Intrathecal IgM Synthesis Is Associated with Spinal Cord Manifestation and Neuronal Injury in Early MS

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Objectives: Intrathecal Immunoglobulin M synthesis (IgM_intrathecal Fraction (IF)16) and spinal MRI lesions are both strong independent predictors of higher disease activity and severity in multiple sclerosis (MS). We investigated whether IgMIF+ is associated with spinal cord manifestation and higher neuroaxonal damage in early MS.

Methods: In 122 patients with a first demyelinating event associations between (1) spinal versus (vs) non-spinal clinical syndrome (2) spinal vs cerebral T2-weighted (T2w) and (3) contrast-enhancing (CE) lesion counts with IgGIF+ (vs IgGF+) or IgMIF+ (vs IgMF+) were investigated by logistic regression adjusted for age and sex, respectively. For serum neurofilament light chain (sNfL) analysis patients were categorized for presence or absence of oligoclonal IgG bands (OCGB), IgGIF and IgMF (>0% vs 0%, respectively): (1) OCGB+/IgGIF+/IgMF+; (2) OCGB+/IgGIF−/IgMF+; (3) OCGB−/IgGIF+/IgMF+; and (4) OCGB−/IgGIF−/IgMF−. Associations between categories 2 to 4 vs category 1 with sNfL concentrations were analyzed by robust linear regression, adjusted for sex and MRI parameters.

Results: Patients with a spinal syndrome had a 8.36-fold higher odds of IgMIF+ (95% CI 3.03–23.03; p < 0.01). Each spinal T2w lesion (odds ratio 1.39; 1.02–1.90; p = 0.037) and CE lesion (OR 2.73; 1.22–6.09; p = 0.014) was associated with an increased risk of IgMIF+ (but not of IgGF+); this was not the case for cerebral lesions. OCGB+/IgGIF+/IgMF+ category patients showed highest sNfL levels (estimate: 1.80; 0.55–3.06; p < 0.01).

Interpretation: Intrathecal IgM synthesis is strongly associated with spinal manifestation and independently more pronounced neuroaxonal injury in early MS, suggesting a distinct clinical phenotype and pathophysiology.

Ann Neurol 2022;91:814–820

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Received Nov 22, 2021, and in revised form Jan 31, 2022. Accepted for publication Mar 7, 2022.

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.26348.
**Introduction**

Intrathecal IgM synthesis is strongly and independently associated with faster conversion from clinically isolated syndrome (CIS) to Multiple Sclerosis (MS),\(^1\),\(^2\) a more severe disease course,\(^3\)–\(^5\) higher brain lesion load,\(^3\)–\(^5\) and higher serum neurofilament light chain (sNfL) levels, reflecting neuro-axonal damage.\(^3\)

Spinal cord lesions are common in early MS and can be found in 30–50% of CIS patients.\(^6\),\(^7\) Their presence is associated with a higher rate of conversion from CIS to MS,\(^8\) even when asymptomatic they appear to be the strongest MRI predictor of physical disability after 5 years and indicated an increased risk of reaching an EDSS score of 3.\(^6\) One study reported higher cerebral and spinal lesion loads in patients with an elevated IgM index.\(^4\)

We aimed to investigate whether presence of IgM Intrathecal Fraction (IF) (IgMIF\(^+\)) is associated with spinal cord manifestation in a first demyelinating event. Furthermore, we analyzed whether IgMIF\(^+\) is associated with higher sNfL levels after adjustment for other modifying factors, suggestive of a specific pathophysiological link between IgMIF\(^+\) and neuro-axonal damage.

**Material and Methods**

**Patients, Inclusion Criteria and Data Collection**

Between 2012 and 2019 we prospectively included 122 patients with a first demyelinating event suggestive of MS recruited into the Swiss MS Cohort and the cerebrospinal fluid (CSF) biobanking study at the University Hospital Basel. 77 (63.2%) fulfilled McDonald criteria 2017 at lumbar puncture (LP) (Table 1). Patients were treatment naïve with a median time from onset of first symptoms to LP of 17 (interquartile range [IQR] 7–53) days.

