Magnetic Resonance Imaging Correlates of Multiple Sclerosis Immunopathological Patterns

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Objective: Histology reveals that early active multiple sclerosis lesions can be classified into 3 main interindividually heterogeneous but intraindividually stable immunopathological patterns of active demyelination (patterns I–III). In patterns I and II, a T-cell- and macrophage-associated demyelination is suggested, with pattern II only showing signs of a humoral immune response. Pattern III is characterized by inflammatory lesions with an oligodendrocyte degeneration. Patterns suggest pathogenic heterogeneity, and we postulated that they have distinct magnetic resonance imaging (MRI) correlates that may serve as biomarkers.

Methods: We evaluated in an international collaborative retrospective cohort study the MRI lesion characteristics of 789 conventional prebiopsy and follow-up MRIs in relation to their histopathologically classified immunopathological patterns (n = 161 subjects) and lesion edge features (n = 112).

Results: A strong association of a ringlike enhancement and a hypointense T2-weighted (T2w) rim with patterns I and II, but not pattern III, was observed. Only a fraction of pattern III patients showed a ringlike enhancement, and this was always atypical. Ringlike enhancement and T2w rims colocalized, and ringlike enhancement showed a strong association with macrophage rims as shown by histology. A strong concordance of MRI lesion characteristics, meaning that different lesions showed the same features, was found when comparing biopsied and nonbiopsied lesions at a given time point, indicating lesion homogeneity within individual patients.

Interpretation: We provide robust evidence that MRI characteristics reflect specific morphological features of multiple sclerosis immunopatterns and that ringlike enhancement and T2w hypointense rims might serve as a valuable noninvasive biomarker to differentiate pathological patterns of demyelination.
Multiple sclerosis (MS) is an inflammatory demyelinating disease with unknown etiology and a striking heterogeneity with regard to clinical characteristics, therapeutic response, and magnetic resonance imaging (MRI) features. Furthermore, histological analysis reveals substantial differences when comparing lesions from different patients. Although all lesions show the common characteristics of demyelination, inflammation, axonal damage, and astrogliosis, major histological differences can be found, allowing their classification into 3 main immunopathological patterns of active demyelination (patterns I–III). Immunopathological patterns among early active MS lesions have been shown to be consistent within an individual over space and time. These patterns suggest different mechanisms of lesion development. Patterns I and II are typically sharply demarcated, and all myelin proteins are lost to an equal extent. However, only pattern II lesions are associated with immunoglobulins and complement deposited along myelin sheaths and present within macrophages, suggesting an antibody- and complement-mediated mechanism of demyelination. In contrast, pattern III lesions are characterized by an ill-defined lesion edge, the presence of apoptotic oligodendrocytes, and a preferential loss of myelin-associated glycoprotein (MAG) compared to other myelin proteins. MAG is located in distal oligodendrocyte processes, and its loss is considered to be a marker of metabolically stressed oligodendrocytes. Changes observed in pattern III lesions suggest oligodendrocyte damage preceding demyelination. Thus far, these immunopathological patterns can only be diagnosed by histology. The MRI correlates are unknown.

MRI is used as a routine diagnostic tool in MS with a broad spectrum of MRI findings. Contrast-enhancing lesions on postcontrast T1-weighted (T1w) MRIs are accepted as a marker of blood–brain barrier breakdown, and may show different patterns of enhancement. Ring enhancing lesions, characterized either by an incomplete, open ring, or by a complete, closed ring, may be found in MS patients. The frequency of patients with such lesions varies considerably, depending on the investigated cohorts, the observation period, the number of MRIs analyzed, and technical approaches, and ranges from 12 to >90% of patients, with the highest percentages found in very active cohorts. Hypointense T2w rims may be observed and are characterized by a border of decreased signal intensity at the lesion edge. Histological–radiological studies have suggested that the pathological correlates are accumulations of macrophages (macrophage rims). A colocalization of ring enhancing lesions with hypointense rims on T2w images has been described.

The aim of the present study was to analyze retrospectively the MRI lesion characteristics in a cohort of 161 MS patients in relation to their histopathologically defined immunopathological patterns as well as histologically defined lesion edge characteristics. With a better understanding of the linkage between MRI features and underlying immunopattterns, MRI might serve as a biomarker that helps clinicians to make more informed treatment decisions.

