

Intrathecal Immunoglobulin M Synthesis is an Independent Biomarker for Higher Disease Activity and Severity in Multiple Sclerosis

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Objective: We aimed to determine in relapsing multiple sclerosis (MS) whether intrathecal synthesis of immunoglobulin (Ig) M and IgG is associated with outcomes reflecting inflammatory activity and chronic worsening.

Methods: We compared cerebrospinal fluid analysis, clinical and magnetic resonance imaging data, and serum neurofilament light chain (sNfL) levels at baseline and follow-up in 530 patients with relapsing MS. Patients were categorized by the presence of oligoclonal IgG bands (OCGB) and intrathecal synthesis of IgG and IgM (intrathecal fraction [IF]: IgG_{IF} and IgM_{IF}). Relationships with the time to first relapse, sNfL concentrations, T2-weighted (T2w) lesions, MS Severity Score (MSSS), and time to initiation of high-efficacy therapy were analyzed in covariate-adjusted statistical models.

Results: By categorical analysis, in patients with IgM_{IF} the median time to first relapse was 28 months shorter and MSSS on average higher by 1.11 steps compared with patients without intrathecal immunoglobulin synthesis. Moreover, patients with IgM_{IF} had higher sNfL concentrations, more new/enlarging T2w lesions, and higher total T2w lesion counts (all $p \leq 0.01$). These associations were absent or equally smaller in patients who were positive for only OCGB or OCGB/IgG_{IF}. Furthermore, quantitative analyses revealed that in patients with IgM_{IF} \geq median, the time to first relapse and to initiation of high-efficacy therapy was shorter by 32 and by 203 months, respectively (both $p < 0.01$), in comparison to patients with IgM_{IF} $<$ median. Dose-dependent associations were also found for IgM_{IF} but not for IgG_{IF} with magnetic resonance imaging-defined disease activity and sNfL.

Interpretation: This large study supports the value of intrathecal IgM synthesis as an independent biomarker of disease activity and severity in relapsing MS.

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The clinical course of multiple sclerosis is heterogeneous and unpredictable on individual grounds at the time of diagnosis. Biomarkers bridging this prognostic gap are urgently needed for personalized therapeutic decision making. The presence of oligoclonal immunoglobulin (Ig) G bands (OCGB) in cerebrospinal fluid (CSF) is a diagnostic hallmark of multiple sclerosis and was recently reintroduced in the early diagnostic algorithm for multiple sclerosis to satisfy the criterion of dissemination in time.¹ Besides this qualitative measure, intrathecal accumulation of B cells and plasma cells can also be detected by intrathecal production of IgG (intrathecal fraction of IgG [IgG_{IF}]),² which has been found in 70 to 86%^{2,3} of patients with multiple sclerosis. In a large clinically isolated syndrome (CIS) cohort, OCGB and the presence of intrathecal IgG_{IF} were associated with 1.4- and 1.6-fold increased⁴ likelihoods of conversion to multiple sclerosis. Unlike the adaptive immune response in lymphatic tissue, where the initial production of IgM is typically supplanted by IgG owing to an isotype switch of B cells, intrathecal production of IgM persists as a characteristic feature of multiple sclerosis.⁵ The intrathecal fraction of IgM (IgM_{IF}) according to Reiber's formula² is present in up to 23% of multiple sclerosis patients.^{4,6,7}

The presence of OCMB has been reported to be associated with a more active inflammatory disease phenotype, both in relapsing and in primary progressive multiple sclerosis.^{8–11}

Moreover, some studies have found that quantitated intrathecal IgM synthesis is associated with a higher likelihood of conversion from CIS to clinically definite multiple sclerosis and a more severe disease course,^{4,7,12,13} but other studies did not confirm this finding.^{6,14,15} Little is known how intrathecal IgM and IgG synthesis are associated independently with magnetic resonance imaging

(MRI) and body fluid markers related to progression and, eventually, the choice of therapy, and whether this relation is dose-dependent.

The aim of this study was to investigate the added value of quantitative estimates of IgM_{IF} and IgG_{IF} to prognosticate the long-term disease activity and severity based on the time interval between first symptoms and a first relapse, the Multiple Sclerosis Severity Score (MSSS), respectively, and the level of longitudinal development of neuroaxonal damage and disease burden as measured by serum neurofilament light chain (sNfL) and MRI lesions. Lastly, we explored how these measures correlated with the physician's decision to initiate or to switch to high-efficacy disease-modifying therapies (heDMT).

Patients and Methods

Patients

We included participants in the Swiss Multiple Sclerosis Cohort Study (SMSC) diagnosed as either CIS or multiple sclerosis,^{1,16,17} who had a lumbar puncture (LP) with complete analysis of OCGB, IgG_{IF}, IgM_{IF}, and the intrathecal fraction of IgA (IgA_{IF}). The initial clinical event and first relapse were defined as new, worsening (in the case of first relapse), or recurrent neurologic symptoms that lasted for ≥ 24 hours without fever, infection, or adverse reaction to a prescribed medication and that were preceded by a stable or improving neurologic status of ≥ 30 days (in the case of first relapse). Institutional review boards at the respective SMSC centers approved the study, and written informed consent was obtained from all participants.

Baseline and Follow-Up Clinical Data, Multiple Sclerosis Data, and Serum Collection

Demographic and clinical variables collected at the baseline visit included sex, date of birth, date of onset of

multiple sclerosis symptoms, and first relapse (2 patients experienced their first relapse with unknown date) where applicable, and current and previous disease-modifying therapies (DMTs). Therapies were categorized into “heDMT” (ocrelizumab, rituximab, alemtuzumab, and natalizumab), “oral” (teriflunomide, dimethyl fumarate, fingolimod, and siponimod), “platform” (interferon beta and glatiramer acetate), and “untreated”. Standardized clinical assessments based on Expanded Disability Status Scale (EDSS) scores were performed by certified raters (<http://www.neurostatus.net>) at baseline and each follow-up visit every 6 or 12 months.^{16,18} Blood samples were collected within 8 days from the clinical visit and processed and stored at -80°C following standardized procedures. The median (interquartile range [IQR]) follow-up from baseline was 5.1 (3.0–7.0) years.

Cerebrospinal Fluid Analysis

OCGBs were assessed by isoelectric focusing followed by immunoblotting, immunofixation, or silver staining (depending on the study center). Testing of OCGBs was considered positive if pattern 2 or 3 (local synthesis of IgG within the central nervous system) was present.¹⁹ CSF and serum concentrations of IgA, IgG, IgM, and albumin were measured nephelometrically. We used the derived CSF-to-serum quotients for Igs and albumin (Q_{IgG} , Q_{IgM} , Q_{IgA} , and Q_{alb}) for calculations of the intrathecal Ig synthesis according to the formulae proposed by Reiber and colleagues.²

The amount of intrathecal IgG, IgM, or IgA synthesis was expressed as the intrathecal fraction as a percentage of the total measured isotype concentration in CSF (IgG_{IF} , IgM_{IF} , and IgA_{IF}).² The integrity of the blood–CSF barrier was determined by calculating the Q_{alb} .²⁰

Serum Neurofilament Light Chain Measurements

sNfL was measured (at baseline and every 6 or 12 months) with the NF-light[®] assay (Quanterix, Billerica, MA, USA).