Brain and spinal MRI scans were performed in 1.5 or 3 T scanners within clinical routine. Brain diagnostic imaging protocol included a 3D Magnetization Prepared-Rapid Gradient Echo (MPRAGE) pre and post contrast, and a 3D Fluid Attenuated Inversion Recovery (FLAIR) sequence. Whole spinal cord diagnostic imaging protocol included T2-weighted and contrast-enhanced T1-weighted spin-echo or turbo-spin-echo sequences.

Baseline cerebral and spinal T2-weighted (T2w) and contrast-enhancing (CE) MRI lesion counts were assessed by two neuroradiologists (TL, JL). The type of clinical syndrome (optic nerve, supratentorial, brainstem/cerebellum, spinal, multifocal) was assessed independently by three neurologists (BD, JO, RS), unaware of CSF results, based on detailed medical history, physical examination (including EDSS), visual, sensory and motor evoked potentials and cerebral and spinal MRI. The study was approved by the local ethical committee and patients were included after written informed consent.

**Cerebrospinal Fluid Analysis**

Oligoclonal IgG bands (OCGBs) were detected by isoelectric focusing followed by immunofixation.\(^9\) CSF and serum concentrations of IgG, IgM and albumin were measured nephelometrically and the calculations of quantitative intrathecal IgG and IgM synthesis based on Reiber formula (IgG\(_{IF}\) and IgM\(_{IF}\) in %).\(^10\)

**Serum Neurofilament Light Chain Measurements**

sNfL was measured in duplicate by single molecule array assay and age-adjusted Z-scores were calculated in reference to a healthy control cohort.\(^11\) Intra- and inter-assay variability (coefficients of variation) was below 10%.

**Statistical Analysis**

Interrater variability of clinical syndrome assessments was determined by Light’s kappa.\(^12\) Patients were categorized by presence (’) or absence (’) of IgG\(_{IF}\) and IgM\(_{IF}\) (Table 1).

**Associations of Intrathecal Ig Synthesis with Clinical Syndrome and MRI Lesions.** Associations of (1) spinal versus (vs) non-spinal clinical syndrome (n = 111; five patients were excluded due to non-classifiable type and 6 due to multifocal clinical syndrome localization) and (2) spinal and cerebral T2w (n = 86 with available cerebral and spinal MRI data) and (3) CE lesion counts (n = 85 with cerebral and spinal MRI data) (independent variables, respectively) were separately investigated by logistic regression adjusted for age and sex with IgG\(_{IF}\)\(^+\) (vs IgG\(_{IF}\)\(^−\)) or IgM\(_{IF}\)\(^+\) (vs IgM\(_{IF}\)\(^−\)) as dependent variable. For analysis (1) additional adjustment for cerebral and spinal T2w and CE lesion counts was performed (n = 75 with cerebral and spinal MRI data). In analyses (2) and (3) cerebral vs spinal lesion counts were analyzed by the same model. To explore the association of IgG\(_{IF}\)\(^+\) independent of IgM\(_{IF}\)\(^+\), additional analyses excluding patients with intrathecal IgM synthesis were performed (Table 1).

**Associations of Intrathecal Ig Synthesis with sNfL.** Associations of (A) IgG\(_{IF}\)\(^+\) (vs IgG\(_{IF}\)\(^−\)) and (B) IgM\(_{IF}\)\(^+\) (vs IgM\(_{IF}\)\(^−\)) (independent variables, respectively) with sNfL Z-scores as dependent variable were analyzed by robust linear regression models,\(^13\) adjusted for sex, cerebral and spinal T2w and CE lesion counts (n = 84 with available cerebral and spinal MRI data, respectively). Accordingly, associations with IgG\(_{IF}\)\(^+\) were additionally analyzed by excluding IgM\(_{IF}\)\(^+\) patients (n = 23).
TABLE 1. Patients’ characteristics stratified by presence or absence of intrathecal IgG and IgM synthesis