Patients and Methods
This retrospective cohort study was approved by the Mayo Clinic Institutional Review Board (IRB #2067-99) and the ethics committee of the University Medical Center Göttingen (#19/09/10), and informed consent was obtained from each subject. The study included analysis of formalin-fixed, paraffin-embedded archival brain biopsy tissue, pre- and postbiopsy MRIs, and medical records from 161 subjects with brain biopsy–proven inflammatory demyelination consistent with MS (n = 121 from the Mayo Clinic Rochester, USA and n = 40 from the University Medical Center Göttingen, Germany). The pathological features of biopsied lesions, which are often tumefactive, demonstrate the hallmarks of classic inflammatory demyelination with myelin loss, relative axonal preservation, variable inflammation, and gliosis, and do not differ in their pathology from other acute lesions. Patients were identified from a large cohort of 821 biopsied patients with inflammatory demyelinating lesions belonging to the MS Lesion Project, a unique international collaborative effort to study the pathologic, clinical, and radiographic correlates of MS lesions. Brain biopsies were performed for clinical differential diagnostics and not for research purposes. Inclusion criteria for the present study were (1) histopathological diagnosis of inflammatory demyelinating lesions consistent with early active demyelinating MS, classified into one of the main immunopathological patterns I–III according to published criteria; (2) at least one MRI obtainable for review; and (3) clinical documentation available for the analysis of basic demographic and clinical data. Excluded were patients with (1) acute disseminated encephalomyelitis, defined as demyelination limited to perivenular areas; (2) neuromyelitis optica spectrum diseases, defined by clinical, serological, and histological criteria; and (3) subjects with other confounding diseases such as neoplasm, infection, vascular disease, or prior brain irradiation.

Neuropathological Analyses
In short, formalin-fixed, paraffin-embedded slides were stained with hematoxylin and eosin, Luxol-fast blue/periodic
acid-Schiff, and Bielschowsky silver impregnation, as well as with antibodies against myelin proteins (anti-proteolipid protein [PLP], anti-myelin basic protein [MBP], anti-myelin oligodendrocyte glycoprotein [MOG], anti–2′,3′-cyclic nucleotide 3′-phosphodiesterase [CNP], anti-MAG), against the terminal complement complex and complement C9neo antigen, and against aquaporin-4. Macrophages were stained with KiM1P antibodies (Prof Radzun, Göttingen, Germany) and anti-CD68 antibodies (Dako, Glostrup, Denmark). For detailed information see Stork et al.21

First, the demyelinating activity was determined based on published criteria with early active demyelinating lesions containing myelin-laden macrophages immunoreactive both for minor (MAG, MOG, CNP) and major (PLP, MBP) myelin proteins.22 Early active demyelinating lesions represent the earliest lesion stages and are a prerequisite for immunopattern classification. Those lesions were then classified into one of the immunopathological patterns I–III (Fig 1).1

The lesion edge was classified as either a sharp or ill-defined lesion edge using (1) the macrophage stainings KiM1P or CD68 and (2) the myelin stainings PLP or CNP. In addition, the presence of a macrophage rim, defined as an accumulation of macrophages at the lesion edge, was determined.

Histopathological analysis was performed by board-certified neuropathologists (W.B., I.M., Y.G.). Tissue sections were analyzed using an Olympus BX41 microscope equipped with a DP20 camera (Olympus Optical, Hamburg, Germany). Figures were prepared in Publisher 2016 (Microsoft, Redmond, WA).

**Radiographic Assessment**

A total of 789 conventional prebiopsy and follow-up MRIs were reviewed using procedures previously reported.7 The majority of individuals had one prebiopsy MRI (median time from a patient’s last prebiopsy MRI to biopsy was 5 days, interquartile range [IQR] = 1—10 days). Due to the retrospective nature of the study, a variety of scanners and imaging techniques were used. T1w, T2w, fluid-attenuated inversion recovery, and T1w + gadolinium sequences were studied. The biopsied lesion, termed the index lesion, and any nonbiopsied lesions were evaluated separately for (1) lesion location (periventricular, frontal, parietal, occipital, temporal, corpus callosum, subcortical, juxtacortical, cortical, subcortical U fiber involvement, deep gray matter involvement, brain stem, cerebellum, spinal cord, other location), number, and size (T2w to T2w margin as well as discernable lesion, meaning the lesion diameter without surrounding edema); (2) the presence and degree of edema (mild = ≤1cm from the lesion; moderate = 1–3cm from the

lesion; marked = ≥3cm from the lesion); (3) the presence and grade of mass effect (mild = sulcal effacement; moderate = minimal subfalcine or uncal herniation of <1cm; marked = subfalcine or uncal herniation >1cm); (4) the presence of a T2w hypointense rim; and (5) the presence and pattern of gadolinium enhancement. T2w hypointense rims were defined by a clearly visible border of decreased signal intensity compared with the lesion center as well as the surrounding edema. The following enhancement patterns were differentiated: homogenous (uniform and solid enhancement throughout the lesion), ringlike (including open ring with the ring opening toward the gray matter, closed ring with a complete circular border, and incomplete ring or arc with only partial surrounding of the lesion border), Baló-like with multiple concentric rings, or heterogeneous (including diffuse and patchy, fluffy/cotton ball–like, nodular with areas of enhancement of >2mm and punctuate with areas of enhancement of <2mm). Single patients could show different enhancement patterns. MRI features were evaluated by 2 raters (R.H.G., I.M.) blinded to the patient’s immunopattern after training sessions to achieve consistency. In addition, 10 randomly selected patients were crossed to assess the agreement for results of MRI variables, and confirmed agreement on all measures of interest.