Intra- and interassay variability were evaluated with 3 native serum samples in each of the runs on independent days. The mean coefficients of variation (CVs) of duplicate determinations for concentration were 5.5% (6.2pg/ml, sample 1), 3.3% (18.9pg/ml, sample 2), and 3.1% (37.3pg/ml, sample 3). Interassay CVs were 7.0% (sample 1), 5.6% (sample 2), and 5.6% (sample 3). For the generation of age-dependent sNfL Z -scores, we used sNfL measurements in 8,865 samples of 4,209 healthy controls (median age = 44.8 years).²¹

Magnetic Resonance Imaging

Brain MRI scans were performed annually in 1.5 (n = 940) or 3T (n = 978) scanners. The imaging

protocol was standardized across centers and included a 3-dimensional magnetization prepared – rapid gradient echo (MPRAGE) and a 3-dimensional fluid attenuated inversion recovery (FLAIR) sequence, which were acquired at a spatial resolution of 1mm.³ The number and occurrence of new/enlarging T2-weighted (T2w) lesions were automatically assessed annually by using a deep learning-based approach²² and a longitudinal evaluation method,²³ respectively, followed by manual quality assessment and correction.

Statistical Analysis

Data are presented as the median and interquartile range (IQR) or mean and standard deviation (SD), and as absolute and relative frequencies in the case of categorical data.

Categorical Analysis. Given that the presence of intrathecal Ig subtypes is not distributed evenly and independently, in a first step the patients were categorized in ascending order for the presence or absence of OCGB, IgG_{IF} (>0% vs 0%) and IgM_{IF} (>0% vs 0%; Fig 1): (1) $\text{OCGB}^{-}/\text{IgG}_{\text{IF}}^{-}/\text{IgM}_{\text{IF}}^{-}$ (ie, absence of OCGB and IgG_{IF} and IgM_{IF}), n = 46; (2) $\text{OCGB}^{+}/\text{IgG}_{\text{IF}}^{-}/\text{IgM}_{\text{IF}}^{-}$, n = 114; (3) $\text{OCGB}^{+}/\text{IgG}_{\text{IF}}^{+}/\text{IgM}_{\text{IF}}^{-}$, n = 229; and (4) $\text{OCGB}^{+}/\text{IgG}_{\text{IF}}^{+}/\text{IgM}_{\text{IF}}^{+}$ (ie, presence of OCGB and IgG_{IF} and IgM_{IF}), n = 111. Eleven of 530 patients (2.1%) were $\text{IgG}_{\text{IF}}^{+}$, $\text{IgM}_{\text{IF}}^{+}$, or $\text{IgA}_{\text{IF}}^{+}$ in combination with OCGB^{-} , whereas 19 of 530 (3.6%) had an $\text{OCGB}^{+}/\text{IgG}_{\text{IF}}^{-}/\text{IgM}_{\text{IF}}^{+}$ combination; all these were excluded from this categorization.

In a second step, we investigated associations between demographic (sex and age) or CSF parameters (OCGB^{+} vs OCGB^{-} ; $\text{IgG}_{\text{IF}}^{+}$ vs $\text{IgG}_{\text{IF}}^{-}$; $\text{IgM}_{\text{IF}}^{+}$ vs $\text{IgM}_{\text{IF}}^{-}$; $\text{IgA}_{\text{IF}}^{+}$ vs $\text{IgA}_{\text{IF}}^{-}$; above categories 2–4 vs category 1) with described endpoints (time to a first relapse, longitudinal sNfL levels, MRI lesions, MSSS, and time to heDMT) by univariable (Cox proportional hazards, linear mixed, negative binomial mixed, and linear) models, respectively.

Next, associations between these 4 categories and time to first relapse and time to first initiation of an heDMT were investigated by multivariable Cox proportional hazards models, adjusted for age at first symptoms, sex, and DMT category (heDMT, oral, platform, or untreated; DMT category was a time-varying covariate). To analyze associations between the CSF categories and longitudinal sNfL concentrations, age-dependent sNfL Z -scores were modeled in healthy controls using a generalized additive model for location, scale, and shape (GAMLSS) to reflect the deviation of a patient’s sNfL value from the mean value of same-age healthy controls.²¹ Serum NfL Z -scores (dependent variable) were analyzed