|                                      | IgGIF+ | IgGIF− | IgGIF+/IgGIF− | IgGIF−/IgGIF− | IgMIF+ | IgMIF− |
|--------------------------------------|--------|--------|---------------|---------------|--------|--------|
| Number                               | 69 (56.6) | 53 (43.4) | 41 (33.6) | 50 (41.0) | 31 (25.4) | 91 (74.6) |
| Sex (male)                           | 17 (24.6) | 17 (24.6) | 11 (26.8) | 16 (32.0) | 7 (22.6) | 27 (29.7) |
| Age (median, IQR, y)                 | 31.0 (26.4, 41.1) | 38.3 (31.2, 48.7) | 32.5 (28.2, 43.5) | 38.3 (32.5, 49.7) | 28.9 (23.9, 37.0) | 36.1 (29.5, 44.6) |
| EDSS at LP (median, IQR)             | 2.0 (2.0, 2.5) | 2.0 (1.0, 2.0) | 2.0 (2.0, 2.5) | 2.0 (1.0, 2.0) | 2.0 (2.0, 2.5) | 2.0 (1.0, 2.5) |
| McDonald criteria 2017 fulfilled at LP | 52 (75.4) | 25 (47.2) | 28 (68.3) | 24 (48.0) | 25 (80.6) | 52 (57.1) |

Clinical syndrome

|                                      |        |        |               |               |        |        |
|--------------------------------------|--------|--------|---------------|---------------|--------|--------|
| Optic nerve                          | 16 (26.7) | 23 (45.1) | 13 (38.2) | 22 (45.8) | 4 (13.8) | 35 (42.7) |
| Supratentorial                        | 7 (11.7) | 4 (7.8) | 6 (17.6) | 4 (8.3) | 1 (3.4) | 10 (12.2) |
| Brainstem /cerebellum                | 11 (18.5) | 12 (23.5) | 8 (23.5) | 11 (22.9) | 4 (13.8) | 19 (23.2) |
| Spinal                               | 26 (43.3) | 12 (23.5) | 7 (20.6) | 11 (22.9) | 20 (69.0) | 18 (22.0) |
| Multifocal†                          | 6 (8.7) | 0 (0) | 4 (9.8) | 0 (0) | 2 (6.5) | 4 (4.4) |
| Unclear†                             | 3 (4.3) | 2 (3.8) | 3 (7.3) | 2 (4.0) | 0 (0) | 5 (5.5) |

CSF characteristics

|                                      |        |        |               |               |        |        |
|--------------------------------------|--------|--------|---------------|---------------|--------|--------|
| OCGB+                                | 69 (100) | 27 (50.9) | 41 (100) | 24 (48.0) | 31 (100) | 65 (71.4) |
| IgGIF+                               | 69 (100) | 0 (0) | 41 (100) | 0 (0) | 28 (90.3) | 41 (45.1) |
| IgMIF+                               | 28 (40.6) | 3 (5.7) | 0 (0) | 0 (0) | 31 (100) | 0 (0) |
| IgAIF+                               | 2 (2.9) | 3 (5.7) | 1 (2.4) | 3 (6.0) | 1 (3.2) | 4 (4.4) |
| Cerebral MRI                         | 68 (98.6) | 52 (98.1) | 41 (100) | 49 (98.0) | 30 (96.8) | 90 (97.8) |
| T2w data available                   | 67 (98.5) | 52 (100) | 41 (100) | 49 (100) | 29 (96.7) | 90 (100) |
| CEL data available                   | 67 (98.5) | 51 (98.1) | 40 (97.6) | 48 (98.0) | 30 (100) | 88 (97.8) |
| T2w lesions number (Median, IQR)     | 9 (3, 16) | 3.5 (1, 12) | 5 (2, 13) | 3 (1, 12) | 11 (6, 18) | 4.5 (1, 13) |
| Any cerebral T2w lesion               | 62 (92.5) | 42 (80.8) | 36 (87.8) | 39 (79.6) | 29 (100) | 75 (83.3) |
| Any cerebral CE lesion                | 27 (40.3) | 13 (25.5) | 15 (37.5) | 11 (22.9) | 14 (46.7) | 26 (29.5) |
| Spinal cord MRI                      | 52 (75.4) | 36 (67.9) | 28 (68.3) | 35 (70) | 25 (80.6) | 63 (69.2) |
| T2w data available                   | 52 (100) | 36 (100) | 28 (100) | 35 (100) | 25 (100) | 63 (100) |
| CEL data available                   | 51 (98.1) | 36 (100) | 27 (96.4) | 35 (100) | 25 (100) | 62 (98.4) |
| T2w lesions, number (Median, IQR)    | 1 (0, 2) | 1 (0, 1) | 1 (0, 1) | 1 (0, 1) | 1 (1, 4) | 1 (0, 1) |
| Any spinal T2w lesion                 | 35 (67.3) | 19 (52.8) | 15 (53.6) | 18 (51.4) | 21 (84.0) | 33 (52.4) |
| Any spinal CE lesion                  | 20 (39.2) | 7 (19.4) | 5 (18.5) | 7 (20.0) | 15 (60.0) | 12 (19.4) |
| Serum NfL Z-score (Median, IQR)      | 1.16 (0.25, 2.28) | −0.10 (−0.94, 1.10) | 0.91 (0.25, 2.31) | −0.10 (−0.98, 1.19) | 1.48 (−0.02, 2.07) | 0.56 (−0.75, 1.73) |