**Clinical Assessment**

Clinical information was obtained via medical record review (82%), personal interview and examination (71%), patient telephone contact (21%), and physician contact (6%) by certified neurologists (C.F.L., I.M.).

Clinical course was classified as first neurological event/monophasic, relapsing–remitting, secondary progressive, primary progressive, or progressive relapsing MS.23 Patients were categorized as having MS at last follow-up by either McDonald 2001 or Poser criteria.24,25 A single neurological episode at last follow-up was classified as clinically isolated demyelinating syndrome (CIS).

**Statistical Analyses**

Our analyses focused on comparing imaging features associated with the index lesion on prebiopsy MRI by immunopattern. When analyzing prebiopsy MRIs, individuals with more than one MRI (46%) were categorized as having or not having the feature of interest associated with the index lesion on any of their prebiopsy MRIs, an approach that maintains the patient as the unit of analysis and does not necessitate a repeated measures analysis. We also analyzed whether a particular imaging finding was ever present for the biopsied or any nonbiopsied lesion on any pre- or postbiopsy MRI. We compared immunopatterns on categorical variables
FIGURE 1: Histology of immunopathological patterns I to III. The first column shows pattern I (A, E, H), the second column pattern II (B, F, I), and the third column pattern III histology (C, D, G, J, K) to illustrate pathologic heterogeneity of early active demyelinating multiple sclerosis lesions. (A, B) Myelin stainings show demyelinating lesions with an equal loss of all myelin proteins in pattern I and II (anti-myelin oligodendrocyte glycoprotein [MOG] in A and anti-cyclic nucleotide phosphodiesterase [CNP] in B). (C, D) In contrast, myelin-associated glycoprotein [MAG] loss (C) is typical for pattern III lesions. MAG is lost (C), whereas MOG (D) is still present in lesion areas. (E–G) Lesions are infiltrated by numerous macrophages (KiM1P). Lesion edges (Figure legend continues on next page.)
using 3-group and pairwise (2-group) chi-squared tests with p values calculated using Monte Carlo simulation. We tested for differences across immunopatterns on numeric variables using Kruskal–Wallis tests. We used logistic regression to assess the relationship between rings and rims on prebiopsy MRI comparing patterns I and II versus pattern III and summarize sensitivity, specificity, positive predictive value, and negative predictive value for this comparison. We did not correct for multiple comparisons.

Results

Patient Demographics and Distribution of Immunopathological Patterns

A total of 161 patients fulfilled inclusion criteria, with 24% of cases having immunopathological pattern I, 56% pattern II, and 20% pattern III (Figs 1 and 2A). Overall, the median age (IQR) at first attack was 37 years (26–45) and 55% of patients were female. Demographic data as well as clinical characteristics stratified by immunopathological patterns are summarized in Table 1, and the distribution of immunopathological patterns is given in Figure 2A. There was no clear difference by immunopathology in terms of age at first attack (median [IQR] age = 37 [30–44] years for pattern I vs 38 [26–46] for pattern II vs 36 [25–45] for pattern III; p = 0.85), proportion female (58% vs 57% vs 45%; p = 0.52), or time from symptom onset to biopsy (median [IQR] weeks = 8 [4–40] vs 8 [5–42] vs 5 [3–13]; p = 0.22). With comparable follow-up across groups (median [IQR] years = 4 [2–6] vs 4 [2–9] vs 4 [2–11]; p = 0.77), 76% of pattern I patients, 66% of pattern II patients, and 48% of pattern III patients fulfilled MS 2001 criteria at last follow-up (p = 0.02). In contrast, a CIS was diagnosed in 27% of pattern III patients, 10% of pattern II patients, and 3% of pattern I patients. Pattern II patients tended to have a lower Expanded Disability Status Scale (EDSS) at the attack leading to biopsy (index attack) compared to patterns I and III (median [IQR] of 4 [3–5] vs 3 [2–4] vs 4 [3–6]; p = 0.05), but a comparable EDSS at last follow-up (3 [2–5] vs 2 [1–4] vs 3 [2–4]; p = 0.44).

Lesion Location, Size, and Number

No significant differences in index lesion location and size were found on prebiopsy MRI when comparing immunopatterns. The biopsied lesion may have involved multiple regions, but occurred most commonly in the frontal (49%), parietal (42%), and periventricular regions (15%). Prebiopsy MRIs showed that some 19% of index lesions (the biopsied lesions) were 0.3–2cm in size, whereas about half (51%) were 2–5cm and 30% were >5cm. Most patients were multifocal (70%), with at least one other lesion in addition to the biopsied lesion on prebiopsy MRI. Overall, 14% had one other T2w lesion, 18% had 2 to 5, 12% had 6 to 10, and 25% had >10. Multiple T1w hypointense nonbiopsied lesions were very common, with 27% of patients having 2 to 5, 14% having 6 to 10, and 17% having >10. The maximum number of other, nonbiopsied, T1w hypointense lesions was lower in pattern III compared to pattern I and II patients (p = 0.04); 41% of pattern III patients had a single T1w hypointense lesion, whereas this number was lower for pattern I and II patients (14 and 15%).

