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Intrathecal IgM synthesis is associated with spinal cord manifestation and neuronal injury in early MS

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Abstract

Objective: Intrathecal Immunoglobulin M synthesis ($IgM_{Intrathecal\ Fraction\ (IF)}^{+}$) and spinal MRI lesions are both strong independent predictors of higher disease activity and severity in multiple sclerosis (MS). We investigated whether IgM_{IF}^{+} 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 IgG_{IF}^{+} (vs IgG_{IF}^{-}) or IgM_{IF}^{+} (vs IgM_{IF}^{-}) 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), IgG_{IF} and IgM_{IF} (>0% vs 0%, respectively): 1) $OCGB^{-}/IgG_{IF}^{-}/IgM_{IF}^{-}$; 2) $OCGB^{+}/IgG_{IF}^{-}/IgM_{IF}^{-}$; 3) $OCGB^{+}/IgG_{IF}^{+}/IgM_{IF}^{-}$; and 4) $OCGB^{+}/IgG_{IF}^{+}/IgM_{IF}^{+}$. 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 IgM_{IF}^{+} (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 IgM_{IF}^{+} (but not of IgG_{IF}^{+}); this was not the case for cerebral lesions. $OCGB^{+}/IgG_{IF}^{+}/IgM_{IF}^{+}$ 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.

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[7] 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 $IgM_{\text{Intrathecal Fraction (IF)}}$ (IgM_{IF}^{+}) is associated with spinal cord manifestation in a first demyelinating event. Furthermore, we analyzed whether IgM_{IF}^{+} is associated with higher sNfL levels after adjustment for other modifying factors, suggestive of a specific pathophysiological link between IgM_{IF}^{+} 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 naive 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 3T 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; 5 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).

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, IgG_{IF} and IgM_{IF} (>0% vs 0%, respectively)[3]:

1) $OCGB^{-}/IgG_{IF}^{-}/IgM_{IF}^{-}$; n=26,

2) $OCGB^{+}/IgG_{IF}^{-}/IgM_{IF}^{-}$; n=24,

3) $OCGB^{+}/IgG_{IF}^{+}/IgM_{IF}^{-}$; n=41, and

4) $OCGB^{+}/IgG_{IF}^{+}/IgM_{IF}^{+}$; n=28

(3 (2.5%) patients had a $OCGB^{+}/IgG_{IF}^{-}/IgM_{IF}^{+}$ 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[13], adjusted for sex, cerebral and spinal 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 5 (4.1%) patients, all IgM_{IF}^{-}, 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 IgM_{IF}^{+} than in IgM_{IF}^{-} 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 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 IgG_{IF}^{+} patients (OR 2.33; 0.97-5.59; p=0.058; n=111), however this trend disappeared after exclusion of IgM_{IF}^{+} patients (OR 0.85; 0.28-2.59; p=0.778; n=82) (**Table 2A**).

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 IgM_{IF}^{+}, 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 IgG_{IF}^{+} (**Table 2A**).

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

Associations of intrathecal Ig synthesis/categories with serum NfL levels

In multivariable analysis patients with IgM_{IF}^{+} had a 1.09 units higher sNfL Z-score vs IgM_{IF}^{-} ones (0.30-1.88; p<0.01; n=84); in IgG_{IF}^{+} vs IgG_{IF}^{-} patients the sNfL Z-score was on average increased by 0.93 units (0.07-1.78, p=0.036; n=84). After excluding IgM_{IF}^{+} patients significance was lost (estimate: 0.88; -0.16-1.91; p=0.102; n=61) (**Table 2B**). $OCGB^{+}/IgG_{IF}^{+}/IgM_{IF}^{+}$ category patients showed the highest sNfL levels (estimate: 1.80; 0.55-3.06; p<0.01; n=22) compared with $OCGB^{-}/IgG_{IF}^{-}/IgM_{IF}^{-}$ (n=15) patients, followed by category $OCGB^{+}/IgG_{IF}^{+}/IgM_{IF}^{-}$ (estimate: 1.17; 0.04-2.31; p=0.047; n=26) and $OCGB^{+}/IgG_{IF}^{-}/IgM_{IF}^{-}$ (estimate: 0.60; 0.59-1.79; p=0.327; n=20) (**Figure 1**; **Table 2B**). These associations were independent of the number of cerebral and spinal T2w and CE MRI lesions.