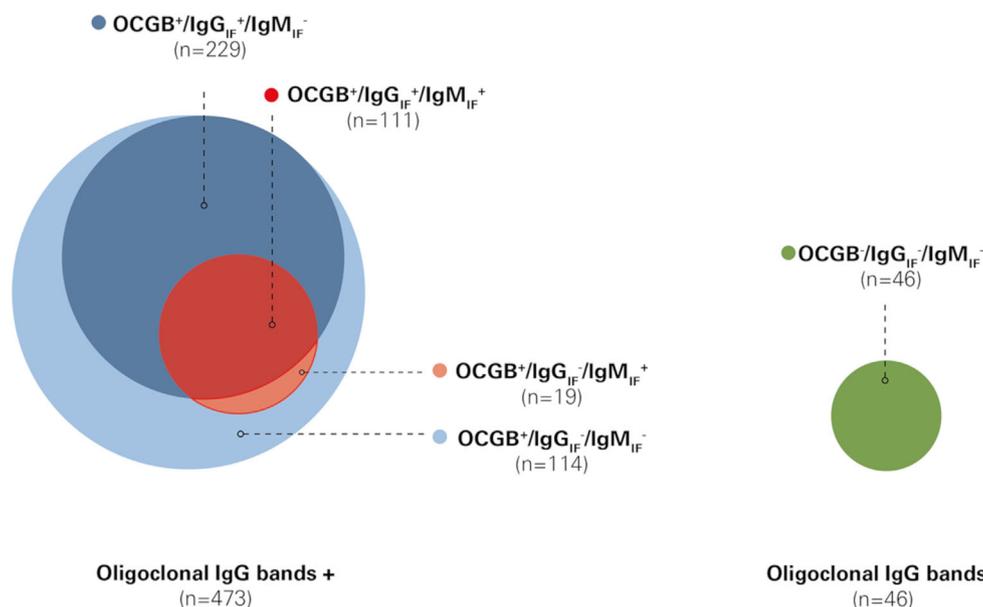


FIGURE 1: Combination of CSF immunoglobulin synthesis and frequencies in patients with and without presence of oligoclonal IgG bands. OCGB were present (+) in 473 patients, and 340 of them had additional IgG_{IF} and 130 showed presence of IgM_{IF}, whereas 46 had no (−) OCGB, IgG_{IF}, and IgM_{IF}. Patients were categorized into the following 4 groups according to the presence or absence of OCGB, IgG_{IF}, and IgM_{IF}: (1) OCGB[−]/IgG_{IF}[−]/IgM_{IF}[−], n = 46 (green); (2) OCGB⁺/IgG_{IF}[−]/IgM_{IF}[−], n = 114 (light blue); (3) OCGB⁺/IgG_{IF}⁺/IgM_{IF}[−], n = 229 (blue); and (4) OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺, n = 111 (red). The presence of IgM_{IF} coincided with that of IgG_{IF} in 85.4% (111 of 130) samples (only 19 of 130 patients [15.6%] had an OCGB⁺/IgG_{IF}[−]/IgM_{IF}⁺ pattern). In OCGB[−], 11 of 530 patients (2.1%) showed IgG_{IF}⁺, IgM_{IF}⁺, or IgA_{IF}⁺ and were excluded from this categorization (not shown in figure). CSF = cerebrospinal fluid; IgG = immunoglobulin G; IgG_{IF}/M_{IF}/A_{IF} = immunoglobulin G/M/A intrathecal fraction; IgM = immunoglobulin M; OCGB = oligoclonal IgG bands; + = presence of OCGB or IgG/IgM by Reiber formula; − = absence of OCGB or IgG/IgM by Reiber formula.

by multivariable linear mixed models adjusted for sex and DMT category at the respective visits for blood sampling.

T2w hyperintense lesion counts and the occurrence of new/enlarging T2w hyperintense lesions were analyzed by multivariable mixed negative binomial models including disease duration or time between MRI scans, respectively as offset, and adjusted for age at first symptoms, sex, and dominant DMT category (DMT category with longest treatment duration) or DMT category at visit, respectively. MSSS (calculated at the last follow-up visit) was analyzed by a multivariable linear model, adjusted for age, sex, and dominant treatment category.

Quantitative Analysis. In a fourth step, we investigated in OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients whether there was an association between the outcomes above and the quantitative amount of IgG_{IF}/IgM_{IF} by (1) splitting at median IgG_{IF}/IgM_{IF}, and (2) including IgG_{IF} and IgM_{IF} as continuous variables in the same (ie, “combined”) model in order to assess their potential individual and independent contributions. For all endpoints, the same model structure described above was used and adjusted for the same covariables.

For time to first relapse and time to start of an heDMT, IgM_{IF} was additionally assessed by splitting at the

median in OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺, and IgG_{IF} by splitting at the median in OCGB⁺/IgG_{IF}⁺/IgM_{IF}[−] patients.

For each model, the estimated effect of the Ig parameter is presented together with its 95% confidence intervals (CIs) and *p*-value. In addition, the estimates (marginal effects) are presented graphically by extracting the predicted values together with their 95% CIs using the R package sjPlot.²⁴

Time to first relapse and time to first initiation of an heDMT are presented in Kaplan–Meier curves.

Model assumptions were assessed using graphical methods. For the Cox proportional hazard models, we inspected Schoenfeld’s residuals. Linear models were assessed by checking residuals and leverages. The residuals were checked by visual inspection using a plot of the residuals versus the fitted values, in addition to a normal Q-Q plot. All analyses were conducted using the statistical software R (version 3.6.3) (R is an open-source, interpreted (statistical) programming language available at www.r-project.org).

Results

Demographic, Clinical, and CSF Characteristics

Five hundred and thirty patients were included in this study (Table 1). OCBs were detected in 473 (89.2%); 340 (71.9%) of those had IgG_{IF}, 27.5% IgM_{IF}, and

TABLE 1. Demographic, Clinical and CSF Characteristics at Lumbar Puncture

Characteristic	All ^a	OCGB ⁻ IgG _{IF} ⁻ IgM _{IF} ⁻	OCGB ⁺ IgG _{IF} ⁻ IgM _{IF} ⁻	OCGB ⁺ IgG _{IF} ⁺ IgM _{IF} ⁻	OCGB ⁺ IgG _{IF} ⁺ IgM _{IF} ⁺
Demographics					
n (%)	530 (100)	46 (8.7)	114 (21.5)	229 (43.2)	111 (20.9)
Female, n (%)	355 (67.0)	31 (67.4)	56 (49.1)	169 (73.8)	78 (70.3)
Age at first symptoms, mean (SD), yr	33.5 (10.7)	36.0 (11.8)	35.3 (11.3)	33.4 (10.0)	31.0 (10.9)
Characteristics at LP					
1 clinical event, n (%)	319 (60.2)	34 (73.9)	69 (60.5)	133 (58.1)	65 (58.6)
≥2 clinical events, n (%)	211 (39.8)	12 (26.1)	45 (39.5)	96 (41.9)	46 (41.4)
Age, mean (SD), yr	35.7 (11.0)	38.3 (13.8)	37.8 (11.5)	35.9 (10.0)	32.7 (11.1)
First symptoms to LP, median (IQR), mo	2.8 (0.4, 20.7)	1.5 (0.3, 10.5)	3.9 (0.4, 18.0)	3.1 (0.4, 25.4)	2.0 (0.3, 19.8)
DMT before LP	18 (3.4)	3 (6.5)	3 (2.6)	10 (4.4)	2 (1.8)
HeDMT before LP	4 (0.7)	1 (2.2)	0 (0)	2 (0.9)	0 (0)
CSF characteristics					
OCGB ⁺ , n (%)	473 (89.2)	0 (0)	114 (100)	229 (100)	111 (100)
IgG _{IF} ⁺ , n (%)	345 (65.1)	0 (0)	0 (0)	229 (100)	111 (100)
IgM _{IF} ⁺ , n (%)	138 (26.0)	0 (0)	0 (0)	0 (0)	111 (100)
IgA _{IF} ⁺ (n, %)	62 (11.7)	0 (0)	7 (6.1)	25 (10.9)	22 (19.8)
Albumin quotient, median (IQR)	4.9 (3.9, 6.5)	5.1 (4.4, 6.7)	6.0 (4.6, 8.3)	5.0 (3.9, 6.4)	4.2 (3.4, 5.0)
White cell count, median (IQR), n/μl	5.0 (2.3, 10.3)	2.0 (1.0, 4.0)	4.0 (2.0, 6.7)	5.6 (3.0, 10.7)	10.0 (5.0, 19.0)

^aEleven of 530 patients were OCGB⁻ and IgG_{IF}⁺ and/or IgM_{IF}⁺ and/or IgA_{IF}⁺, and 19 of 530 patients had an OCGB⁺/IgG_{IF}⁻/IgM_{IF}⁺ pattern; these were excluded from the categorization in columns 3–6 (OCGB⁻/IgG_{IF}⁻/IgM_{IF}⁻ or OCGB⁺/IgG_{IF}⁻/IgM_{IF}⁻ or OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁻ or OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺).

CSF = cerebrospinal fluid; heDMT = high-efficacy disease-modifying treatment; IgG/M/A_{IF} = immunoglobulin G/M/A intrathecal fraction; IQR = interquartile range; LP = lumbar puncture; OCGB = oligoclonal IgG bands.