Lesion Enhancement Patterns

A comparison was performed between the 3 immunopathological patterns for the presence and pattern of gadolinium enhancement of the index lesion on prebiopsy MRI. Enhancement of the index lesion was found in the majority of cases (94%). Pattern III cases were twice as likely to show no enhancement (14%) compared to pattern I (7%) and pattern II (3%), but this difference was not statistically significant (p = 0.14; see Fig 2B). A detailed analysis of the gadolinium enhancement patterns differentiating homogenous, heterogeneous, ringlike, or Baló-like enhancement (for details see Patients and Methods section) showed that 56% of patients overall had a ringlike enhancement. A strong association of a ringlike enhancement with patterns I and II on prebiopsy MRI was found (see Fig 2C–F; pattern I vs III, p = 0.02; pattern II vs III, p < 0.001). A ringlike enhancement of index lesion was found in the majority of pattern I (59%) and pattern II patients (65%), and only in a minority of pattern III patients (23%, n = 5).

Among patients with multifocal lesions, 70% had gadolinium-enhancing lesions in addition to the biopsied lesion on prebiopsy MRI, with multiple enhancing lesions common (16% having 1, 20% having 2–5, 13% having 6–10, and 21% having more). The difference in the frequency of ringlike enhancement was also observed when considering the index or other, nonbiopsied lesions on prebiopsy imaging (69% in pattern I, 72% in pattern II, 24% in pattern III; p < 0.001).
With proportionally fewer pattern III patients showing ringlike enhancing lesions, these patients were more likely to show a heterogeneous enhancement pattern on the index lesion or other, nonbiopsied lesions (72% vs 47% for pattern I and 52% for pattern II; \( p = 0.13 \)). Multiple concentric rings were only seen in pattern III patients (\( n = 2 \)) and are typical for Baló’s concentric sclerosis, a distinctive disease with concentric demyelination.

**Atypical Ring Enhancement of Pattern III Patients**

Because ringlike enhancing lesions were much less common in pattern III patients, we evaluated whether the ring patterns observed in these individuals were in any way distinctive. Including all MRIs (pre- as well as postbiopsy) and index lesions as well as other lesions, we identified a total of 6 of 33 patients with ringlike enhancement.
However, in each single case unusual ring features were present, as demonstrated in Figure 3. Instead of showing a smooth-contoured, single ring as is typical for pattern I and II lesions (see Fig 2D–F), lesions showed complex concentric ring/arc enhancement (enhancement was not classified as typical Baló-like), thick, irregular contoured arc enhancement, an incomplete fluffy ring of enhancement, or hazy, irregularly formed arcs that may coalesce (see Fig 3). Thus, ringlike enhancement in pattern III was always atypical and distinguishable from ringlike enhancement in pattern I and II patients.

**T2w Hypointense Rims**

We analyzed for presence of hypointense rims on T2w images on prebiopsy MRIs, visible surrounding the lesions, and their association with immunopathological

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**TABLE 1. Demographic Summary**

| Characteristic                        | I, n = 38 | II, n = 90 | III, n = 33 | p    |
|---------------------------------------|-----------|------------|-------------|------|
| Participants with clinical data, n (%)| 31 (82%)  | 72 (80%)   | 30 (91%)    | 0.37 |
| Women, n (%)                          | 22 (58%)  | 51 (57%)   | 15 (45%)    | 0.52 |
| Age at first attack, median (IQR)     | 37 (30–44)| 38 (26–46) | 36 (25–45)  | 0.85 |
| Disease duration, median (IQR)        |           |            |             |      |
| Weeks from onset to biopsy            | 8 (4–40)  | 8 (5–42)   | 5 (3–13)    | 0.22 |
| Years from onset to last follow-up    | 4 (2–6)   | 4 (2–9)    | 4 (2–11)    | 0.77 |
| Course prior to biopsy, n (%)         |           |            |             |      |
| First neurological event              | 17 (45%)  | 45 (50%)   | 17 (52%)    |      |
| Relapsing–remitting                   | 13 (34%)  | 20 (22%)   | 7 (21%)     |      |
| Secondary progressive                 | 0         | 2 (2%)     | 0           |      |
| Primary progressive                   | 0         | 2 (2%)     | 1 (3%)      |      |
| Progressive relapsing                 | 0         | 0          | 1 (3%)      |      |
| Unavailable/uncertain                 | 8 (21%)   | 21 (23%)   | 7 (21%)     |      |
| Course at last follow-up, n (%)       |           |            |             |      |
| Monophasic                            | 9 (24%)   | 13 (14%)   | 12 (36%)    |      |
| Relapsing–remitting                   | 17 (45%)  | 42 (47%)   | 9 (27%)     |      |
| Secondary progressive                 | 3 (8%)    | 10 (11%)   | 3 (9%)      |      |
| Primary progressive                   | 0         | 0          | 1 (3%)      |      |
| Progressive relapsing                 | 0         | 2 (2%)     | 1 (3%)      |      |
| Unavailable                           | 9 (24%)   | 23 (26%)   | 7 (21%)     |      |
| Diagnosis at last follow-up, n (%)    |           |            |             | 0.02 |
| MS                                    | 29 (76%)  | 59 (66%)   | 16 (48%)    |      |
| CIS                                   | 1 (3%)    | 9 (10%)    | 9 (27%)     |      |
| Unavailable                           | 8 (21%)   | 22 (24%)   | 8 (24%)     |      |
| EDSS, median (IQR)                    |           |            |             |      |
| At index attack                       | 4 (3–5)   | 3 (2–4)    | 4 (3–6)     | 0.05 |
| At last follow-up                     | 3 (2–5)   | 2 (1–4)    | 3 (2–4)     | 0.44 |