n and percentage if not otherwise noted.

†31 Patients with IgMIF+ (IgMIF+/IgGIF+; n = 28 and IgMIF+/IgGIF−; n = 3) were excluded.

Five patients with a multifocal syndrome had a brainstem/cerebellum and spinal manifestation (IgMIF+/IgGIF+; n = 2 and IgMIF−/IgGIF−; n = 3) and one patient had an optic nerve and supratentorial localization (IgMIF+/IgGIF+: n = 1).

In five patients the clinical syndrome could not be unequivocally assigned (supratentorial vs optic nerve (n = 1); supratentorial vs brainstem/cerebellum (n = 3) and supratentorial vs spinal (n = 1); IgMIF+/IgGIF−: n = 3 and IgMIF−/IgGIF−: n = 2).

CE = contrast-enhancing lesion; EDSS = Expanded Disability Status Scale; Ig G/MIF = immunoglobulin G/M intrathecal fraction; IQR = Interquartile range; LP = lumbar puncture; MRI = Magnetic resonance imaging; n = number; OCGB = oligoclonal IgG bands; OCGB/IgMIF+/IgGIF− = presence of OCGB/IgMIF/ IgGIF; NfL Z-score = serum neurofilament light chain Z-score; T2w = T2-weighted; y = years.
Associations of Intrathecal Ig Categories with sNfL. As intrathecal synthesis of Ig subtypes is not evenly and independently distributed and to analyze it in relation to the same reference, the patients were categorized in ascending order for presence or absence of OCGB, IgGIF and IgMIF (>0% vs 0%, respectively):

1. OCGB+/IgGIF+/IgMIF+/C0; n = 26,
2. OCGB+/IgGIF+/IgMIF+/C0; n = 24,
3. OCGB+/IgGIF+/IgMIF+; n = 41, and
4. OCGB+/IgGIF+; n = 28.

(3 (2.5%) patients had a OCGB+/IgGIF+/IgMIF+ profile and were excluded from analysis).

Using category 1 as reference, associations of the CSF Ig categories 2) to 4) (independent variables) with sNfL Z-scores (dependent variable) were analyzed by robust linear regression models, adjusted for sex, cerebral and spinal T2w and CE lesion counts.