12.3% IgA_{IF} (65.1, 26.0, and 11.7%, respectively, of the entire cohort; see Fig 1).

At the time of LP, 319 (60.2%) patients had experienced 1 clinical attack and 211 (39.8%) ≥2 clinical attacks (see Table 1). CSF variables between patients with LP before versus after first relapse were similar (Table S1).

The following results were based on multivariable analyses; all corresponding univariable analyses showed congruent results (Table S2).

Time Interval between First Symptoms and First Relapse. Three hundred and ninety-two (74.0%) patients

had a first relapse (second clinical attack), whereas 138 (26.0%) patients remained relapse free during a median disease duration of 9.0 years at last follow-up (IQR 5.3–13.3).

OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients had a 94% higher risk of experiencing a first relapse (hazard ratio [HR] 1.94 [CI: 1.24, 3.05], $p < 0.01$) compared with patients without intrathecally produced Ig (OCGB⁻/IgG_{IF}⁻/IgM_{IF}⁻). In contrast, the IgG_{IF} status was not associated with time to first relapse, showing similar, smaller hazard ratios for OCGB⁺/IgG_{IF}⁺ and OCGB⁺/IgG_{IF}⁻ patients (Table 2A). Accordingly, the median time to first relapse

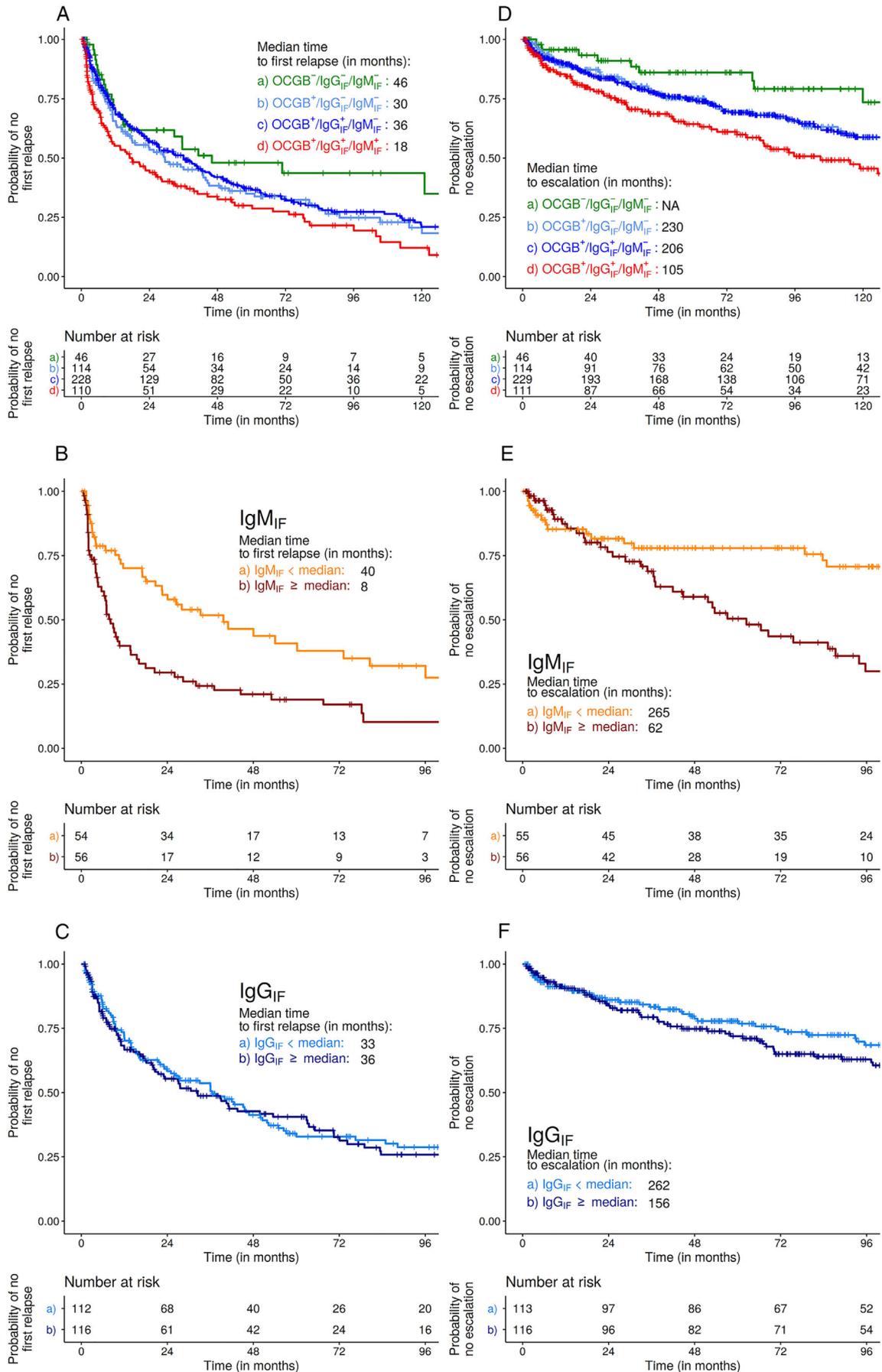
TABLE 2. Multivariable Models for the Following Outcomes: (A) Time to First Relapse, (B) sNfL Z-scores, (C) sNfL Z-scores in Untreated Patients, (D) Total T2w Lesion Count, (E) New/Enlarging T2w Lesion Count, (F) Multiple Sclerosis Severity Score and (G) Time to First Initiation of a heDM

A. Time to First Relapse				
	n	HR	CI	p
Male versus female	498	0.93	0.74, 1.17	0.528
Age	498	0.99	0.98, 1.00	0.237
OCGB ⁺ /IgG _{IF} ⁻ /IgM _{IF} ^{-a}	114	1.45	0.93, 2.27	0.102
OCGB ⁺ /IgG _{IF} ⁺ /IgM _{IF} ^{-a}	228	1.40	0.92, 2.14	0.115
OCGB ⁺ /IgG _{IF} ⁺ /IgM _{IF} ^{+a}	110	1.94	1.24, 3.05	<0.01
B. sNfL Z-scores				
	n	Est.	CI	p
Male versus female	3045	0.03	-0.18, 0.23	0.782
OCGB ⁺ /IgG _{IF} ⁻ /IgM _{IF} ^{-a}	667	0.10	-0.27, 0.47	0.594
OCGB ⁺ /IgG _{IF} ⁺ /IgM _{IF} ^{-a}	1394	0.12	-0.22, 0.46	0.502
OCGB ⁺ /IgG _{IF} ⁺ /IgM _{IF} ^{+a}	716	0.41	0.04, 0.78	0.032
C. sNfL Z-scores in Untreated Patients				
	n	Est.	CI	p
Male versus female	456	0.51	0.10, 0.92	0.016
OCGB ⁺ /IgG _{IF} ⁻ /IgM _{IF} ^{-a}	100	0.71	0.06, 1.36	0.033
OCGB ⁺ /IgG _{IF} ⁺ /IgM _{IF} ^{-a}	181	0.79	0.21, 1.38	<0.01
OCGB ⁺ /IgG _{IF} ⁺ /IgM _{IF} ^{+a}	88	1.18	0.53, 1.83	<0.01
D. T2w Lesion Count				
	n	IRR	CI	p
Male versus female	1795	1.16	0.91, 1.48	0.226
Age	1795	1.02	1.01, 1.03	<0.01
OCGB ⁺ /IgG _{IF} ⁻ /IgM _{IF} ^{-a}	389	1.66	1.08, 2.57	0.021
OCGB ⁺ /IgG _{IF} ⁺ /IgM _{IF} ^{-a}	809	1.59	1.06, 2.37	0.023
OCGB ⁺ /IgG _{IF} ⁺ /IgM _{IF} ^{+a}	414	2.53	1.63, 3.93	<0.01

