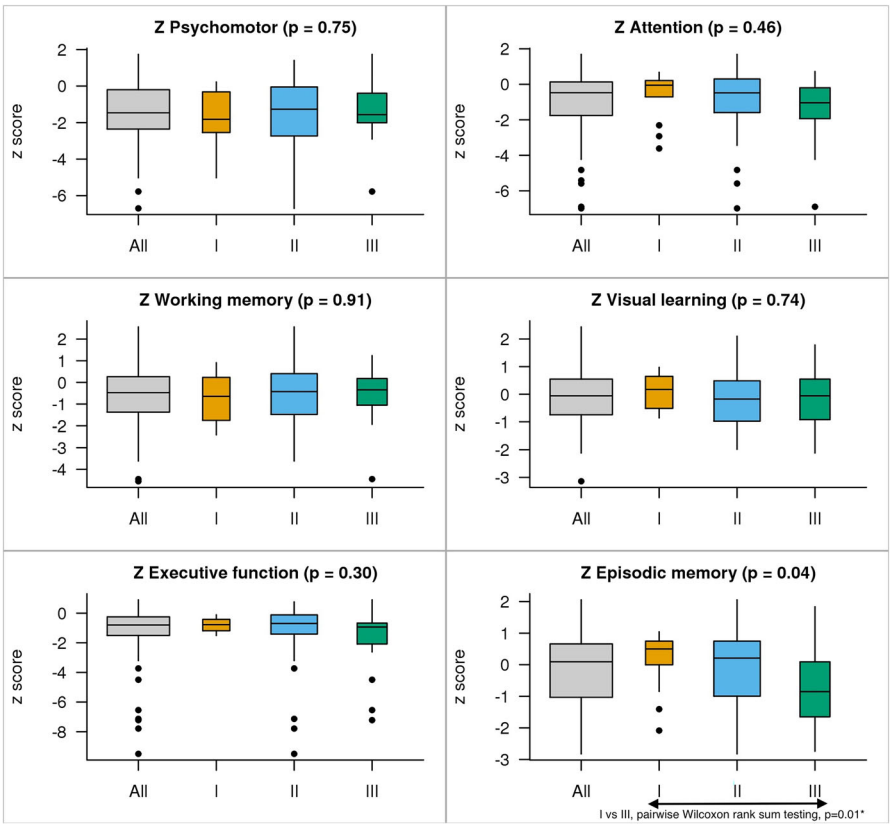

Figure 5. CogState performance across the immunopatterns.

Several possible reasons may underlie the apparent lack of clinical-radiographic-cognitive distinction between different immunopatterns in our study cohort.

First, while active white matter lesions are pathological substrates for relapses; MS progression is most likely driven by mechanisms that converge across immunopatterns, regardless of pathogenic mechanisms that drive the acute demyelinated plaque. Therefore, long-term outcomes, which are largely dependent on progression, would be similar regardless of different early active plaque immunopatterns.[9] The mechanisms of MS progression, independent of immunopattern, include: the smoldering slowly expanding plaque; cortical demyelination; diffuse NAWM pathology; axonal injury with chronic energy failure related to denuded axon requiring increasing energy demands; superimposed aging processes shared across all white matter immunopatterns.[29–33]

Selection bias may also have contributed to the findings. The current study cohort includes biopsy-proven CNS inflammatory demyelinating disease patients who were available for face-to-face follow-up. The study flow diagram for the current cohort is described in detail in a recent paper from our group.[34] Consequently, it is limited to patients with overall favorable outcome following the attack that prompted biopsy and omits a significant proportion of those with severe disability or mortality related to aggressive onset of the disease. Therefore, our conclusions are representative of the survivors of the attack that led to brain biopsy. It has already been suggested that patients with IP-III have a worse prognosis in the short-term, reaching higher disability during index attack[9] and being less likely to respond to treatment.[10,11] However, so far it has not been proven that either survival or long-term disability are worse in patients with IP-III compared to other immunopatterns. In fact, just recently we demonstrated that long-term survival after biopsy was similar regardless of immunopattern.[9]

Also, participants had to be physically able and interested in traveling to a tertiary referral center for an extensive follow-up evaluation. While many clinical and personal factors are associated with this selection process, we do not believe immunopattern would have a direct effect. That is, we expect it is not the immunopattern *per se* that could influence participation, but the clinical manifestations of the disease. For example, immunopattern could have an indirect effect if factors associated with

participation were also associated with immunopattern. However, we found that clinical and imaging characteristics were largely comparable across immunopatterns and concluded that our data were informative about long-term outcomes. Importantly, the distribution across immunopatterns in our follow-up study was similar to baseline distribution.[8] Finally, statistical power could also be a limiting factor since IP groups in our cohort are relatively small.

The study cohort is heterogenic with regards to the follow-up period, with a median of long-term follow-up of 11 years, ranging from a minimum of 0 (no follow-up at all) to a maximum of 40 years. This variability makes the comparison between patients in the different immunopattern groups challenging. It needs to be pointed out that when we stratified patient subgroups by disease duration (<5, 5–10 and >10 years), it did not influence the results.

Another limitation is the lack of baseline cognitive and radiographic data. Unfortunately, this was not possible due to the study design – the patients were recruited for a face-to-face follow-up after a median of 11 years after the primary event that led to biopsy and, eventually, pathology review at the Mayo Clinic.