Discussion

Our study showed that the presence of IgM_{IF}^{+}, but not IgG_{IF}^{+} is independently (also of observed higher overall lesion counts in IgM_{IF}^{+} 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 IgM_{IF}^{+}, while there was no association with cerebral lesion count. Conversely, for IgG_{IF}^{+} no topographical associations were found. IgM_{IF}^{+} 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 IgM_{IF} (but not IgG_{IF}) 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 IgM_{IF}^{+} patients could be explained by higher local spinal IgM_{IF} 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 patients[17] 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 IgM_{IF} 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 IgM_{IF}^{+} 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].

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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, LK, CG, DL, RS, JL, JK.

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Potential Conflicts of interest:

The authors report no potential conflicts of interests.

References

1. Huss A, Abdelhak A, Halbgebauer S, et al. Intrathecal immunoglobulin M production: A promising high-risk marker in clinically isolated syndrome patients. *Ann Neurol.* 2018;83(5):1032-1036.
2. Pfuhl C, Grittner U, Giess RM, et al. Intrathecal IgM production is a strong risk factor for early conversion to multiple sclerosis. *Neurology.* 2019;93(15):e1439-e1451.
3. Oechtering J, Schaedelin S, Benkert P, et al. Intrathecal Immunoglobulin M Synthesis is an Independent Biomarker for Higher Disease Activity and Severity in Multiple Sclerosis. *Ann Neurol.* 2021.
4. Ozakbas S, Cinar BP, Ozcelik P, et al. Intrathecal IgM index correlates with a severe

disease course in multiple sclerosis: Clinical and MRI results. *Clin Neurol Neurosurg.* 2017;160:27-29.

5. Villar LM, Masjuan J, Gonzalez-Porque P, et al. Intrathecal IgM synthesis is a prognostic factor in multiple sclerosis. *Ann Neurol.* 2003;53(2):222-226.
6. Arrambide G, Tintore M, Auger C, et al. Lesion topographies in multiple sclerosis diagnosis: A reappraisal. *Neurology.* 2017;89(23):2351-2356.
7. Brownlee WJ, Altmann DR, Alves Da Mota P, et al. Association of asymptomatic spinal cord lesions and atrophy with disability 5 years after a clinically isolated syndrome. *Mult Scler.* 2017;23(5):665-674.
8. Patrucco L, Rojas JI, Cristiano E. Assessing the value of spinal cord lesions in predicting development of multiple sclerosis in patients with clinically isolated syndromes. *J Neurol.* 2012;259(7):1317-1320.
9. Andersson M, Alvarez-Cermeno J, Bernardi G, et al. Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. *J Neurol Neurosurg Psychiatry.* 1994;57(8):897-902.
10. Reiber H. Cerebrospinal fluid--physiology, analysis and interpretation of protein patterns for diagnosis of neurological diseases. *Mult Scler.* 1998;4(3):99-107.
11. Disanto G, Barro C, Benkert P, et al. Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. *Ann Neurol.* 2017;81(6):857-870.
12. Conger AJ. Kappa and Rater Accuracy: Paradigms and Parameters. *Educ Psychol Meas.* 2017;77(6):1019-1047.
13. Yohai V, Stahel WA, Zamar RH. A procedure for robust estimation and inference in linear regression; in Stahel, WA and Weisberg, SW. Directions in robust statistics and diagnostics, Part II, 1991.
14. Reali C, Magliozzi R, Roncaroli F, et al. B cell rich meningeal inflammation associates with increased spinal cord pathology in multiple sclerosis. *Brain Pathol.*

2020;30(4):779-793.

15. Reiber H. Dynamics of brain-derived proteins in cerebrospinal fluid. *Clin Chim Acta.* 2001;310(2):173-186.

16. Sellebjerg F, Christiansen M, Garred P. MBP, anti-MBP and anti-PLP antibodies, and intrathecal complement activation in multiple sclerosis. *Mult Scler.* 1998; 4(3):127-31.