E. New/Enlarging T2w Lesion Count				
	n	IRR	CI	p
Male versus female	838	0.93	0.55, 1.58	0.789
Age	838	0.98	0.96, 1.00	0.072
OCGB ⁺ /IgG _{IF} ⁻ /IgM _{IF} ^{-a}	164	1.84	0.73, 4.62	0.195
OCGB ⁺ /IgG _{IF} ⁺ /IgM _{IF} ^{-a}	387	1.61	0.70, 3.69	0.258
OCGB ⁺ /IgG _{IF} ⁺ /IgM _{IF} ^{+a}	195	3.13	1.29, 7.58	0.011
F. Multiple Sclerosis Severity Score				
	n	Est.	CI	p
Male versus female	499	0.70	0.30, 1.10	<0.01
Age	499	0.06	0.04, 0.08	<0.01
OCGB ⁺ /IgG _{IF} ⁻ /IgM _{IF} ^{-a}	114	0.73	0.01, 1.45	0.047
OCGB ⁺ /IgG _{IF} ⁺ /IgM _{IF} ^{-a}	228	0.86	0.19, 1.52	0.012
OCGB ⁺ /IgG _{IF} ⁺ /IgM _{IF} ^{+a}	111	1.11	0.38, 1.84	<0.01
G. Time to First Initiation of an heDMT				
	n	HR	CI	p
Male versus female	500	1.06	0.79, 1.42	0.715
Age	500	0.99	0.97, 1.00	0.037
OCGB ⁺ /IgG _{IF} ⁻ /IgM _{IF} ^{-a}	114	2.02	0.99, 4.14	0.055
OCGB ⁺ /IgG _{IF} ⁺ /IgM _{IF} ^{-a}	229	2.09	1.04, 4.16	0.037
OCGB ⁺ /IgG _{IF} ⁺ /IgM _{IF} ^{+a}	111	2.95	1.44, 6.02	<0.01

^aComparator group: OCGB⁻/IgG_{IF}⁻/IgM_{IF}⁻: n = 46 patients.
 CI = 95% confidence interval; heDMT = high-efficacy disease-modifying treatment; HR = hazard ratio; IgG_{IF}/M_{IF} = immunoglobulin G/M intrathecal fraction; OCGB⁺ = presence of oligoclonal IgG bands.

was shorter in OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ versus OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁻ and OCGB⁺/IgG_{IF}⁻/IgM_{IF}⁻ patients (median time to first relapse 18 vs 36 vs 30 months, respectively; 46 months in OCGB⁻/IgG_{IF}⁻/IgM_{IF}⁻ patients; Fig 2A).



(Figure legend continues on next page.)

In the quantitative analysis, patients with IgM_{IF} levels above the median had their first relapse earlier than those with values below the median (HR 1.86 [CI 1.18, 2.93], $p < 0.01$, $n = 110$; see Fig 2B), whereas the quantity of IgG_{IF} in OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁻ patients was not related to the time to the first relapse (HR 1.12 [CI 0.82, 1.52], $p = 0.492$, $n = 228$; see Fig 2C). In the model combining IgM_{IF} and IgG_{IF}, patients with IgM_{IF} above versus below the median showed an increased hazard of a first relapse, which was not seen for IgG_{IF} (Table 3A).

Serum Neurofilament Light Chain Concentrations. sNfL was measured in 520 patients with 3,260 longitudinal samples (median [IQR] number of samples per patient: 6 [4–8]).

OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients had higher sNfL Z-scores when compared with patients without intrathecal Ig synthesis (OCGB⁻/IgG_{IF}⁻/IgM_{IF}⁻; estimate 0.41 [CI 0.04, 0.78], $p = 0.032$; ie, OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients had on average 0.41 standard deviations higher sNfL concentrations). In those patients with presence of only OCGB⁺ or OCGB⁺/IgG_{IF}⁺, sNfL Z-scores were not elevated (Fig 3A; Table 2B).

In the combined model, sNfL Z-scores were higher in patients with IgM_{IF} above versus below the median, whereas for IgG_{IF} this was not the case. Similar results were seen when using IgM_{IF} and IgG_{IF} as continuous variables (Table 3B).

In untreated patients (494 samples in 197 patients), similar results were seen. The highest sNfL Z-scores were found in OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients (estimate 1.18 [CI 0.53, 1.83], $p < 0.01$), followed by only OCGB⁺ or OCGB⁺/IgG_{IF}⁺ patients (see Fig 3B; Table 2C).

Again, in the combined model the patients with IgM_{IF} above versus below the median or with higher IgM_{IF} when analyzed as a continuous variable had higher sNfL Z-scores, which was not seen for IgG_{IF} (Table 3C).

MRI: Total and New/Enlarging T2w Lesions. T2w lesion count data were available from 488 patients and 1,918 MRIs (number of MRIs per patient: 4 [2–5]).

OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients had on average 2.5 times more T2w lesions compared with patients without intrathecal Ig (incidence rate ratio [IRR] 2.53 [CI 1.63, 3.93], $p < 0.01$, Table 2D; see Fig 3C). In the combined model, we found numerically stronger effects for IgM than for IgG (Table 3D).

Information on new/enlarging T2w lesions from annual scans was available from 305 patients and 905 MRIs. OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients had the highest number of new/enlarging T2w lesions (IRR 3.13 [CI 1.29, 7.58], $p = 0.011$), with no such associations seen in OCGB⁺ only and in OCGB⁺/IgG_{IF}⁺ patients (Table 2E; see Fig 3D).

Patients with IgM_{IF} above versus below the median or analyzed as a continuous variable had a higher incidence of new/enlarging T2w lesions when analyzed in the same model with IgG_{IF} (Table 3E). A similar effect was not seen for IgG_{IF} (see Table 3E).