There is a large body of evidence suggesting that diverse immune and possibly toxic mechanisms are reflected in pathological inter-individual heterogeneity of MS lesions. It has even been suggested that MS could be a syndrome with a final common pathway of CNS demyelination and destruction, rather than a single disease.[35] In pattern III lesions mitochondrial involvement and hypoxia-like tissue injury are suggested to play a role,[36] resulting in the preferential loss of MAG, as seen in ischemic stroke.[37] Importantly, neuropathological changes in NAWM, suggestive of pre-demyelinating lesions, in prior studies were found exclusively in IP-III cases.[36]

It remains to be established whether different immunopatterns are linked with specific MS phenotypes, including cognitive disability. Cognitive performance of MS patients in different immunopatterns had not been studied to date. Cognitive dysfunction is common in MS, affecting 40–60% of all patients, which is not necessarily correlated with disability.[38] The neuropathological substrate of cognitive dysfunction in MS is unknown. The role of focal inflammation has been suggested based on MRI studies, as reflected by the relationship between poor cognitive performance and the presence of active MRI lesions.[39,40] Cortical lesions have also been implicated,[41,42] as well as deep gray matter involvement.[41] In this study we verified whether immunopathological heterogeneity at disease onset, as defined by immunopattern, was reflected in the long-term cognitive outcomes. We found a slight, yet statistically significant, difference between IP-I and IP-III in episodic memory scores. Despite statistical significance, this finding is interpreted with caution given the small magnitude of the difference. No other clinically meaningful differences in cognition between immunopatterns were found.

In the same long-term follow-up biopsy-proven CNS-IDD cohort we have recently analyzed correlations between radiographic features and cognition.[34] We found that cognitive impairment was overall correlated not only with total WM lesion volume but also with the extent of biopsy-related tissue damage. Also, we observed that executive function correlated solely to callosal volume and DTI-measured callosal integrity, and working memory was associated with cortical volume instead.[43]

Here, we used multivariable modeling to further examine predictors of working memory, episodic memory, and attention domains. After adjusting for age and sex, cortical volume appeared to be more closely related to cognitive performance than WM lesion volume and immunopattern was not associated with these domains. Specifically, a 10% reduction in cortical volume was associated with a 0.9 z-score unit reduction in working memory ($P = 0.006$), a 0.4 unit reduction in episodic memory ($P = 0.11$), and a 1.1 unit reduction in attention ($P = 0.01$). On the other hand, a 10% increase in WM lesion volume was associated with much smaller effect sizes: 0.03 z-score units lower for working memory ($P = 0.03$), 0.02 units lower for episodic memory ($P = 0.08$), and 0.02 units lower for attention ($P = 0.28$). Although we did not show any immediate effect of IP on cognition, we confirmed that cortical involvement may be of the essence for MS-related cognitive deficits. Importantly, ours was a white matter based pathological study and it is not known whether cortical pathology differs across the immunopatterns. This would suggest that factors independent of the white matter plaque are key drivers of MS-related cognitive impairment. It has been shown that cortical involvement is a strong predictor of long-term cognitive function. Just recently, Haider et al. demonstrated that cortical MTR was the strongest factor to explain cognitive outcome measures in an initially clinically isolated syndrome cohort that was followed up for 30 years.[44] Another study showed that cortical atrophy was the strongest predictor of cognitive decline over 5 years.[45] On the other hand, different studies suggested that it was white matter lesions-associated disruption of cortico-subcortical tracts that was a predominant driver of information processing speed decline.[46]

It needs to be acknowledged that each MS plaque might influence cognitive processes through multiple mechanisms that also vary within disease duration. Immunopattern defines early active white matter lesions

only. The fact that we did not establish a clear link between the immunological features of early active demyelination and long-term development of cognitive decline may suggest that in early MS cognitive impairment is driven by immunopattern-independent cortical lesions. In later MS stages cognitive disability may rather depend on mechanisms associated with disease progression, which we believe is less associated with plaque activity but rather with chronic axonal damage.[9]

Also, it needs to be underscored that although all patients included in this study had pathology consistent with MS, 24% of the cohort did not meet clinical McDonald criteria for MS diagnosis, including one case (1%) of Radiologically Isolated Syndrome, and 17 cases (23%) of Isolated Demyelinating Syndrome. Despite pathological consistency with MS, these isolated cases could be associated with different biological mechanisms leading to demyelinating lesions formation.

Finally, there is a need for additional radiographic-pathological correlative studies to define in vivo markers to monitor the dynamic pathology of MS. Moreover, the analysis of pathological correlates of cognitive deficits in MS would be key in designing future therapeutic strategies and clinical trials in this regard as the effect of immunotherapies on cognition is still the matter of debate.[47] Availability and accurate characterization of MS lesions of interest is crucial for better understanding disease mechanisms.

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Author Contributions

Alicja Kalinowska-Lyszczarz: acquisition/analysis, drafting manuscript/figures. Jan-Mendelt Tillema: conception/design, acquisition/analysis, drafting manuscript/figures. William Oliver Tobin: conception/design, acquisition/analysis, drafting manuscript/figures. Yong Guo: acquisition/analysis. Imke Metz: acquisition/analysis. Wolfgang Brück: acquisition/analysis. Hans Lassmann: acquisition/analysis. Stephen D. Weigand: acquisition/analysis, drafting manuscript/figures. John D. Port: conception/design, acquisition/analysis, drafting manuscript/figures. Monica Giraldo-Chica: acquisition/analysis. Claudia F. Lucchinetti: conception/design, drafting manuscript/figures. Dr. Lucchinetti had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of Interests

All authors have no financial interests or potential conflicts of interest to report with regards to the current study.

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Data Availability Statement

Anonymized data are available from the corresponding author (CFL) from any qualified investigator, upon reasonable request.

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