*Probability value not shown due to the number of categories.
FIGURE 3: Magnetic resonance imaging (MRI) characteristics of pattern III lesions with atypical ringlike enhancement. All 6 pattern III patients with a ringlike enhancement pattern showed atypical features, as demonstrated in this figure. (A) Axial T1-weighted (T1w) MRI with gadolinium showing complex concentric arc enhancement (upper image) with faint complex T2w hypointense rims (lower image). (B) Thick arc of enhancement (upper image) without a clear associated T2w rim (lower image). (C) Several hazy arcs and atypical ring enhancement (upper image) without clear T2w rims (lower image). (D) Faint concentric (Figure legend continues on next page.)
patterns. The index lesion showed a hypointense T2w rim in 43% of patients. Again, a strong association was found with immunopatterns ($p = 0.003$). Some 44% of pattern I patients and 53% of pattern II patients showed a hypointense T2w rim compared to pattern III patients (13%). (B) The MRI shows 2 typical hypointense T2w rims, characterized by a border of decreased signal intensity at the lesion edge compared to the hyperintense lesion center and surrounding edema.

Colocalization of Ringlike Enhancement and Hypointense T2w Rims

In the cohort as a whole, 39% of patients had an index lesion with both ringlike enhancement and a T2w rim, and these two features were strongly linked (odds ratio $[\text{OR}] = 14.0$, 95% confidence interval $[\text{CI}] = 5.2–42.7$; $p < 0.001$; index lesions evaluated on prebiopsy MRIs).

Specifically, among individuals with a ring enhancing index lesion on prebiopsy MRI, 69% also had T2w rims in the index lesion (Fig 4A, B). On the other hand, among individuals with no ring enhancing lesion, 88% had no rim. This relationship held in each of the immunopatterns, with the OR (95% CI) being approximately 4 (1–33) in pattern I cases, 11 (3–45) in pattern II cases, and 19 (1–1,167) in pattern III cases. Although varying to some degree, these ORs were not clearly different ($p = 0.51$).

Prior studies suggested that hypointense T2w rims are associated with a macrophage rim. Therefore, we correlated the histological lesion edge characteristics with radiological findings.

**Macrophenage Rims and Their Correlation with Ring Enhancing Lesions and Hypointense T2w Rims**

For a subset of patients who were examined radiologically, histological lesion edge characteristics could be specified in histological analyses. First, we correlated the presence of a macrophage rim as shown in histological sections with the presence of a ring enhancing index lesion on the MRI closest to biopsy (index MRI; Table 2 [top], Fig 5A, C). A macrophage rim was defined as a clearly visible, bandlike increase of macrophages at the lesion edge compared to the lesion center (see Figs 1F and 5C). A clear association of a macrophage rim and ring enhancement of the index lesion could be observed ($p = 0.04$). We found 73% of patients with a macrophage rim showed ring enhancement of the index lesion on index MRI, whereas among those without a macrophage rim, ring enhancement was less common (43%). Next, we analyzed whether the histologically determined macrophage rim also correlated with the presence of a radiologically determined hypointense T2w rim. A trend for an association was found ($p = 0.07$), with 60% of patients with a macrophage rim showing a hypointense T2w rim on MRI versus 32% without a macrophage rim (see Table 2 [bottom], Fig 5B, C).

In addition, we correlated macrophage rims as shown in histological sections with the immunopatterns. Macrophage rims were present in about one fourth of rings with a prominent edge of enhancement (upper image) and associated concentric T2w rims (lower image). (E) Complex multifocal concentric arc enhancement (upper image) with associated concentric T2w rims (lower image). Images in D and E were taken from the same patient, with MRIs 2 years apart. (F) Incomplete fluffy ring of enhancement. (G) Coalescence of multiple atypical ring enhancing lesions.
pattern I and II patients (25 and 26%) in contrast to pat-
tern III patients, of whom none of the 26 patients showed
a macrophage rim ($p = 0.01$).