Disability (Multiple Sclerosis Severity Score). The MSSS was on average higher by 1.11 points in OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients (estimate 1.11 [CI 0.38, 1.84], $p < 0.01$), followed by those with OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁻ (estimate 0.86 [CI 0.19, 1.52], $p = 0.012$) and OCGB⁺/IgG_{IF}⁻/IgM_{IF}⁻ (estimate 0.73 [CI 0.01, 1.45], $p = 0.047$), when compared with those without intrathecal Ig synthesis (Table 2F; see Fig 3E). No dose-dependent effects of IgM_{IF}⁺ (nor of IgG_{IF}⁺) were found in relation to MSSS (Table 3F).

Initiation of High-Efficacy Disease-Modifying Treatments HeDMT was started in 223 of 530 patients (42.1%; no previous DMT: 66 [29.6%]; escalation from platform therapies: 81 [36.3%]; escalation from oral treatments: 76 [34.1%]). OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients featured the highest probability of escalation to heDMT (HR 2.95 [CI 1.44, 6.02],

FIGURE 2: Kaplan–Meier analyses for probability of a first relapse (A–C) and escalation to a high efficacy treatment (D–F). (A) Median time to first relapse was shorter in OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ (red line) than in OCGB⁻/IgG_{IF}⁻/IgM_{IF}⁻ patients (green line; 18 vs 46 months, HR 1.94 [CI 1.24, 3.05], $p < 0.01$). Time to first relapse in OCGB⁺/IgM_{IF}⁻ patients was not impacted by the IgG_{IF} status (light blue line = OCGB⁺/IgG_{IF}⁻/IgM_{IF}⁻, median time 30 months; dark blue line = OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁻, 36 months). (B) In OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients ($n = 110$), we saw a dose effect of the amount of IgM_{IF}: patients with higher IgM_{IF} experienced a first relapse earlier than those with lower IgM_{IF} (above [red line] vs below [orange line] median IgM_{IF}: HR 1.86 [CI 1.18, 2.93], $p < 0.01$, $n = 110$). (C) For OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁻ patients, no such dose effect for IgG_{IF} was found (HR 1.12 [CI 0.82, 1.52]; $p = 0.492$, $n = 228$). (D) Time to switch to heDMT was shortest in the OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ group (red line; median 105 months, HR 2.95 [CI 1.44, 6.02], $p < 0.01$; $n = 111$) versus OCGB⁻/IgG_{IF}⁻/IgM_{IF}⁻ (green line), whereas in OCGB⁺/IgG_{IF}⁻/IgM_{IF}⁻ (light blue line) it was 230 months, and in OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁻ (dark blue line) it was 206 months. (E) Patients with increasing IgM_{IF} levels showed an increased hazard for escalation to heDMT (above [red line] vs below [orange line] median IgM_{IF}: HR 2.28 [CI 1.30, 3.99]; $p < 0.01$; $n = 111$). (F) No increased hazard of switching to an heDMT was found for IgG_{IF}⁺ (above [dark blue line] vs below [light blue line] median: HR 1.38 [CI 0.92, 2.09]; $p = 0.124$; $n = 229$). CI = 95% confidence interval; heDMT = high-efficacy disease-modifying treatment; HR = hazard ratio; IgG_{IF}/M_{IF} = immunoglobulin G/M intrathecal fraction; OCGB = oligoclonal IgG bands; + = presence of OCGB or IgG_{IF}/IgM_{IF}; - = absence of OCGB or IgG_{IF}/IgM_{IF}.

TABLE 3. Multivariable Models for Dose-dependent Effects of IgM_{IF} and IgG_{IF} in OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ Patients for the Following Outcomes: (A) Time to First Relapse, (B) sNfL Z-scores, (C) sNfL Z-scores in Untreated Patients, (D) Total T2w Lesion Count, (E) New/Enlarging T2w lesion count, (F) Multiple Sclerosis Severity Score and (G) Time to First Initiation of a heDMT

A. Time to First Relapse	HR	CI	p
1. IgM _{IF} (> vs <median)	1.85	1.17, 2.92	<0.01
2. IgG _{IF} (> vs <median)	1.13	0.73, 1.75	0.578
3. IgM _{IF} (per 1%)	1.01	1.00, 1.02	0.064
4. IgG _{IF} (per 1%)	1.01	1.00, 1.02	0.180
B. sNfL Z-scores (n = 716)	Est.	CI	p
1. IgM _{IF} (> vs <median)	0.43	0.10, 0.77	0.013
2. IgG _{IF} (> vs <median)	0.01	-0.32, 0.34	0.951
3. IgM _{IF} (per 1%)	0.01	0.00, 0.02	0.034
4. IgG _{IF} (per 1%)	0.00	-0.01, 0.01	0.909
C. sNfL Z-scores in untreated patients (n = 88)	Est.	CI	p
1. IgM _{IF} (> vs <median)	0.92	0.18, 1.66	0.019
2. IgG _{IF} (> vs <median)	-0.15	-0.88, 0.59	0.696
3. IgM _{IF} (per 1%)	0.02	0.00, 0.04	0.032
4. IgG _{IF} (per 1%)	0.00	-0.02, 0.02	0.855
D. T2w Lesion Count (n = 414)	HR	CI	p
1. IgM _{IF} (> vs <median)	1.63	0.96, 2.75	0.071
2. IgG _{IF} (> vs <median)	1.02	0.61, 1.72	0.940
3. IgM _{IF} (per 1%)	1.01	1.00, 1.02	0.271
4. IgG _{IF} (per 1%)	1.01	0.99, 1.02	0.385
E. New/Enlarging T2w Lesion Count (n = 195)	HR	CI	p
1. IgM _{IF} (> vs <median)	2.86	1.25, 6.54	0.013
2. IgG _{IF} (> vs <median)	1.04	0.49, 2.20	0.913
3. IgM _{IF} (per 1%)	1.02	1.01, 1.02	<0.01
4. IgG _{IF} (per 1%)	1.00	1.00, 1.01	0.417

F. Multiple Sclerosis Severity

Score (n = 111)	HR	CI	p
1. IgM _{IF} (> vs <median)	0.59	-0.23, 1.40	0.156
2. IgG _{IF} (> vs <median)	-0.08	-0.87, 0.71	0.843
3. IgM _{IF} (per 1%)	0.01	-0.01, 0.03	0.201
4. IgG _{IF} (per 1%)	0.00	-0.01, 0.02	0.680

