JAMA Neurology | Original Investigation

Prognostic Factors for Multiple Sclerosis Symptoms in Radiologically Isolated Syndrome

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IMPORTANCE Understanding the risk factors for symptom development will allow clinicians to stratify people with radiologically isolated syndrome (pwRIS) more effectively and tailor their management strategies accordingly.

OBJECTIVE To identify prognostic factors at radiologically isolated syndrome (RIS) diagnosis associated with the development of multiple sclerosis (MS) symptoms.

DESIGN, SETTING, AND PARTICIPANTS This cohort study was performed in samples collected between July 2004 and September 2022 and included 33 MS centers. All pwRIS who meet the 2017 McDonald criteria for dissemination in space with a sample collected near the diagnostic magnetic resonance imaging were included. No patients who met eligibility criteria were excluded. The data were analyzed from July 2024 to November 2024.

EXPOSURE Body fluid biomarkers and environmental factors in pwRIS.

MAIN OUTCOMES AND MEASURES The main outcome was the development of MS symptoms. Analyses involved univariable and multivariable Cox proportional hazards models, including age, sex, and treatment following RIS diagnosis, as additional independent variables.

RESULTS The study included 273 pwRIS (mean age, 38.6 [SD 11.6] years; 207 women [75.8%] and 66 men [24.2%]) with a median follow-up of 5.0 [IQR, 2.5-7.7] years. A total of 101 pwRIS developed MS symptoms (37.0%). The presence of immunoglobulin G oligoclonal bands (OBs) (hazard ratio [HR], 5.09; 95% CI, 2.36-10.97; $P < .001$), immunoglobulin M OBs (HR, 2.58; 95% CI, 1.61-4.14; $P < .001$), and a κ free light chain index of 6.1 or more (HR, 2.79; 95% CI, 1.37-5.67; $P = .005$) were associated with MS symptoms. High cerebrospinal fluid neurofilament light chain (NfL) levels (HR, 1.31; 95% CI, 1.18-1.45; $P < .001$) and high serum NfL *z* scores (HR, 1.42; 95% CI, 1.16-1.72; $P = .005$) were also associated with an increased risk of MS symptoms. In contrast, high anti-cytomegalovirus titers (HR, 0.59; 95% CI, 0.38-0.93; $P = .02$) and high ultraviolet radiation exposure in the year before (HR, 0.52; 95% CI, 0.37-0.74; $P < .001$) and the year after (HR, 0.54, 95% CI, 0.38-0.75; $P < .001$) diagnosis reduced the risk of MS symptoms. For all these prognostic factors, the multivariable analysis yielded similar results. The combination of high serum NfL *z* scores and positive immunoglobulin G OBs conferred a 5-year risk of clinical symptoms of 58.3% (95% CI, 45.9-67.9). This risk increased to 81.6% (95% CI, 60.9-91.4) in pwRIS who were younger and positive for immunoglobulin M OBs.

CONCLUSIONS AND RELEVANCE The study elucidates the prognostic factors that significantly impact the risk of developing MS symptoms in pwRIS at diagnosis, thereby, enhancing the potential for tailored clinical interventions.

JAMA Neurol. 2025;82(7):722-733. doi:10.1001/jamaneurol.2025.1481
Published online June 2, 2025. Corrected on October 13, 2025.

Supplemental content

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Individuals with incidental magnetic resonance imaging (MRI) findings similar to those in patients with multiple sclerosis (MS) who are asymptomatic or have nonspecific symptoms and no clinical history of neurological symptoms suggestive of central nervous system demyelination are labeled as having radiologically isolated syndrome (RIS).[1] Over the years, the definition of RIS has been based on several radiological criteria and recommendations (RIS 2009 criteria,[2] MAGNIMS 2017 recommendations,[3] and RIS Consortium 2023 criteria[4]). RIS represents the earliest preclinical manifestation within central nervous system demyelinating disorders, as people with RIS (pwRIS) are at high risk of developing MS symptoms, fulfilling a relapsing-remitting phenotype (RRMS), or less frequently, a primary progressive phenotype (PPMS).[5] Follow-up studies of pwRIS indicated that the risk of neurological symptoms suggestive of MS at 5 years and 10 years was 34% and 51%, respectively.[6,7] While several biological markers have a prognostic role in pwRIS, studies underscore the need for further research.[8] Given that some pwRIS develop symptoms and evidence show that early introduction of disease-modifying therapies reduce the risk of a first clinical event,[9,10] reliable baseline prognostic factors at RIS diagnosis will be crucial for identifying high-risk pwRIS who could benefit from early treatment.

Key Points

Question Which biomarkers are associated with the development of multiple sclerosis (MS) symptoms in people with radiologically isolated syndrome (pwRIS)?

Findings This study provides a comprehensive evaluation of biomarkers in a large cohort of pwRIS, measured close to the time of RIS diagnosis, thereby, more accurately reflecting the status of pwRIS at this initial stage. This design enabled the identification of biomarkers most strongly associated with the subsequent development of MS symptoms in pwRIS and facilitated the establishment of a biomarker combination predictive of prognosis within the first 5 years following RIS diagnosis.

Meaning These results demonstrate that the identification of pwRIS who are at higher risk of developing clinical symptoms is crucial not only for prognosis but also for guiding treatment decisions, such as determining which individuals may benefit most from early initiation of disease-modifying therapies or enrollment in clinical trials.

Methods

For detailed methods, refer to the eMethods in Supplement 1.

pwRIS

This is a multicenter, observational cohort study of pwRIS. The inclusion criteria were pwRIS fulfilling the 2017 McDonald criteria for dissemination in space (MAGNIMS 2017 recommendations[3]) at RIS diagnostic MRI and cerebrospinal fluid (CSF) and/or blood sample collection performed within 6 months of RIS diagnosis. Individuals with follow-up of less than 1 year were excluded. Ethical approval was obtained and all participants gave written informed consent. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines.

Data Collection

Baseline data (at RIS diagnosis) included age, sex, family history of MS, and reason for diagnostic MRI. Radiological findings were recorded, including the presence of periventricular, cortical/juxtacortical, infratentorial, spinal cord (SC) lesions, and contrast-enhancing lesions (CEL). Follow-up data included new T2 lesions and CEL, and information on whether individuals received treatment after RIS diagnosis. Follow-up MRI was conducted in accordance with the clinical practice of each center. The date of the first MRI showing dissemination in time, either by the presence of new T2 lesions or CEL, was recorded.

Outcomes

The primary outcome was the development of MS symptoms, including relapses or a progressive course.[11] A secondary outcome was neurological disability, measured by time to Expanded Disability Status Scale (EDSS) 3.0 and 4.0, confirmed at 2 time points.