### TABLE 3. Associations of intrathecal Ig synthesis (1) and intrathecal Ig categories (2) with sNfL Z-scores

|                      | n   | Est | CI      | p   |
|----------------------|-----|-----|---------|-----|
| 1. Ig synthesis      |     |     |         |     |
| IgGIF+ (vs IgGIF−)  | 84  | 0.93| 0.07, 1.78 | 0.036 |
| IgGIF+ (vs IgGIF−)  | 61  | 0.88| −0.16, 1.91 | 0.102 |
| IgMIF+ (vs IgMIF−)  | 84  | 1.09| 0.30, 1.88  | <0.01 |
| 2. Ig categories    |     |     |         |     |
| OCGB+/IgGIF−/IgMIF− | 20  | 0.60| −0.59, 1.79 | 0.327 |
| OCGB+/IgGIF+/IgMIF− | 26  | 1.17| 0.04, 2.31  | 0.047 |
| OCGB+/IgGIF−/IgMIF+ | 22  | 1.80| 0.55, 3.06  | <0.01 |

*a adjusted for sex, cerebral and spinal T2w and CE lesion counts (respectively).

b n = 23 patients with presence of IgMIF were excluded from this analysis.

c vs reference group OCGB+/IgGIF+/IgMIF− (n = 15).

CE = contrast-enhancing; CI = 95% confidence interval; Est = Estimate; Ig G/MIF = immunoglobulin G/M intrathecal fraction; n = number; OCGB = oligoclonal IgG bands; p = p-value; sNfL Z-score = serum neurofilament light chain Z-score; T2w = T2-weighted; vs = versus; + = presence of OCGB or IgGIF/IgMIF−; − = absence of OCGB or IgGIF/IgMIF.
T2w and CE lesion counts (n = 83 with available cerebral and spinal MRI data, respectively). All analyses were conducted using the statistical software R (version 3.6.3).

Results

Association of Intrathecal Ig Synthesis with Clinical Syndrome

In 103 (84.4%) patients the independent categorization of clinical syndromes was identical and in 14 (11.5%) consensus was reached between the raters. In five (4.1%) patients, all IgMIF−, the clinical syndrome could not be unequivocally assigned (Table 1). The independent agreement between the raters on type of clinical syndrome according Light’s kappa was 0.86 (95%CI 0.79–0.92).12

Spinal syndromes were >3-fold more frequent in IgMIF+ than in IgMIF− patients (69.0% vs 22%; p < 0.01; Table 1). Accordingly, patients with a spinal syndrome had a 8.36-fold higher odds of an intrathecal IgM synthesis compared to those with non-spinal syndromes (95%CI
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3.03–23.03; p < 0.01; n = 111; OR 9.73; 2.51–31.67; p < 0.01 after additional adjustment for T2w and CE lesion numbers). Numerically this was also observed for IgGIF+ patients (OR 2.33; 0.97–5.59; p = 0.058; n = 111), however this trend disappeared after exclusion of IgMIF+ patients (OR 0.85; 0.28–2.59; p = 0.778; n = 82) (Table 2).

**Associations of Intrathecal Ig Synthesis with MRI Lesion Counts**

Every spinal (OR 1.39; 1.02–1.90; p = 0.037; n = 86) T2w lesion was associated with a 1.39-fold increased risk of IgMIF+, while such an association was not found for cerebral lesions (OR 1.02, 0.98–1.06; p = 0.433; n = 86), and not for IgGIF+ (Table 2).

Presence of spinal but not cerebral CE lesions was associated with a higher likelihood of an IgMIF production (2.73-fold per lesion (1.22–6.09; p = 0.014; n = 85)) which was not seen for IgGIF+ (OR 2.40; 0.93–6.18; p = 0.071; n = 85) after exclusion of IgMIF+ patients: OR 1.17; 0.44–3.15; p = 0.75; n = 61) (Table 2).

**Associations of Intrathecal Ig Synthesis/ Categories with Serum NfL Levels**

In multivariable analysis patients with IgMIF+ had a 1.09 units higher sNfL Z-score vs IgMIF− ones (0.30–1.88; p <0.01; n = 84); in IgGIF+ vs IgGIF− patients the sNfL Z-score was on average increased by 0.93 units (0.07–1.78, p = 0.036; n = 84). After excluding IgMIF+ patients significance was lost (estimate: 0.88; −0.16–1.91; p = 0.102; n = 61) (Table 3).