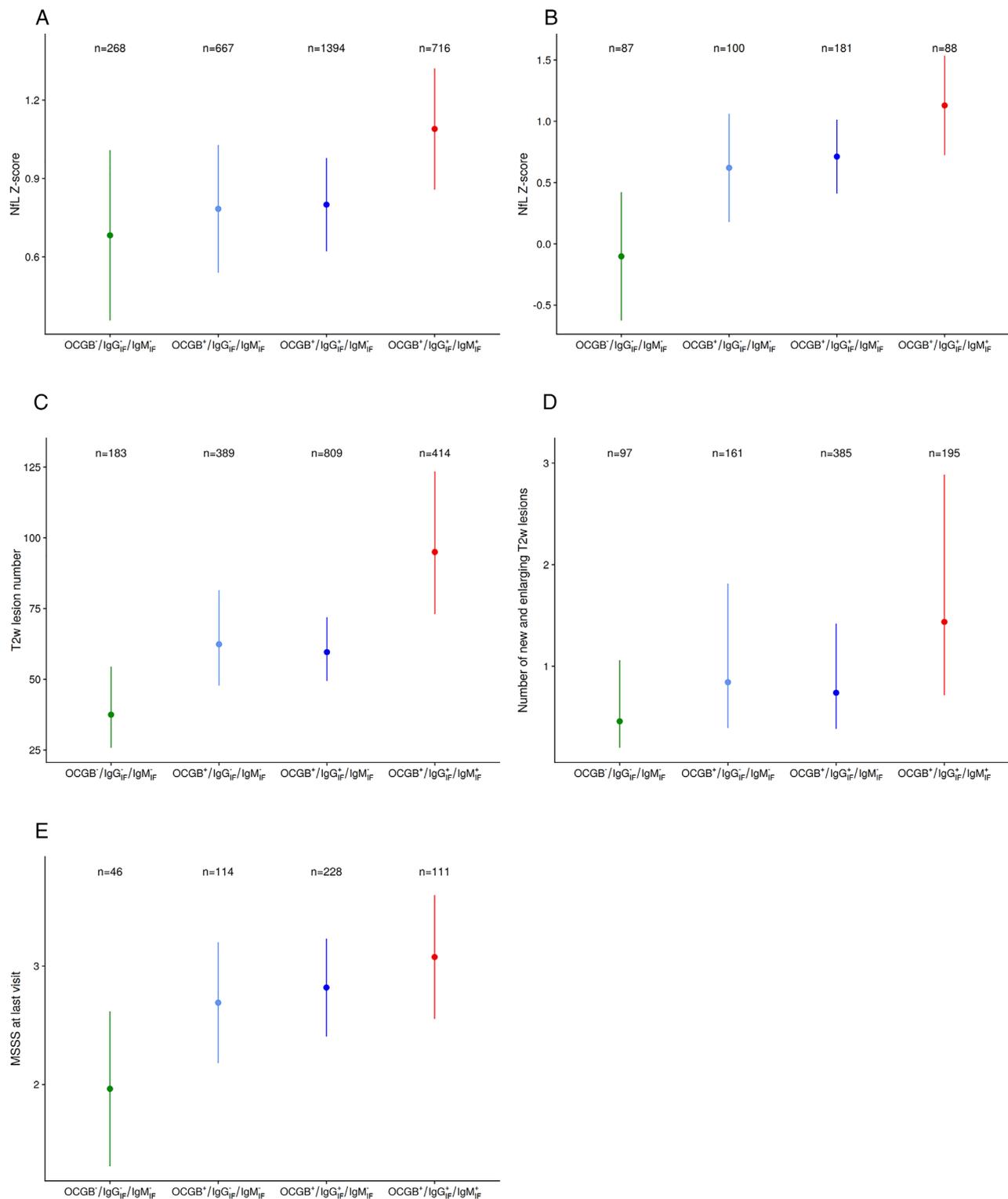
G. Time to First

Initiation of an heDMT (n = 111)	HR	CI	p
1. IgM _{IF} (> vs <median)	2.35	1.33, 4.16	<0.01
2. IgG _{IF} (> vs <median)	0.87	0.51, 1.48	0.603
3. IgM _{IF} (per 1%)	1.02	1.01, 1.03	<0.01
4. IgG _{IF} (per 1%)	1.000	0.99, 1.01	0.944

Lines 1 and 2: Model 1: IgM_{IF} and IgG_{IF} split by median. Lines 3 and 4: Model 2: IgM_{IF} and IgG_{IF} as continuous variables.

CI = 95% confidence interval; heDMT = high-efficacy disease-modifying treatment; HR = hazard ratio; IgG_{IF}/M_{IF} = immunoglobulin G/M intrathecal fraction; OCGB⁺ = presence of oligoclonal IgG bands.

$p < 0.01$) compared with patients without intrathecal Ig synthesis. In those with either only OCGB⁺ or OCGB⁺/IgG_{IF}⁺, these effects were weaker (Table 2G). Accordingly, the time to initiation of heDMT was shortest in the OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ (105 months) versus OCGB⁻/IgG_{IF}⁻/IgM_{IF}⁻ patients (given that only 26.8% were treated with heDMT in this group, the median time to switch could not be determined), whereas in OCGB⁺/IgG_{IF}⁻/IgM_{IF}⁻ and in OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁻ patients it was 230 and 206 months, respectively (see Fig 2D); similar to the analysis of the time interval to first relapse, the time to heDMT in OCGB⁺/IgM_{IF}⁻ patients was not impacted by the IgG_{IF} status. Again, this association was dose-dependent for IgM_{IF}, but not for IgG_{IF}. In OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients, IgM_{IF}⁺ levels above the median were associated with a >2-fold increased hazard for escalation to heDMT (HR 2.28 [CI 1.30, 3.99], $p < 0.01$, $n = 111$) versus those below the median (see Fig 2E), whereas this was not the case for IgG_{IF}⁺ in patients with only OCGB⁺/IgG_{IF}⁺ (HR 1.38 [CI 0.92, 2.09], $p = 0.12$, $n = 229$; see Fig 2F). In the combined model, similar results were found (Table 3G).



(Figure legend continues on next page.)

Discussion

The prediction of the individual course of multiple sclerosis based on current clinical and MRI measures provides limited accuracy for therapeutic decision making. OCGB are present in up to 98% of multiple sclerosis patients and have been

reinstated as a diagnostic criterion in the 2017 MS diagnostic criteria.¹ However, the presence of OCGB as a prognostic biomarker is relevant only in the minority of multiple sclerosis patients where they are absent, because this might go along with a milder disease course.²⁵

OCMB have shown prognostic value,^{9,11,26–28} but owing to the difficulties in standardizing the methodology, this measure is not widely used. In contrast, the quantitation of intrathecal Ig synthesis using the Reiber formulae is a fully standardized analytical method.

Here, we have identified that the presence and, furthermore, the quantity of IgM_{IF} is a stronger and more independent prognostic biomarker than OCGB and IgG_{IF} for acute disease activity (time interval from disease onset to first relapse) and the chronic course of disability (MSSS), and for biological and radiological measures (sNfL Z-scores and MRI activity).

Additionally, we demonstrate that the presence of intrathecal IgM production is associated with a first choice of heDMT or an earlier switch to heDMT, as the physician's consequence for the need for a more aggressive therapy. Moreover, this association holds true also when the quantity of IgM_{IF} is considered; in this case, we observed that the quantity of IgM_{IF} is related to a shorter time to DMT escalation and to "time to first relapse," in addition to increased sNfL concentrations and disease activity as determined by MRI measures. In contrast, the presence of IgG_{IF}, or the quantity of IgG_{IF} in addition to OCGB has not shown significant additional prognostic value (see Tables 2 and 3; Figs 2 and 3). In line with prior results,⁶ intrathecal IgA_{IF} was relatively rare in the present study cohort (present in 62 [11.7%] of patients; see Table 1) and was not associated with any of the outcomes (see Table S2), suggesting that its presence might lack a relevant pathogenic role in multiple sclerosis.