Concordance of Ring Enhancement and T2w Rims between Biopsied and Nonbiopsied Lesions within Patients

We also investigated whether different lesions from a sin-
gle patient demonstrate the same MRI characteristics.

We analyzed the concordance of the ring enhance-
ment of the index lesion on prebiopsy MRIs with the
enhancement of any other, nonbiopsied lesions and found
a strong association (OR = 4.8, 95% CI = 1.8–14.3; $p = 0.001$). When the index lesion was ring enhancing,
61% of patients showed other ring enhancing lesions. Vice
versa, if the index lesion showed no ringlike enhancement,
in 78% of patients no other lesions showed a ringlike
enhancement pattern.

Next, we studied the concordance of hypointense T2w rims in biopsied and nonbiopsied lesions and again
found a strong association (OR = 6.5, 95% CI = 2.1–
23.3; $p < 0.001$). In patients with an index lesion on
prebiopsy MRI having a hypointense T2w rim, in 50% of
the patients, nonbiopsied lesions also showed such a rim.
Furthermore, in patients whose biopsied lesion did not
show a hypointense T2w rim, 89% also showed no other
lesions with hypointense T2w rims.

Lesion Edge Characteristics by Histology and Their Correlation with Immunopathological Patterns

Next, we correlated sharp versus ill-defined lesion edges as shown by (1) a macrophage staining or (2) a myelin
staining (PLP or CNP) with the immunopathological pat-
terns, as prior studies suggested that pattern I and II
lesions are characterized by sharp lesion edges in contrast
to pattern III lesions with diffuse lesion edges. Nearly all
pattern I (92%) and the majority of pattern II (58%) but
only a minority of pattern III (38%) lesions revealed sharp
lesion edges in macrophage stainings ($p < 0.001$). Sharp
lesion edges as defined by myelin staining were found in
58% of pattern I, 36% of pattern II, and 18% of pattern
III lesions ($p = 0.01$).

No relationship was found between sharp lesion
edges as depicted by macrophage or myelin stainings and
a ringlike enhancement or a hypointense T2w rim as
shown by MRI (data not given).

Edema and Mass Effect

The majority (76%) of index lesions showed edema on
prebiopsy MRIs. Any edema, ranging from mild to mar-
ked, was most frequent in pattern I index lesions (84%),
followed by pattern II (78%) and pattern III (58%), but
the differences were not statistically significant ($p = 0.06$
overall). Also, no statistically significant differences were
found for a mass effect ($p = 0.43$), which occurred in
about half of the patients overall (pattern I, 55%; patterns
II and III, 41%).

Logistic Regression Modeling to Predict Immunopatterns

We fit a logistic regression model comparing combined
patterns I and II versus pattern III and using prebiopsy
rings and prebiopsy T2w rims as the predictors. These
parameters were chosen because they showed the most
prominent differences when comparing immunopatterns,
and because they are easily identifiable on MRI in clinical
routine.

In logistic regression modeling, having both rings and
rims on prebiopsy MRI versus neither feature was associated
with a >10-fold increase in the odds of the individual being
pattern I or II compared to being pattern III (OR = 10.5,
95% CI = 2.5–43.8; $p = 0.001$). Equivalently, compared
to having both rings and rims, the absence of these features
on prebiopsy MRI was associated with a >10-fold increase
in the odds of the individual being pattern III compared to
being pattern I or pattern II.

| MRI Finding | CD68/KiM1P Rim |  |  |  |
|-------------|---------------|---|---|---|
|             | No            | Yes |  |  |
| Ring on index lesion on index MRI vs CD68/KiM1P rim  | 35 (57%) | 4 (27%) | 0.04 |
| Index lesion shows no ring | 26 (43%) | 11 (73%) |  |  |
| T2w hypointense rims on index lesion on index MRI vs CD68/KiM1P rim |  |  |  |  |
| Index lesion shows no T2w rim | 38 (68%) | 6 (40%) | 0.07 |
| Index lesion shows T2w rim | 18 (32%) | 9 (60%) |  |  |

MRI = magnetic resonance imaging; T2w = T2-weighted.
Diagnostic Performance of Ringlike Enhancing Lesions and Hypointense T2w Rims

Table 3 summarizes sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for discriminating between patterns I or II versus pattern III based on index lesion findings on prebiopsy MRI. With ringlike enhancement more common in patterns I and II, the sensitivity to detect patterns I or II based on this feature was 63%, whereas specificity was higher at 77%. Using a T2w hypointense rim as a marker of patterns I or II indicated only 50% sensitivity but high specificity (87%). Having either a ring or rim pattern was 70% sensitive and 76% specific, whereas having both a ring and rim was only 45% sensitive but 86% specific. As a whole, these imaging features had a very high PPV (93–95%) but low NPV (≤36%). In other words, ring or rim features provided an extremely strong indication of patterns I or II, but the absence of these features, although associated with greatly elevated odds of pattern III, leaves the underlying immunopattern uncertain.