Determination of Baseline Body Fluid Biomarkers

The following immunoglobulin-related biomarkers and indices were determined in baseline paired serum and CSF samples: immunoglobulin G (IgG) oligoclonal bands (OBs) (positive if 2 or more bands were detected in the CSF but not in serum), index (positive if 0.7 or higher), immunoglobulin M (IgM) OBs, IgM index (represented as a continuous or categorical variable, with values more than 0.1 considered positive), IgM intrathecal fraction[12] (positive if more than 0), and the κ free light chain (KFLC) index (represented as continuous or categorical variable, with values 6.1 or higher considered positive).[13] IgG and IgM OBs were determined by isoelectric focusing and immunoblotting, as previously described.[14,15]

CSF levels of neurofilament light chain (cNfL), glial fibrillary acidic protein (cGFAP), chemokine ligand 13 (cCXCL13), and chitinase 3-like 1 (cCHI3L1) were measured using Simoa immunoassays and with an in-house assay for cCHI3L1.[16] CSF myelin basic protein (cMBP) concentrations were measured by enzyme-linked immunosorbent assay (ELISA). Serum levels of NfL (sNfL), GFAP (sGFAP), and CHI3L1 (sCHI3L1) were also measured using the same Simoa assays as those used for CSF. Assay variability is shown in eTable 1 in Supplement 1.

Determination of Baseline Biomarkers Related to Environmental Factors

Serum IgG responses to Epstein-Barr (EBV)–encoded nuclear antigen-1 (EBNA1) and viral capsid antigen (VCA), as well as human herpes virus-6 (HHV-6), varicella-zoster virus (VZV), and human cytomegalovirus (HCMV), were measured in pwRIS and 152 healthy donors (HD) ELISA. Serum levels of 25-hydroxyvitamin D were measured by chemiluminescence, and levels less than 20 ng/mL were considered low.[17]

Data on cumulative ultraviolet radiation (UVR) exposure the year before and the year after RIS diagnosis were gener-

Table 1. Demographic, Clinical, and Radiological Characteristics of People With Radiologically Isolated Syndrome (RIS) Included in the Study

Characteristics	No. (%)						
	Whole cohort	MS symptoms		EDSS 3.0		EDSS 4.0	
		No	Yes	No	Yes	No	Yes
No	273 (100)	172 (63.0)	101 (37.0)	78 (77.2)	23 (22.8)	85 (84.2)	16 (15.8)
Age, y, mean (SD)[a]	38.6 (11.6)	39.4 (11.7)	37.1 (11.5)	36.3 (11.6)	39.9 (11.0)	36.2 (11.7)	41.8 (9.3)
Sex							
Female	207 (75.8)	126 (73.3)	81 (80.2)	61 (78.2)	20 (87.0)	68 (80.0)	13 (81.3)
Male	66 (24.2)	46 26.7)	20 (19.8)	17 (21.8)	3 (13.0)	17 (20.0)	3 (18.7)
Follow-up time, y, median (IQR)[b]	5.0 (2.5-7.7)	3.8 (2.1-7.4)	5.8 (3.7-8.1)	5.4 (3.3-7.2)	8.0 (6.0-10.8)	5.5 (3.4-7.9)	7.0 (5.7-8.8)
Time to sample collection, mo, median (IQR) [c,d]	1.6 (0.4-3.3)	1.4 (0.5-3.1)	1.7 (0.2-3.5)	1.9 (0.1-3.5)	1.1 (0.2-3.7)	1.8 (0.1-3.5)	1.0 (0.4-3.6)
Time to outcome, y, mean (SD)[e]	NA	NA	2.4 (2.6)	NA	4.8 (3.7)	NA	5.2 (3.6)
Patients with periventricular lesions[f]	267 (97.8)	168 (97.7)	99 (98.0)	244 (97.6)	23 (100)	251 (97.7)	16 (100)
Patients with cortico/yuxtacortical lesions[f]	220 (80.9)	135 (78.5)	85 (85.0)	197 (79.1)	23 (100)	204 (79.7)	16 (100)
Patients with infratentorial lesions[f]	121 (44.5)	71 (41.3)	50 (50.0)	38 (49.4)	12 (52.2)	42 (50.0)	8 (50.0)
Patients with spinal cord lesions[f,g]	64 (36.0)	42 (32.8)	22 (44.0)	17 (41.5)	5 (55.6)	18 (40.9)	4 (66.7)
Patients with CEL[f,g]	47 (21.7)	22 (14.5)	25 (38.5)	20 (39.2)	5 (35.7)	21 (38.2)	4 (40.0)
Patients with new T2 lesions at follow-up[i,j]	143 (62.7)	58 (42.6)	85 (92.4)	66 (91.7)	19 (95.0)	72 (92.2)	13 (92.9)
Patients with CEL at follow-up[j]	53 (23.9)	12 (9.2)	41 (44.6)	32 (45.7)	9 (40.9)	34 (44.7)	7 (43.8)
Patients treated during follow-up[j]	30 (11.0)	21 (12.2)	9 (8.9)	56 (71.8)	15 (65.2)	66 (72.9)	11 (68.8)

Abbreviations: CEL, contrast-enhancing lesions; EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; NA, not applicable.

[a] Age was calculated as the difference between the date of the RIS diagnostic magnetic resonance imaging (MRI) and the date of birth.

[b] Follow-up time was calculated as the difference between the date of the RIS diagnostic MRI and the date of the last visit.

[c] Refers to the time between the date of the RIS diagnostic MRI and the cerebrospinal fluid/blood collection.

[d] In 4 patients, the time to sample collection was 7 months.

[e] Time to outcome was calculated as the difference between the date of cerebrospinal fluid/blood collection and the corresponding outcome.

[f] Refers to the baseline RIS diagnostic MRI.

[g] Information not available for 95 individuals (34.8%) and 56 individuals (20.5%), respectively.

[h] Refers to follow-up MRI performed after RIS diagnosis and before the development of MS symptoms.

[i] Information not available for 45 individuals (16.5%) and 51 individuals (18.7%), respectively.

[j] Refers to individuals who received treatment between RIS diagnosis and the development of the corresponding outcomes, or between RIS diagnosis and the time of last follow-up for individuals not reaching the outcomes. The 30 people with RIS who received treatment before MS symptoms were treated with interferon β (n = 12), glatiramer acetate (n = 7), dimethyl fumarate (n = 7), azathioprine (n = 1), teriflunomide (n = 1), cladribine (n = 1), and rituximab (n = 1). The 9 people with RIS who developed MS symptoms despite treatment received interferon β (n = 5), glatiramer acetate (n = 3), and dimethyl fumarate (n = 1).

ated using satellite-derived information from the National Aeronautics and Space Administration. These data enable estimates of cumulative UVR exposure specific to the geographical coordinates of an individual's residence and a defined time frame.[18]

Serum cotinine levels were measured by ELISA, and levels higher than 14 ng/mL were used to indicate smoking status. Interleukin 6 (IL-6), tumor necrosis factor, resistin, and adiponectin were quantified in serum by ELISA. eTable 1 in the Supplement 1 summarizes assay variability.

Statistical Analysis

Age- and sex-normalized *z* scores for sNfL and sGFAP were calculated using a large control reference.[19,20] Cox proportional hazards models were used to investigate the association between prognostic factors and time to MS symptoms, as well as time to EDSS 3.0 and 4.0. Each predictor was modeled in univariable and multivariable models, including age, sex, and treatment as additional independent variables.