OCGB+/IgGIF+/IgMIF+ category patients showed the highest sNfL levels (estimate: 1.80; 0.55–3.06; p <0.01; n = 22) compared with OCGB+/IgGIF−/IgMIF− (n = 15) patients, followed by category OCGB+/IgGIF+/IgMIF− (estimate: 1.17; 0.04–2.31; p = 0.047; n = 26) and OCGB+/IgGIF−/IgMIF− (estimate: 0.60; 0.59–1.79; p = 0.327; n = 20) (Figure: Table 3). These associations were independent of the number of cerebral and spinal T2w and CE MRI lesions.

**Discussion**

Our study showed that the presence of IgMIF+, but not IgGIF+ is independently (also of observed higher overall lesion counts in IgMIF+ positive patients) associated with clinical spinal cord syndromes in patients with a first demyelinating event. Furthermore, the number of spinal T2w and CE lesions was quantitatively associated with the presence of IgMIF+, while there was no association with cerebral lesion count. Conversely, for IgGIF+ no topographical associations were found. IgMIF+ patients had the highest sNfL levels after full adjustment for known factors to influence sNfL concentrations including T2w and CE MRI lesions, suggesting an important role of intrathecal IgM synthesis in the pathogenesis of neuro- axonal damage in early MS.

In secondary progressive MS (SPMS), local B-cell-rich-meningeal inflammation and formation of tertiary follicles have been shown to be associated with the extent of spinal pathology,14 which may be mediated by intrathecal immunoglobulin production as part of the persistent humoral immune response in MS. Patients with an intrathecal IgM synthesis showed a faster disease progression, and as well a shorter time to onset of SPMS.4,5

Leptomeningeally produced proteins have higher concentrations in lumbar vs ventricular CSF which may result from their steady release due to a local outside/in concentration gradient at the border with the subarachnoid space.15 We have recently shown that the quantity of intrathecal IgMIF (but not IgGIF) is associated with the level of MS disease activity in a dose-dependent manner for clinical and MRI outcome measures.3 Therefore the higher extent of spinal inflammatory activity in IgMIF+ patients could be explained by higher local spinal IgMIF concentrations. Intrathecal synthesis of IgM (but not of IgG) was associated with early activation of the complement cascade, specifically of complement factor C3,16 which is in line with the pentameric IgM being the most efficient isotype for complement activation. In this context it is important that the contribution of antibodies and their capacity for complement activation for initial plaque development has been observed in some MS patients17 and that the complement system plays a role in demyelination and axonal injury.18,19 We therefore postulate that the specific preponderance for lesion formation in the spinal cord in presence of IgMIF indicates a distinct phenotype and pathophysiology with involvement of antibodies in demyelination and axonal injury in early MS. Future studies should also investigate the impact of IgMIF+ on the extent of spinal pathology especially in progressive MS disease stages. This population may specifically profit from therapies that are able to target the intrathecal B-cell pool responsible for IgM production.20

**Acknowledgements**

The authors express their deep thankfulness to patients and relatives for their participation and support, study nurses for their motivated collaboration and recruitment efforts and the administrative personnel of the Swiss Multiple Sclerosis Cohort. This investigation was supported by the Swiss Multiple Sclerosis Society (research grant 2021/10) and Swiss National Science Foundation (grant 320030_189140 / 1). The Swiss MS Cohort study...
received funding from the Swiss Multiple Sclerosis Society and grant funding from Biogen, Bristol Myers Squibb, Celgene, Merck, Novartis, Roche, and Sanofi. Open access funding provided by Universitat Basel.

Author Contributions
Conception and design of study: JO, JK. Acquisition and analysis of data: JO, TL, SS, BD, AM, AO, SM, EW, AB, MK, TD, PB, IH, AR, SM, LA, PL, AS, CP, CG, DL, RS, JL, JK. Drafting of the manuscript: JO, SS, DL, JK.

Potential Conflicts of Interest
The authors report no potential conflicts of interests.

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