Discussion

Histopathology of early active MS lesions revealed 4 histopathological patterns of demyelination that are intraindividually stable, but differ between patients, which suggests different disease pathomechanisms leading to lesion formation.\(^1\)–\(^3\),\(^29\) The fourth pattern is possibly caused by a genetic or metabolic disturbance of oligodendrocytes and restricted to very few and exceptional patients. Three main immunopathological patterns thus remain, and these can only be identified by histology. Noninvasive biomarkers for their identification are urgently needed, and this study provides the first evidence for their differentiation by routine MRI.

We compared the MRI characteristics of 161 patients who were classified according to their immunopathological patterns by histology and found that the majority of pattern I and pattern II patients but only a minority of pattern III patients showed ring enhancing lesions. In addition, ring enhancing lesions of pattern III patients did not reveal a smooth-contoured, thin, and regular ring or arc as was typical for pattern I and II patients. Instead, when present at all, they were atypical rings such as irregularly thick rings and arcs. In addition, 2 patients showed concentric rings that indicate Baló’s concentric sclerosis. It is known that Baló’s concentric sclerosis lesions are always pattern III lesions,\(^1\),\(^30\) and we could confirm this in our study.

Although seen somewhat less often than ringlike enhancement, T2w hypointense rims were much more common in pattern I and II cases compared to

FIGURE 5: Colocalization of ringlike enhancement and hypointense T2-weighted (T2w) rims, and their association with macrophage rims as shown by histology. A strong colocalization of ring enhancing lesions with hypointense T2w rims was found (A, B). A gadolinium-enhanced magnetic resonance image with 3 ring enhancing lesions is demonstrated (A), with all of them showing hypointense T2w rims (B). Ringlike enhancement was strongly associated with macrophage rims as shown by histology. (C) A typical macrophage rim with an accumulation of macrophages at the lesion edge (KiM1P) is demonstrated. Scale bar in C, 200μm.
pattern III cases. Thus, by evaluating the pattern of MRI contrast enhancement or presence of T2w hypointense rims, we now have for the first time a noninvasive biomarker that helps to differentiate pathological patterns of demyelination.

These 2 imaging features were not independent markers. Rather, the ring enhancement was strongly associated with hypointense T2w rims. In our study, 69% of ring enhancing lesions showed a hypointense T2w rim. Such a colocalization was described previously.7,9,15–17 The frequency of hypointense rims in the literature ranges from 9% up to 54% in MS patients.9,17 In prior studies, a colocalization of ring enhancing lesions with T2w hypointense rims was found in 32.5% of MS lesions and 54% of patients studied.9 As it was suggested that the pathological correlate of hypointense T2w rims are macrophage accumulations at the lesion edge, we correlated macrophage rims as shown in histological sections with ring enhancement as well as hypointense T2w rims identified by MRI.14,15 Our data suggest macrophage rims are associated with both ringlike enhancement and T2w rims on MRI.

We postulate that lesion pathogenesis of pattern I and II lesions begins with a breakdown of the blood–brain barrier (BBB). Smaller lesions typically show a nodular and centrifugal contrast enhancement, and this turns into a ringlike and centripetal enhancement when lesions expand.14,31 This BBB damage is followed by an extravasation of hematogenous macrophages, which form a macrophage rim at the lesion edge, and this is seen as a hypointense rim in T2w MRI images. These rims typically demonstrate numerous MRP14-positive macrophages, indicating a recent hematogenous origin of macrophages (own observations). In contrast, the expression of the microglia marker TMEM119 was only seen in a small percentage of cells.32 The hypointense T2w rims may reflect the presence of an iron-rich macrophage layer containing paramagnetic free radicals, and their detection may be increased with a higher field strength.9,14,33,34 These rims also show a decreased diffusivity compared to the lesion center in diffusion-weighted imaging, which is well explained by the high density of macrophages.9 Finally, the BBB is restored, although the T2w rim may still be visible.9 In the long run, macrophage rims may either dissolve or lesions may become chronic active lesions, defined as chronic lesions with slowly ongoing, smoldering demyelination at the lesion edge.35

Pattern I and II lesions are characterized by T-cell and macrophage infiltration, and the myelin damage in pattern I lesions may be induced by macrophage toxins.29 Pattern II lesions in addition show antibody and complement deposits within lesions, suggesting a complement mediates lysis of antibody-targeted myelin.29 Thus, peripheral immune cells and antibodies may be predominantly responsible for the myelin damage in pattern I and II lesions.

In contrast, we hypothesize that central nervous system (CNS) endogenous pathomechanisms in pattern III plaques may play a role in lesion development. Lesions are also characterized by T-cell and macrophage infiltration, but activated microglia profoundly outnumber hematogenously recruited macrophages in active lesions, and a distal oligodendrogiopathy associated with apoptotic oligodendrocytic cell death is characteristic.1,32 Such pathological changes reflect a cascade of oxidative injury.
mitochondrial damage, and subsequent virtual hypoxia, and comparable histopathological changes have been observed in viral CNS infections, with toxic agents such as the mitochondrial toxin cuprizone and with ischemic damage.