Results

Clinical Characteristics of the RIS Cohort

eFigure 1 in Supplement 1 illustrates a schematic flowchart summarizing the study design. A total of 273 pwRIS with RIS diagnoses were recruited from 33 MS centers from July 2004 to September 2022 (**Table 1**). At baseline, the mean (SD) age was 38.6 (11.6) years and 207 were women (75.8%). Median (IQR) follow-up time was 5.0 (2.5-7.7) years. The main reason for MRI was headache (32.2%) (eTable 2 in Supplement 1). A family history of MS was present in 24 of 213 pwRIS (11.3%). In baseline RIS diagnostic MRI, 267 pwRIS had periventricular lesions (97.8%), 220 had cortico/yuxtacortical lesions (80.9%), 121 had infratentorial lesions (44.5%), and 64 had SC lesions (36.0%). CEL were present in 47 individuals (21.7%). A total of 232 pwRIS had follow-up MRI (85%). Of these, 143 developed new T2 lesions (62.7%) and 53 developed CEL (23.9%) after a mean (SD) time of 1.8 (2.3) years.

The median time between RIS diagnostic MRI and sample collection was 1.6 (0.4-3.3) months.

Body Fluid Biomarkers and Development of MS Symptoms

As shown in Table 1, 101 pwRIS (37%) developed MS symptoms after a mean (SD) time of 2.4 (2.6) years. Of these, 91 were diagnosed with RRMS (90.1%), while 10 met criteria for PPMS (9.9%). Among pwRIS developing RRMS, the most common clinical topography was the SC (46.2%) (eTable 3 in Supplement 1). Due to the low incidence of PPMS, analyses were conducted for the entire cohort. After RIS diagnosis and before MS symptoms, 30 pwRIS received treatment (11%); of these, 9 developed MS symptoms (30%), and 21 did not by their last follow-up (70%).

Figure 1A and eTable 4 in Supplement 1 show the association between demographic and radiological variables and MS symptoms. Younger age (hazard ratio [HR], 1.54; 95% CI, 1.04-2.28; $P = .03$), CEL at baseline (HR, 2.96; 95% CI, 1.78-4.92; $P < .001$), and the development of new T2 lesions (HR, 8.87; 95% CI, 4.10-19.21; $P < .001$) or CEL during follow-up (HR, 3.29; 95% CI, 2.18-4.97; $P < .001$) were associated with MS symptoms. Multivariable analysis yielded consistent results, except for age, which, despite a comparable effect size, was no longer statistically significant (Figure 1A; eTable 4 in Supplement 1). A family history of MS was not associated with MS symptoms (Figure 1A). Regarding immunoglobulin-related biomarkers, 73.5% and 22.1% of pwRIS were positive for IgG and IgM OBs, respectively (**Table 2**). The IgG and IgM indices were positive in 58.1% and 43.6%, respectively, and the IgM intrathecal fraction was positive in 15.4%. For KFLC, 71.5% of pwRIS had an index value of 6.1 or higher. As shown in Table 2 and Figure 1B, IgG and IgM OBs were strongly associated with MS symptoms (IgG OBs: HR, 5.09; 95% CI, 2.36-10.97; $P < .001$ and IgM OBs: HR, 2.58; 95% CI, 1.61-4.14; $P < .001$). Having an IgG index of 0.7 or higher and a KFLC index of 6.1 or higher was also strongly associated with MS symptoms (IgG index: HR, 2.90; 95% CI, 1.76-4.78; $P < .001$ and KFLC index: HR, 2.79; 95% CI, 1.37-5.67; $P = .005$). Weaker associations were found for the IgM and KFLC indices when expressed as continuous variables (IgM index: HR, 1.22; 95% CI, 1.04-1.44; $P = .02$ and KFLC index: HR, 1.12; 95% CI, 1.01-1.25; $P = .04$), whereas having an IgM index higher than 0.1 or an IgM intrathecal fraction higher than 0 was not associated with MS symptoms. Multivariable analysis yielded similar results (Figure 1B; Table 2).

In relation to CSF biomarkers, doubling of baseline cNfL and cGFAP levels was associated with increased MS symptom risk (cNfL levels: HR, 1.31; 95% CI, 1.18-1.45; $P < .001$ and cGFAP levels: HR, 1.14; 95% CI, 1.01-1.28; $P = .04$), remaining significant in multivariable models (Figure 1B; Table 2). Other biomarkers, like cCHI3L1, cCXCL13, and cMBP, showed no association (Figure 1B; Table 2). In blood, only high sNfL *z* scores were associated with MS symptoms (HR, 1.42; 95% CI, 1.16-1.72; $P = .005$), with consistent multivariable findings. sGFAP *z* scores and sCH3L1 levels were not associated with MS risk (Figure 1B; Table 2).

Kaplan-Meier survival analysis revealed that IgG and IgM OBs, as well as high IgG and KFLC indices, were all associated with a shorter time to MS symptoms (all $P < .001$ except for KFLC index, $P = .003$; **Figure 2**). Additionally, the presence of cNfL levels higher than 223.43 pg/mL and an sNfL *z* score higher than 1.67 were associated with a shorter time to MS symptoms ($P < .001$; Figure 2).

Except for the correlation between cGFAP and sGFAP *z* scores, all other CSF and serum biomarkers showed significant correlations with each other and between the 2 compartments (eFigure 2 in Supplement 1).

Environmental Factors and Development of MS Symptoms

eTable 5 in Supplement 1 shows the seroprevalence rates for viruses in pwRIS, which were significantly higher for EBV-encoded antigens compared with HD. Antibody responses to EBV-VCA, but not to EBV-EBNA1, were significantly higher in pwRIS compared with HD ($P = .02$) (eFigure 3 in Supplement 1). EBV-VCA and EBV-EBNA1 titers were comparable between pwRIS who developed MS symptoms and those who did not (eFigure 3 in Supplement 1). Figure 1C and eTable 6 in Supplement 1 show the association between antibody responses in seropositive pwRIS and MS symptoms. Only higher HCMV titers were associated with a lower risk of MS symptoms (HR, 0.59; 95% CI, 0.38-0.93; $P = .02$) in univariable and multivariable analyses.

Low vitamin D levels were observed in 46.5% of pwRIS (eTable 6 in Supplement 1). Neither vitamin D levels nor low vitamin D status were associated with MS symptoms in univariable or multivariable analyses (Figure 1C; eTable 6 in Supplement 1). High UVR exposure the year before (HR, 0.52; 95% CI, 0.37-0.74; $P < .001$) and the year after (HR, 0.54; 95% CI, 0.38-0.75; $P < .001$) study inclusion were associated with a lower risk of MS symptoms, consistent in multivariable analysis (Figure 1C; eTable 6 in Supplement 1).