17. Lucchinetti C, Bruck W, Parisi J, et al. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol.* 2000;47(6):707-717.

18. Mead RJ, Singhrao SK, Neal JW, et al. The membrane attack complex of complement causes severe demyelination associated with acute axonal injury. *J Immunol.* 2002;168(1):458-465.

19. Piddlesden SJ, Lassmann H, Zimprich F, et al. The demyelinating potential of antibodies to myelin oligodendrocyte glycoprotein is related to their ability to fix complement. *Am J Pathol.* 1993;143(2):555-564.

20. Weber M, Von Büdingen HC, Bar-Or A, et al. Modulation of cerebrospinal fluid immunoglobulins by ocrelizumab treatment. MS Virtual 2020, P0110.

Figure legend:

Figure 1. Serum NfL-Z-scores stratified by CSF immunoglobulin categories: **A.** unadjusted and **B.** estimates from a multivariable model (marginal effects).

A. $OCGB^{+}/IgG_{IF}^{+}/IgM_{IF}^{+}$ patients had the highest median serum (s)NfL levels (Z-score: 1.65; IQR 0.21-2.17), followed by $OCGB^{+}/IgG_{IF}^{+}/IgM_{IF}^{-}$ (0.91; 0.25-2.31), $OCGB^{+}/IgG_{IF}^{-}/IgM_{IF}^{-}$ (0.18; -0.38-1.39) and $OCGB^{-}/IgG_{IF}^{-}/IgM_{IF}^{-}$ patients (-0.56; -1.38-0.93).

B. Estimates (marginal effects) as derived from the multivariable analyses for sNfL Z-scores as dependent variable adjusted for sex, spinal and cerebral total T2w and CE lesion counts (n=83; see **Table 2B**; **2.**). $OCGB^{+}/IgG_{IF}^{+}/IgM_{IF}^{+}$ patients displayed the highest sNfL levels (estimate: 1.80; 95%CI 0.55-3.06; $p<0.01$; i.e. 1.80 units (standard deviations) higher sNfL Z-scores than $OCGB^{-}/IgG_{IF}^{-}/IgM_{IF}^{-}$ patients), followed by $OCGB^{+}/IgG_{IF}^{+}/IgM_{IF}^{-}$ (estimate: 1.17; 0.04-2.31; p=0.047) and $OCGB^{+}/IgG_{IF}^{-}/IgM_{IF}^{-}$ (estimate 0.60; -0.59-1.79; p=0.327) compared to $OCGB^{-}/IgG_{IF}^{-}/IgM_{IF}^{-}$ patients.