This study showed that pattern III lesions less often showed contrast enhancement of the index lesion; the lesion enhancement was heterogeneous in the majority of cases, and pattern III patients showed a single lesion significantly more often than pattern I and II patients. Correspondingly, pattern III patients more often had a clinically isolated syndrome at last follow-up. All these features may reflect the CNS endogenous evolution of lesions, secondarily followed by an opening of the BBB.

Prior studies showed an intraindividual homogeneity of active lesions when multiple demyelinating lesions were analyzed by histology at a given time point, and also when serial biopsies or biopsies followed by an autopsy were studied. This MRI analysis also demonstrated a high concordance of ring enhancing lesions and hypointense T2w rims comparing biopsied and nonbiopsied lesions. Thus, this MRI analysis supports the findings of lesion homogeneity within individual patients.

It is important to note that when comparing different patients, the heterogeneity of demyelinating lesions is found in early disease stages that are typically characterized by a relapsing–remitting disease course. Histologically, in these disease stages early active demyelinating lesions prevail, representing the earliest lesion stages, a prerequisite for classification into patterns I to III. In contrast, longstanding established MS lesions are typically uniform in all patients, and chronic inactive and/or smoldering, slowly expanding lesions predominate. At this point, the disease course is typically chronic progressive. Lesion heterogeneity among active demyelinating lesions in early disease stages converges into a uniform chronic lesion in all patients in late disease stages.

Some studies provide evidence for the clinical significance of immunopathological patterns. Studies demonstrated that the treatment response to apheresis therapies (including plasma exchange and immunoabsorption) is associated with immunopathological patterns. Apheresis is a second-line therapy for MS relapses. More than half of pattern II patients who showed signs of a humoral immune response benefited from apheresis treatments. About one third of pattern I patients also improved after apheresis treatments, whereas pattern III patients never responded. Moreover, a large study investigating the plasma exchange treatment response in 153 patients with inflammatory demyelinating diseases found that ring enhancing lesions were associated with a beneficial plasma exchange response, with an OR of 4.0. Thus, ring enhancing lesions are not only associated with patterns I and II as shown in the present study, but they are also associated with a beneficial apheresis treatment response. In conclusion, pathological mechanisms of lesion development may well be mirrored by MRI lesion characteristics, and MRI may serve as a valuable biomarker to help treatment decisions.

A limitation of our study is its retrospective nature, resulting in MRIs taken at different centers with varying protocols and at different time points with respect to the biopsies. Nonetheless, the high number of MRIs available (n = 789) in general had a good quality of standard sequences, allowing a thorough lesion characterization. However, factors such a field strength, resolution, degree of T1 and 2 weighting, and timing of MRI after gadolinium application as well as dose of gadolinium might influence the detection rate of ring enhancing lesions and T2w rims. Thus, optimization and standardization of those factors might result in an increased detection rate of these imaging features. On the other hand, most patients who are biopsied are characterized by an atypical clinical presentation, such as tumefactive lesions. It thus seems likely that ring enhancing lesions are overrepresented. The question is often raised as to whether biopsied patients are representative of the general MS population. A prior study of biopsy cases (n = 91) showed that 90% of patients developed clinical definite or probable MS after a median follow-up of 4.4 years despite atypical initial clinical presentations (ie, tumefactive lesions on MRI suggesting tumors). The clinical course and disability during follow-up were compared with a nonbiopsied prevalence MS cohort (n > 200) matched for disease duration, sex, and age, and no differences could be found. We also performed a clinical–radiographic study that included 168 patients with tumefactive biopsied inflammatory demyelinating lesions. In this cohort, 70% of patients developed definite MS at last follow-up, 83% presented with multiple lesions, and 55% fulfilled Barkhof radiographic criteria for MS at the time of last MRI. We thus conclude that although patients presented with atypical clinical symptoms that led to biopsy, they nevertheless comprise a representative cohort of MS patients and that results from biopsied patients can be extrapolated to prototypic MS.

In conclusion, analyzing MRI correlates of MS immunopathological patterns of demyelination shows that ring enhancement and hypointense T2w rims are significantly associated with patterns I and II, but not pattern III. Ring enhancing lesions and hypointense T2w rims colocalize, and ringlike enhancing lesions are associated with a macrophage rim as shown by histology. In contrast, pattern III lesions often show a heterogeneous contrast...
enhancement. We thus provide evidence that MRI reflects specific morphological features of MS immunopathology, and for the first time we now have identified a noninvasive biomarker that helps to differentiate and predict pathological patterns of demyelination.

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Author Contributions
I.M., W.B., and C.F.L. contributed to the conception and design of the study. All authors contributed to the acquisition and analysis of data. Drafting of manuscript and figures was done by I.M., S.D.W., N.L.Z., W.B., and C.F.L.

Potential Conflicts of Interest
Nothing to report.

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