Smoking was reported in 31.4% of pwRIS based on serum cotinine levels (eTable 6 in Supplement 1). As shown in eFigure 4 in Supplement 1, UVR exposure before and after study inclusion was higher in smokers. In univariable analysis, smoking was associated with lower risk of MS symptoms (HR, 0.51; 95% CI, 0.31-0.85; $P = .01$); this association was no longer statistically significant, although it remained a trend in multivariable analysis after adjusting for UVR exposure and smoking status (Figure 1C; eTable 6 in Supplement 1).

None of the obesity-related biomarkers and proinflammatory cytokines were associated with MS symptoms (eTable 6 in Supplement 1).

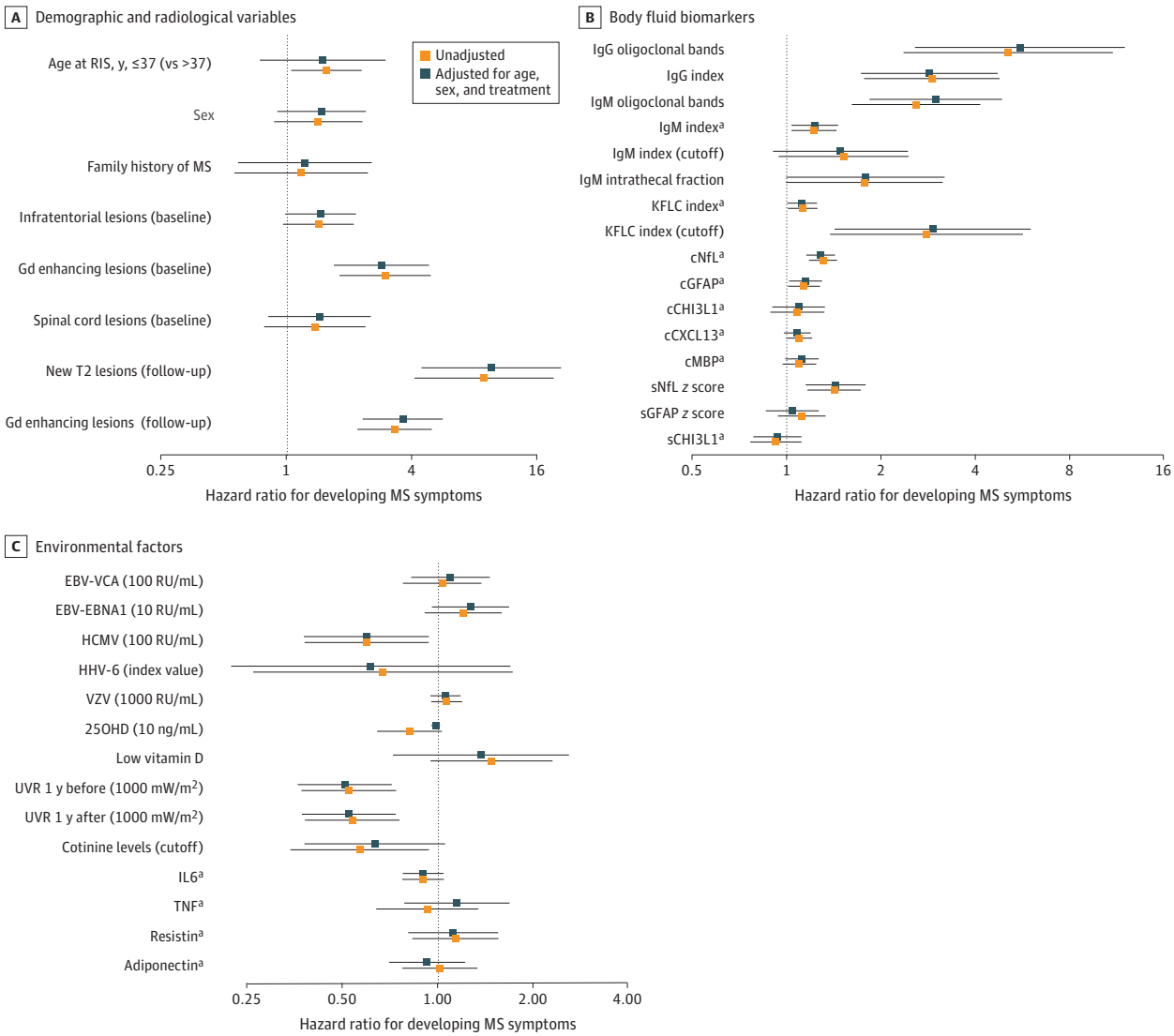
eFigure 5 in Supplement 1 provides a graphical summary of the prognostic factors associated with MS symptoms in pwRIS.

Exploratory Extended Analyses

eFigure 6 in Supplement 1 shows the risk of MS symptoms in the 207 females and 66 males included in the study. Most of the associations remained significant in females but were less consistently associated with MS symptom development in the smaller, less represented group of males.

As shown in eFigure 7 in the Supplement 1, having headache or dizziness as the reason for diagnostic MRI was asso-

Figure 1. Association Between Prognostic Factors and Development of Multiple Sclerosis (MS) Symptoms in People With Radiologically Isolated Syndrome (RIS)



The graphs display hazard ratios with 95% CIs for demographic and radiological variables (A), body fluid biomarkers (B), and environmental factors (C). For Epstein-Barr virus (EBV)–viral capsid antigen (VCA), EBV-Epstein-Barr nuclear antigen 1 (EBNA1), human cytomegalovirus (HCMV), varicella-zoster virus (VZV), 25-hydroxyvitamin D (25OHD), and ultraviolet radiation (UVR), the hazard ratio indicates the increased or decreased risk of MS symptoms per the number of units shown in parentheses. For vitamin D levels and low vitamin D, the multivariable analysis also included vitamin D supplementation and date of sampling (periodic function) as covariates. For cotinine levels, in addition to age, sex, and treatment, the multivariable analysis also included UVR exposure and whether people with RIS quit smoking or remained nonsmokers after RIS diagnosis as covariates. cCHI3L1 indicates cerebrospinal fluid chitinase 3-like 1; cCXCL13, cerebrospinal fluid chemokine ligand 13; cGFAP, cerebrospinal fluid glial fibrillary acidic protein; cMBP, cerebrospinal fluid myelin basic protein; cNfL, cerebrospinal fluid neurofilament light chain; Gd, gadolinium; HHV-6, human herpes virus-6; IgG, immunoglobulin G; IgM, immunoglobulin M; KFLC, κ free light chains; RU, relative units; sGFAP, serum glial fibrillary acidic protein; sNfL, serum neurofilament light chain; sCHI3L1, serum chitinase 3-like 1; TNF, tumor necrosis factor.

[a]Hazard ratio represents the risk of MS symptoms per doubling of biomarker concentration.

ciated with a higher risk of MS symptoms compared with the incidental findings category.

eFigure 8 in Supplement 1 represents a sensitivity analysis excluding the 30 pwRIS who received treatment before MS symptoms, which yielded similar results.

eFigure 9 in Supplement 1 depicts the results of the multivariable Cox regression analysis incorporating age, sex, treatment, CEL, and SC lesions as covariates in the subgroup of 163 pwRIS with available data (59.7%). Infratentorial lesions at baseline and the development of new T2 lesions or CEL during follow-up were associated with MS symptoms. Among the body fluid biomarkers and environmental factors, IgG OBs, both KFLC indices, and smoking were associated with MS symptoms in this patient subgroup.