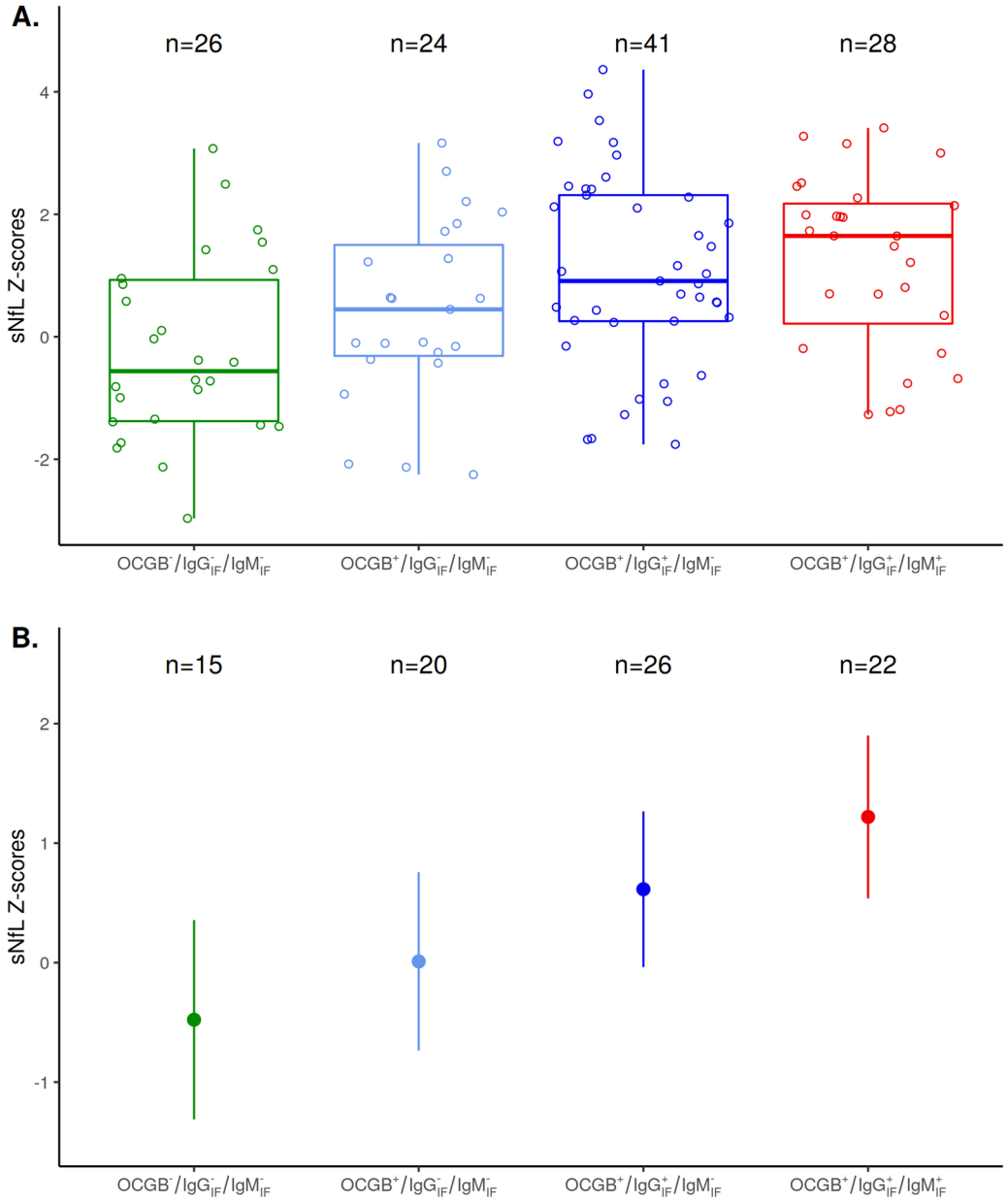

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Table 1. Patients` characteristics stratified by presence or absence of intrathecal IgG and IgM synthesis.

	IgG_{IF}^{+}	**IgG_{IF}^{-}**	**IgG_{IF}^{+} [1]**	**IgG_{IF}^{-} [1]**	**IgM_{IF}^{+}**	**IgM_{IF}^{-}**
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 (32.1)	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, 2.6)	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 (18.3)	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[2]	6 (8.7)	0 (0)	4 (9.8)	0 (0)	2 (6.5)	4 (4.4)
Unclear[3]	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)
IgG_{IF}^{+}	69 (100)	0 (0)	41 (100)	0 (0)	28 (90.3)	41 (45.1)
IgM_{IF}^{+}	28 (40.6)	3 (5.7)	0 (0)	0 (0)	31 (100)	0 (0)
IgA_{IF}^{+}	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)

[1] 31 Patients with IgM_{IF}^{+} ($IgM_{IF}^{+}/IgG_{IF}^{+}$; n= 28 and $IgM_{IF}^{+}/IgG_{IF}^{-}$; n=3) were excluded.
[2] 5 patients with a multifocal syndrome had a brainstem/cerebellum and spinal manifestation ($IgM_{IF}^{+}/IgG_{IF}^{+}$: n= 2 and $IgM_{IF}^{-}/IgG_{IF}^{+}$: n=3) and 1 patient had a optic nerve and supratentorial localization ($IgM_{IF}^{-}/IgG_{IF}^{+}$: n=1).
[3] In 5 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); ($IgM_{IF}^{-}/IgG_{IF}^{+}$: n=3 and $IgM_{IF}^{-}/IgG_{IF}^{-}$: n=2)
n and percentage if not otherwise noted.