Our results are somewhat discordant with recent data from the German multiple sclerosis cohort, in which IgG_{IF} but not IgM_{IF} was associated with risk and shorter time to EDSS worsening within 4 years.⁶ A potential explanation for this discrepancy might be the different statistical approaches: unconfirmed EDSS worsening was analyzed for individual Ig isotypes, whereas we adjusted for the presence of OCGB, IgG_{IF}, and IgM_{IF} in combined models. Furthermore, a longer observation time (median 9.0 vs 4 years since disease onset), a larger patient cohort with disease worsening data (530 vs 330 patients) in conjunction with different statistical approaches (no imputation of data vs imputation of 27–41% of EDSS data at year 4, additionally shortening observation interval) in our study versus the German multiple sclerosis cohort study might be additional factors.⁶

The contribution of antibodies and their capacity to activate complement during the initial development of a multiple sclerosis plaque has been demonstrated in autopsy studies. Complement C3b colocalizes with IgG and IgM deposits on oligodendrocytes and axons, leading to demyelination and axonal injury.^{29,30} Intrathecal synthesis of IgM (but not IgG or IgA) was shown to be associated with early activation of the complement cascade, with intrathecal activation of complement component C3, which is in line with IgM being the most efficient isotype for complement activation.³¹

An indication of a pathogenic role of intrathecal Ig synthesis could be its longitudinal decrease under some heDMTs in parallel to clinical improvement or stabilization of disease. Continuous treatment with the B cell-targeting drugs rituximab and ocrelizumab can lead to

FIGURE 3: Estimates (marginal effects) as derived from the multivariable analyses for serum neurofilament light chain Z-scores (A, all patients; B, untreated patients), MRI disease activity (C, number of T2w hyperintense lesions; D, number of new/enlarging lesions) and Multiple Sclerosis Severity Score (E) stratified by CSF immunoglobulin profiles. (A) sNfL Z-scores in all patients (n = 491 with 3,045 longitudinal samples). After adjustment for covariates, OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients had higher sNfL Z-scores when compared with patients without any intrathecal Ig synthesis (Est. 0.41 [CI 0.04, 0.78], p = 0.032). In patients with presence of only OCGB⁺ or OCGB⁺/IgG_{IF}⁺, sNfL Z-scores were not significantly elevated (Est. 0.10 [CI -0.27, 0.47], p = 0.594 and Est. 0.12 [CI -0.22, 0.46], p = 0.502). (B) sNfL Z-scores in untreated patients (n = 186 with 456 longitudinal samples). The highest sNfL Z-scores were found in OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients (Est. 1.18 [CI 0.53, 1.83], p < 0.01), and weaker effects on a similar level for those patients with only OCGB⁺ or OCGB⁺/IgG_{IF}⁺ (Est. 0.71 [CI 0.06, 1.36], p = 0.033 and Est. 0.79 [CI 0.21, 1.38], p < 0.01) were found. (C) Total T2w lesion count (n = 458 patients with 1,795 longitudinal MRIs). OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients had the highest T2 lesion counts (IRR = 2.53 [CI 1.63, 3.93], p < 0.01). Patients with only OCGB⁺ or OCGB⁺/IgG_{IF}⁺ showed smaller but similar effect sizes (IRR = 1.66 [CI 1.08, 2.57], p = 0.021 and IRR = 1.59 [CI 1.06, 2.37], p = 0.023, respectively). (D) New/enlarging T2w lesion count (n = 282 patients with 838 longitudinal MRIs). OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients had the highest counts of new/enlarging T2 lesions (IRR = 3.13 [CI 1.29, 7.58], p = 0.011), with no effects in the other 2 categories (OCGB⁺ and OCGB⁺/IgG_{IF}⁺: IRR = 1.84 [CI 0.73, 4.62], p = 0.195 and IRR = 1.61 [CI 0.70, 3.69], p = 0.258, respectively). (E) Multiple Sclerosis Severity Score (MSSS) (n = 499 patients). The MSSS was on average 1.11 points [CI 0.382, 1.843] higher in OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁺ patients (Est. 1.112 [CI 0.382, 1.843], p < 0.01), followed by those with OCGB⁺/IgG_{IF}⁺/IgM_{IF}⁻ (Est. 0.855 [CI 0.190, 1.520], p = 0.012) and OCGB⁺/IgG_{IF}⁻/IgM_{IF}⁻ (Est. 0.727 [CI 0.009, 1.445], p = 0.047), compared with those patients without intrathecal immunoglobulin synthesis. Plots show the estimates (marginal effects) and median together with their 95% CI. CI = 95% confidence interval; CSF = cerebrospinal fluid; MRI = magnetic resonance imaging; Est. = estimate; IgG_{IF}/M_{IF} = immunoglobulin G/M intrathecal fraction; IRR = incidence rate ratio; OCGB = oligoclonal IgG bands; sNfL Z-score = serum neurofilament light chain Z-score; T2w = T2-weighted; + = presence of OCGB or IgG_{IF}/IgM_{IF}; - = absence of OCGB or IgG_{IF}/IgM_{IF}.

hypogammaglobulinemia,^{32–34} an effect that is more prominent and occurs faster for IgM than for IgG. Only small case series describe the course of intrathecal Ig synthesis under rituximab or natalizumab therapy in multiple sclerosis: IgG_{IF} decreased transiently under rituximab and returned to levels prior to therapy during months 6 to 9.³⁵ Accordingly, intrathecal IgG synthesis (OCGB^{36,37}; IgG_{IF} [Reiber formula]³⁸) under natalizumab therapy decreased in some patients, but returned after treatment cessation to initial levels.³⁷ In contrast, intrathecal IgM synthesis and its relation to treatment effects have not been evaluated; an aspect we are now actively evaluating.

Limitations

Given that most patients entered the SMSC after the lumbar puncture had been performed, a correlation of intrathecal Ig synthesis with complete longitudinal EDSS scores from disease start was not possible in our study. In addition, our results are restricted mainly to CSF analysis in an early phase of multiple sclerosis; an extension of the CSF profiling into later stages of relapsing multiple sclerosis to gain a better understanding of the role of intrathecal IgM synthesis and its value as biomarker is warranted.

Conclusions

Our study strongly supports the value of a quantitative analysis of IgM levels in CSF as a prognostic biomarker, independent of other Ig measures, which might be useful for therapeutic decision making in the early phases of disease.

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Author Contributions

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A.M., S.M., K.B., H.W., T.L., J.L., O.Y., T.S., T.D., A.R., C.Z., C.G., L.K., C.G., D.L., and J.K.

Drafting of the manuscript: J.O., D.L., and J.K.

Potential Conflicts of Interest

Nothing to report.

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