Table 2. Cox Regression Models Showing the Association Between Body Fluid Biomarkers and Multiple Sclerosis (MS) Symptoms in People With Radiologically Isolated Syndrome

Biomarkers	No.[a]	No. of positive patients, %[b]	HR (95% CI) univariable	*P* value	HR (95% CI) multivariable	*P* value
Immunoglobulin-related						
IgG oligoclonal bands	268	197 (73.5)	5.09 (2.36-10.97)	<.001	5.54 (2.56-11.98)	<.001
IgG index (cut-off)	234	136 (58.1)	2.90 (1.76-4.78)	<.001	2.85 (1.72-4.71)	<.001
IgM oligoclonal bands	199	44 (22.1)	2.58 (1.61-4.14)	<.001	2.99 (1.84-4.86)	<.001
IgM index[c]	149	0.1 (0.1-0.2)	1.22 (1.04-1.44)	.02	1.23 (1.04-1.46)	.02
IgM index (cut-off)	149	65 (43.6)	1.52 (0.94-2.45)	.09	1.48 (0.90-2.44)	.12
IgM intrathecal fraction	149	23 (15.4)	1.77 (0.99-3.15)	.05	1.78 (1.00-3.19)	.05
KFLC index[c]	144	24.0 (5.7-92.4)	1.12 (1.01-1.25)	.04	1.12 (1.00-1.25)	.04
KFLC index (cutoff)	144	103 (71.5)	2.79 (1.37-5.67)	.005	2.92 (1.42-6.00)	.004
CSF biomarkers						
cNfL, pg/mL[c]	226	88.5 (35.7-336.0)	1.31 (1.18-1.45)	<.001	1.28 (1.15-1.43)	<.001
cGFAP, pg/mL[c]	226	495.2 (252.6-1433.3)	1.14 (1.01-1.28)	.04	1.15 (1.02-1.30)	.03
cCHI3L1, ng/mL[c]	230	95.5 (65.8-172.9)	1.08 (0.89-1.32)	.44	1.09 (0.90-1.32)	.38
cCXCL13, pg/mL[c]	194	5.0 (2.0-20.4)	1.10 (1.00-1.21)	.06	1.08 (0.98-1.19)	.12
cMBP, pg/mL[c]	201	1625.3 (936.0-2506.5)	1.10 (0.97-1.24)	.14	1.12 (0.99-1.26)	.07
Serum biomarkers						
sNfL (*z* scores)	215	1.6 (1.2)	1.42 (1.16-1.72)	<.001	1.43 (1.15-1.79)	<.001
sGFAP (*z* scores)	215	0.7 (1.2)	1.12 (0.94-1.33)	.22	1.04 (0.86-1.27)	.68
sCHI3L1, ng/mL[c]	208	32.2 (24.7-42.3)	0.92 (0.77-1.11)	.41	0.93 (0.78-1.11)	.45

Abbreviations: cCHI3L1, cerebrospinal fluid chitinase 3-like 1; cCXCL13, cerebrospinal fluid chemokine ligand 13; cGFAP, cerebrospinal fluid glial fibrillary acidic protein; cMBP, cerebrospinal fluid myelin basic protein; cNfL, cerebrospinal fluid neurofilament light chain; CSF, cerebrospinal fluid; HR, hazard ratio; IgG, immunoglobulin G; IgM, immunoglobulin M; KFLC, κ free light chains; sCHI3L1, serum chitinase 3-like 1; sGFAP, serum glial fibrillary acidic protein; sNfL, serum neurofilament light chain.

[a] Refers to the number of patients with available information.

[b] Refers to the percentage of positive patients for categorical variables and median (interquartile range) or mean (SD) values for continuous variables.

[c] HR represents the risk of MS symptoms per doubling of biomarker concentration. Multivariable analysis was adjusted for age, sex, and treatment during follow-up.

Body Fluid Biomarkers and Development of Neurological Disability

Of the 101 pwRIS who developed MS symptoms, 23 reached an EDSS of 3.0 (22.8%) and 16 reached an EDSS of 4.0 (15.8%) after a mean (SD) time of 4.8 (3.7) years and 5.2 (3.6) years, respectively (Table 1). Four pwRIS reached an EDSS of 6.0 (4.0%) and 2 developed secondary progressive MS (2.0%). Due to the low numbers, these outcomes were not included in the analysis. During follow-up, 71 (70.3%) and 77 (76.2%) pwRIS received treatment, with 15 (21.1%) and 11 (14.3%) reaching an EDSS of 3.0 and 4.0.

Younger age was associated with a lower risk of an EDSS of 4.0 in univariable analysis (HR, 0.19; 95% CI, 0.05-0.70; *P* = .01) and this association remained in multivariable analysis (eTable 7 in Supplement 1). SC lesions increased the risk of an EDSS of 3.0 (HR, 4.88; 95% CI, 1.07-22.38; *P* = .04) and 4.0 (HR, 14.15; 95% CI, 1.14-176.45; *P* = .04) in multivariable analysis. None of the body fluid biomarkers were associated with EDSS outcomes (eTable 8 in Supplement 1).

Environmental Factors and Development of Neurological Disability

Only elevated anti-VZV titers were associated with an increased risk of an EDSS of 3.0 in multivariable analysis (HR, 1.41; 95% CI, 1.07-1.85; *P* = .02) and an EDSS of 4.0 in univariable (HR, 1.43; 95% CI, 1.11-1.85; *P* = .006) and multivariable analyses (HR, 2.10; 95% CI, 1.38-3.20; *P* < .001) (eTable 9 in Supplement 1). High anti-VZV titers were associated with a shorter time to an EDSS of 4.0 (HR, 3.70; 95% CI, 1.30-10.59; *P* = .009; eFigure 10 in Supplement 1). Serum vitamin D levels, low vitamin D status, UVR exposure, and cotinine levels were not associated with the EDSS outcomes (eTable 9 in Supplement 1).

High serum IL-6 levels were associated with an increased risk of an EDSS of 4.0 only in univariable analysis (HR, 1.49; 95% CI, 1.04-2.12; *P* = 04; eTable 9 in Supplement 1). High serum resistin levels were associated with an increased risk of an EDSS of 3.0 (HR, 2.79; 95% CI, 1.28-6.08; *P* = .009) and 4.0 (HR, 3.34; 95% CI, 1.28-8.75; *P* = .01) in multivariable analyses (eTable 9 in Supplement 1), and with a shorter time to an EDSS of 3.0 (HR, 2.60; 95% CI, 1.04-6.54; *P* = .04; eFigure 10 in Supplement 1).