Abbreviations: CEL: contrast-enhancing lesion; EDSS: Expanded Disability Status Scale; Ig G/M_{IF}: immunglobulin G/M intrathecal fraction; IQR: Interquartile range; LP: lumbar puncture; MRI: Magnetic resonance imaging; n: number; OCGB: oligoclonal IgG bands; OCGB/IgM_{IF} /IgG_{IF}+: presence of OCGB/IgM_{IF}/ IgG_{IF}; ;sNfLZ-score: serum neurofilament light chain Z-score; T2w: T2-weighted; y: years.

Table 2A. Associations of clinical syndrome (**1.**), T2w (**2.**) and CE lesions (**3.**) with intrathecal Ig synthesis

	IgG_{IF}^{+} (vs IgG_{IF}^{-})				IgG_{IF}^{+} (vs IgG_{IF}^{-})[1]				IgM_{IF}^{+} (vs IgM_{IF}^{-})			
	n	**OR**	**CI**	**p**	**n**	**OR**	**CI**	**p**	**n**	**OR**	**CI**	**p**
1. Clinical Syndrome*												
Spinal vs non spinal*	111	2.33	0.97, 5.59	0.058	82	0.85	0.28, 2.59	0.778	111	8.36	3.03, 23.03	**<0.01**
Spinal vs non spinal**	75	1.94	0.64; 5.89	0.241	54	0.95	0.24 ; 3.74	0.939	75	9.73	2.51 ; 37.67	**<0.01**
2. T2w lesions*												
Cerebral (per lesion)	86	1.03	0.98, 1.08	0.304	63	1.01	0.97, 1.06	0.587	86	1.02	0.98, 1.06	0.433
Spinal (per lesion)		1.26	0.89, 1.79	0.193		1.09	0.73, 1.62	0.672		1.39	1.02, 1.90	**0.037**
3. CE lesions*												
Cerebral (per lesion)	85	1.16	0.85, 1.60	0.348	61	1.12	0.81, 1.55	0.494	85	1.04	0.84, 1.28	0.718
Spinal (per lesion)		2.40	0.93, 6.18	0.071		1.17	0.44, 3.15	0.750		2.73	1.22, 6.09	**0.014**

[1]1.) n=29; 2.) n=23 and 3.) n=24 patients with presence of IgM_{IF} were excluded from this analysis.
***** adjusted for age and sex.
****** adjusted for age, sex, total (cerebral and spinal) T2w and CE lesion counts.

Table 2B. Associations of intrathecal Ig synthesis (**1.**) and intrathecal Ig categories (**2.**) with sNfL Z-scores.

	n	**Est**	**CI**	**p**
1. Ig synthesis*				
IgG_{IF}^{+} (vs IgG_{IF}^{-})	84	0.93	0.07, 1.78	**0.036**
IgG_{IF}^{+} (vs IgG_{IF}^{-})[1]	61	0.88	-0.16, 1.91	0.102
IgM_{IF}^{+} (vs IgM_{IF}^{-})	84	1.09	0.30, 1.88	**<0.01**
2. Ig categories*				
$OCGB^{+}/IgG_{IF}^{-}/IgM_{IF}^{-}$ [2]	20	0.60	-0.59, 1.79	0.327
$OCGB^{+}/IgG_{IF}^{+}/IgM_{IF}^{-}$ [2]	26	1.17	0.04, 2.31	**0.047**
$OCGB^{+}/IgG_{IF}^{+}/IgM_{IF}^{+}$ [2]	22	1.80	0.55, 3.06	**<0.01**

[1] n=23 patients with presence of IgM_{IF} were excluded from this analysis.
[2] vs reference group $OCGB^{-}/IgG_{IF}^{-}/IgM_{IF}^{-}$ (n=15).
***** adjusted for sex, cerebral and spinal T2w and CE lesion counts (respectively).

Abbreviations: CE: contrast-enhancing; CI: 95% confidence interval; Est: Estimate; Ig G/M_{IF}: immunoglobulin G/M intrathecal fraction; n: number; OCGB: oligoclonal IgG bands; OR: Odds ratio; p: p-value; sNfL Z-score: serum neurofilament light chain Z-score; T2w: T2-weighted; vs: versus; $^{+}$: presence of OCGB or IgG_{IF}/IgM_{IF}; $^{-}$: absence of OCGB or IgG_{IF}/IgM_{IF}.