Prediction of MS Symptoms Within the First 5 Years Following an RIS Diagnosis

Table 3 shows the risk of MS symptoms per year during the first 5 years after an RIS diagnosis. The presence of an sNfL *z* score higher than 1.67 and positive IgG OBs conferred a 5-year risk of MS symptoms of 58.3% (95% CI, 45.9%-67.9%). This risk increased to 78.1% (95% CI, 58.6%-88.4%) in pwRIS with IgM OBs, and up to 81.6% (60.9%-91.4%) for those aged 37 years or younger. Similar risks were observed when using the KFLC index in place of IgG OBs (eTable 10 in Supplement 1). eTables 11 through 14 in Supplement 1 show the risk of MS symptoms in

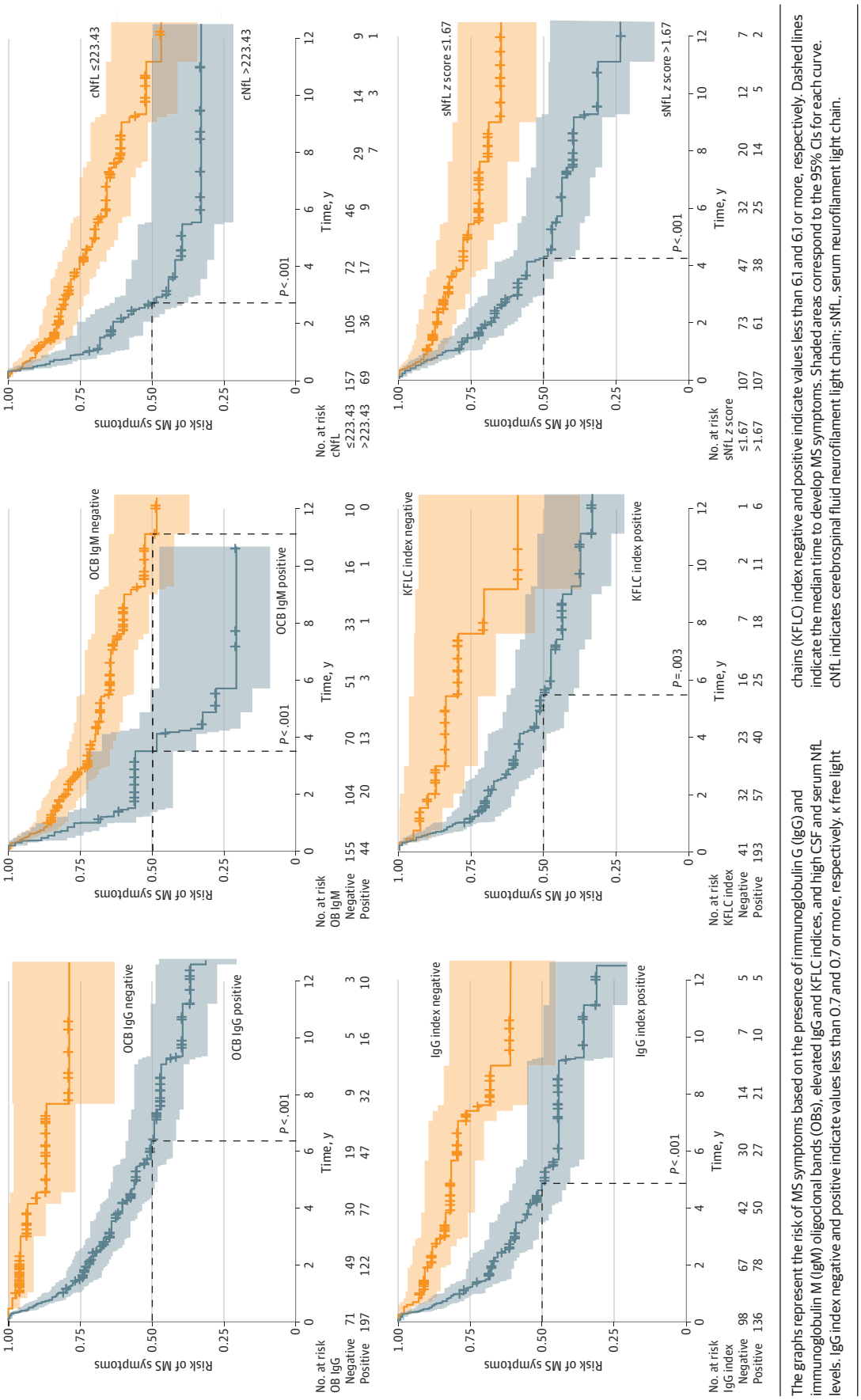


Figure 2. Kaplan-Meier Curves for Time to MS in People With Radiologically Isolated Syndrome (RIS)

The graphs represent the risk of MS symptoms based on the presence of immunoglobulin G (IgG) and immunoglobulin M (IgM) oligoclonal bands (OBs), elevated IgG and KFLC indices, and high CSF and serum NfL levels. IgG index negative and positive indicate values less than 0.7 and 0.7 or more, respectively. κ free light chains (KFLC) index negative and positive indicate values less than 6.1 and 6.1 or more, respectively. Dashed lines indicate the median time to develop MS symptoms. Shaded areas correspond to the 95% CIs for each curve. cNfL indicates cerebrospinal fluid neurofilament light chain; sNfL, serum neurofilament light chain.

Table 3. Risk of Development of Multiple Sclerosis (MS) Symptoms in People With Radiologically Isolated Syndrome (RIS) Within the First 5 Years Following an RIS Diagnosis

Factors				Risk of MS symptoms, % (95% CI)				
sNfL[a]	IgG OBs[b]			1st Year	2nd Year	3rd Year	4th Year	5th Year
Low	Neg			3.1 (0.3-5.8)	4.9 (0.7-9.0)	6.6 (1.0-11.8)	8.0 (1.2-14.4)	9.2 (1.4-16.4)
High	Neg			7.2 (1.0-13.0)	11.2 (1.8-19.7)	14.9 (2.6-25.6)	18.0 (3.2-30.5)	20.5 (3.7-34.3)
Low	Pos			11.3 (5.9-16.4)	17.5 (10.1-24.3)	22.8 (13.7-31.0)	27.4 (16.9-36.6)	30.9 (19.3-40.8)
High	Pos			24.8 (16.3-32.4)	36.5 (26.4-45.2)	45.9 (34.6-55.2)	53.1 (41.1-62.7)	58.3 (45.9-67.9)
sNfL[a]	IgG OBs[b]	IgM OBs[c]		1st Year	2nd Year	3rd Year	4th Year	5th Year
Low	Neg	Neg		3.4 (0.4-6.4)	5.5 (0.7-10.0)	7.2 (1.0-13.0)	8.7 (1.3-15.5)	10.1 (1.6-18.0)
High	Neg	Neg		7.5 (0.9-13.6)	11.8 (1.7-20.8)	15.4 (2.4-26.6)	18.3 (3.0-31.2)	21.2 (3.6-35.6)
Low	Pos	Neg		10.3 (4.8-15.4)	16.0 (8.3-23.1)	20.8 (11.3-29.3)	24.7 (13.7-34.2)	28.3 (16.2-38.7)
High	Pos	Neg		21.5 (12.6-29.5)	32.3 (20.9-42.0)	40.6 (27.4-51.3)	46.8 (32.5-58.1)	52.4 (37.3-63.8)
Low	Neg	Pos		6.9 (0.0-13.3)	10.9 (0.1-20.5)	14.2 (0.3-26.2)	17.0 (0.4-30.8)	19.7 (0.5-35.2)
High	Neg	Pos		14.7 (0.6-26.8)	22.6 (1.3-39.3)	29.0 (2.0-48.5)	34.0 (2.5-55.3)	38.6 (2.8-61.2)
Low	Pos	Pos		19.9 (8.3-30.1)	30.1 (13.9-43.3)	38.0 (18.5-52.9)	44.0 (22.2-59.7)	49.5 (25.5-65.7)
High	Pos	Pos		39.1 (23.5-51.5)	55.0 (36.7-68.0)	65.6 (46.3-77.9)	72.6 (53.1-83.9)	78.1 (58.6-88.4)
sNfL[a]	IgG OBs[b]	Age, y		1st Year	2nd Year	3rd Year	4th Year	5th Year
Low	Neg	>37		2.6 (0.2-4.9)	4.1 (0.4-7.7)	5.5 (0.6-10.2)	6.8 (0.8-12.5)	7.9 (1.0-14.3)
High	Neg	>37		5.4 (0.3-10.3)	8.5 (0.7-15.8)	11.4 (1.0-20.7)	13.9 (1.4-24.9)	15.9 (1.6-28.2)
Low	Pos	>37		10.2 (5.1-15.0)	15.7 (8.6-22.3)	20.7 (11.8-28.7)	25.0 (14.6-34.1)	28.3 (16.9-38.2)
High	Pos	>37		20.3 (11.2-28.6)	30.4 (18.4-40.7)	38.8 (24.6-50.4)	45.6 (29.9-57.8)	50.6 (34.0-63.1)
Low	Neg	≤37		3.8 (0.3-7.1)	5.9 (0.6-11.0)	8.0 (0.9-14.5)	9.8 (1.1-17.7)	11.2 (1.3-20.2)
High	Neg	≤37		7.8 (1.1-14.1)	12.2 (2.0-21.4)	16.2 (2.7-27.7)	19.6 (3.4-33.1)	22.4 (4.0-37.2)
Low	Pos	≤37		14.5 (6.4-21.8)	22.1 (10.8-31.9)	28.6 (14.7-40.3)	34.2 (18.0-47.2)	38.4 (20.6-52.2)
High	Pos	≤37		28.2 (17.7-37.3)	41.1 (28.6-51.4)	51.1 (37.1-62.0)	58.8 (43.9-69.8)	64.2 (48.9-75.0)
sNfL[a]	IgG OBs[b]	IgM OBs[c]	Age, y	1st Year	2nd Year	3rd Year	4th Year	5th Year
Low	Neg	Neg	>37	3.0 (0.2-5.7)	4.8 (0.5-9.0)	6.4 (0.7-11.8)	7.7 (0.9-14.1)	9.1 (1.2-16.3)
High	Neg	Neg	>37	6.0 (0.2-11.4)	9.4 (0.6-17.5)	12.4 (0.9-22.6)	14.9 (1.2-26.7)	17.3 (1.5-30.6)
Low	Pos	Neg	>37	9.6 (4.4-14.6)	15.1 (7.6-21.9)	19.6 (10.3-27.9)	23.3 (12.6-32.7)	26.8 (14.9-37.1)
High	Pos	Neg	>37	18.4 (9.0-26.8)	27.9 (15.1-38.7)	35.3 (20.1-47.7)	41.1 (24.1-54.3)	46.5 (28.2-60.1)
Low	Neg	Pos	>37	5.9 (0.0-11.8)	9.4 (0.0-18.1)	12.3 (0.0-23.4)	14.8 (0.0-27.6)	17.2 (0.0-31.7)
High	Neg	Pos	>37	11.5 (0.0-22.2)	17.9 (0.0-33.1)	23.2 (0.0-41.5)	27.4 (0.0-47.8)	31.5 (0.0-53.5)
Low	Pos	Pos	>37	18.3 (7.0-28.1)	27.7 (11.8-40.8)	35.2 (15.9-50.1)	41.0 (19.2-56.9)	46.3 (22.2-62.9)
High	Pos	Pos	>37	33.2 (15.9-46.9)	47.8 (25.6-63.3)	58.0 (33.1-73.6)	65.2 (39.0-80.1)	71.2 (44.1-85.1)
Low	Neg	Neg	≤37	4.1 (0.2-7.8)	6.5 (0.6-12.1)	8.6 (0.8-15.8)	10.4 (1.1-18.8)	12.1 (1.3-21.8)
High	Neg	Neg	≤37	8.0 (0.9-14.7)	12.6 (1.8-22.3)	16.5 (2.5-28.5)	19.7 (3.1-33.4)	22.8 (3.7-38.1)
Low	Pos	Neg	≤37	12.9 (4.7-20.4)	19.9 (8.2-30.2)	25.7 (11.1-37.9)	30.3 (13.4-43.9)	34.7 (15.7-49.3)
High	Pos	Neg	≤37	24.1 (13.2-33.6)	35.9 (22.0-47.3)	44.8 (28.8-57.2)	51.4 (33.9-64.2)	57.3 (38.8-70.2)
Low	Neg	Pos	≤37	8.0 (0.0-15.7)	12.6 (0.0-23.8)	16.4 (0.0-30.4)	19.6 (0.0-35.5)	22.7 (0.0-40.5)
High	Neg	Pos	≤37	15.4 (0.6-28.0)	23.6 (1.3-40.8)	30.2 (2.0-50.2)	35.3 (2.5-57.1)	40.2 (2.9-63.2)
Low	Pos	Pos	≤37	24.0 (8.1-37.2)	35.7 (13.6-52.2)	44.6 (18.2-62.5)	51.2 (21.8-69.6)	57.1 (24.9-75.5)
High	Pos	Pos	≤37	42.3 (24.8-55.7)	58.7 (38.5-72.3)	69.3 (48.3-81.8)	76.2 (55.3-87.3)	81.6 (60.9-91.4)

Abbreviations: IgG OBs, immunoglobulin G oligoclonal bands; IgM OBs, immunoglobulin M oligoclonal bands; OBs, oligoclonal bands; sNfL, serum neurofilament light chain.

[a] For sNfL, low and high refer to sNfL *z* scores 1.67 or lower and more than 1.67, respectively.

[b] IgG OB neg and pos refer to individuals with negative and positive IgG oligoclonal bands, respectively.

[c] IgM OB neg and pos refer to individuals with negative and positive IgM oligoclonal bands, respectively.

pwRIS, first stratified by the presence or absence of CEL at baseline. The 5-year risk of MS symptoms in pwRIS without CEL, with an sNfL *z* score higher than 1.67, positive IgG and IgM OBs, and 37 years or younger was 86.6% (95% CI, 61.2-95.3). When using the KFLC index instead of IgG OBs, the risk was 83.1% (95% CI, 51.8%-94.1%).

Discussion

This study aimed to identify prognostic factors at RIS diagnosis associated with MS symptoms, a critical step since disease-modifying therapies can delay symptom onset in pwRIS.[9,10]

To achieve this, we analyzed a large cohort of pwRIS with a 5-year median follow-up, focusing on body fluid biomarkers, radiological, and environmental factors previously linked to MS risk or prognosis. Confirming previous findings,[6,7,21] younger age and baseline or follow-up CEL were associated with increased MS symptom risk. Other baseline radiological factors, like infratentorial and SC lesions, were not associated with MS symptoms. While infratentorial lesions showed a nonsignificant trend in both the univariable and age-, sex-, and treatment-adjusted analyses, SC lesions were not associated, likely due to the missing data for this variable. A positive MS family history was not associated with symptom risk.

IgG and IgM OBs and the IgG index emerged as strong predictors of MS symptoms, shortening the time to onset. While IgG OBs have been well studied,[22] the IgG index has often been analyzed alongside IgG OBs under the category of positive CSF[6,7] or reported as unrelated to MS symptom development.[8] To our knowledge, no previous studies have reported on the prognostic role of IgM OBs in pwRIS. Other IgM-related parameters, like the IgM index or the IgM intrathecal fraction, did not outperform IgM OBs. Studies on KFLC in pwRIS are scarce and limited to small cohorts of pwRIS.[8] In this context, in a recent study a KFLC index higher than 9.8 predicted new T2 lesions, but not future clinical events.[23] Interestingly, in our study a KFLC index of 6.1 or higher was strongly associated with MS symptoms.

Both cNfL and sNfL levels were strongly associated with MS symptoms, corroborating prior studies in smaller RIS cohorts.[22,24] Notably, high sNfL *z* scores significantly reduced the time to MS symptoms. There have been no previous reports on GFAP levels in pwRIS. GFAP was weakly associated with MS symptoms in CSF but not in blood, consistent with the lack of correlation between cGFAP and sGFAP. Biomarkers related to inflammation (CXCL13), demyelination (MBP), and astrocyte/microglia activation (CHI3L1) did not show a prognostic role in pwRIS.

Concerning viruses, elevated anti-HCMV titers were associated with a decreased risk of MS symptoms, supporting its suggested protective role.[25] Other herpes viruses, like HHV-6 and VZV, did not influence MS symptom development.

Low sunlight exposure and low serum vitamin D levels are established MS risk factors.[26] High UVR in the year before and after RIS diagnosis markedly reduced MS symptom risk, while serum vitamin D levels and low vitamin D status were not associated with MS symptoms, consistent with prior research.[27]

Unexpectedly, smoking, a well-known risk factor for MS,[26] appeared protective against symptom development in univariable analysis. This association was no longer significant, although it remained as a trend, when adjusting for UVR exposure, suggesting a higher prevalence of smokers in regions with greater solar exposure or a behavioral link, as smokers may spend more time outdoors. However, in the multivariate analysis, including radiological variables in a patient subgroup, smoking remained significant. These results should be interpreted with caution due to the small sample size, but they emphasize the need for future studies to explore the role of smoking in pwRIS.

Obesity is linked to a state of chronic, low-grade inflammation promoting MS development.[28] Levels of proinflammatory cytokines (IL-6, tumor necrosis factor), and proinflammatory (resistin) and anti-inflammatory adipokines (adiponectin) were not associated with MS symptoms.

A secondary study outcome was disability. Findings were less consistent due to the low number of pwRIS who reached this outcome. None of the body fluid biomarkers were associated with the time to an EDSS of 3.0 or 4.0. Prognostic factors at the first clinical demyelinating event (CIS) may better predict disability than those collected at RIS diagnosis. An intriguing finding, to be confirmed in further studies, was the association between elevated anti-VZV titers and disability. In this context, the immune response to VZV is part of the measles-rubella-zoster reaction, which was found to predict MS conversion in patients with CIS.[29] High serum levels of resistin increased the risk of disability in pwRIS. Resistin, primarily secreted by macrophages, enhances IL-6 production.[28] In our study, IL-6 levels were also associated with disability. Interestingly, in a recent study[30] serum resistin levels were negatively associated with atrophy measures in patients with PPMS, suggesting a link between resistin levels and neurodegeneration.

Lastly, we assessed MS symptom risk in pwRIS within 5 years of RIS diagnosis using the combination of 2 highly predictive variables: IgG OBs (or the KFLC index) and sNfL *z* scores. Younger age and, in particular, the presence of IgM OBs further increased the risk. Notably, the risk was close to 90% in pwRIS without CEL at baseline, who represent approximately 80% of the pwRIS population. These findings can aid clinicians in identifying pwRIS at high risk for MS symptoms and selecting candidates for early treatment to delay symptom onset.

The forthcoming 2024 McDonald criteria will classify many pwRIS meeting the 2017 McDonald criteria for dissemination in space as having MS, emphasizing the asymptomatic phase of MS with radiological but no clinical signs. However, identifying pwRIS at higher risk of developing clinical symptoms remains critical for early intervention.

Limitations

The study has limitations. The multicenter and retrospective design limits the acquisition of standardized MRI protocols. Radiological features were based on local reports without a centralized analysis. A subset of patients lacked SC MRI or follow-ups, and nonconventional sequences detecting specific lesions, like purely cortical lesions, the central vein sign, or paramagnetic rims, were not performed. These factors likely reduced the predictive value of MRI. Despite these challenges, its strength lies in the comprehensive biomarker analysis from biological samples collected near the RIS diagnostic MRI.

Conclusions

In summary, our data highlight the potential of liquid biomarkers to identify the risk of developing MS symptoms and to support treatment decisions in the clinical management of pwRIS.

ARTICLE INFORMATION

Accepted for Publication: April 3, 2025.

Published Online: June 2, 2025. doi:10.1001/jamaneurol.2025.1481

Correction: This article was corrected on October 13, 2025, to change the nonbyline authors to collaborators.

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Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Fissolo, Gutierrez, Gasior, Kuhle, Comabella.
Critical review of the manuscript for important intellectual content: Fissolo, Schaedelin, Villar, Lünemann, Correale, Rejdak, Schwab, Vilaseca, Held, García-Merino, Bittner, Troiano, Furlan, Tumani, Pérez-Miralles, Rosenstein, Galimberti, Álvarez-Bravo, Thouvenot, Llufriu, Khoury, Hoepner, Martínez-Yélamos, Hegen, Drulovic, Téllez, Khalil, Oechtering, Pérez-Sempere, Rodríguez-Antigüedad, Martínez-Rodríguez, Strijbis, Killestein, Eichau, Colombo, Zettl, Falk, Veiga González, Ferrer, Quiroga-Varela, Schaller-Nagengast, Bachhuber, Midaglia, Sánchez-López, Costa-Frossard, Monreal, Chan, Paul, Rovira, Tintore, Lycke, Zipp, Hemmer, Kuhle, Montalban, Comabella.
Statistical analysis: Schaedelin, Lünemann, Schwab, Kuhle, Comabella.
Obtained funding: Lünemann, Hemmer, Comabella.
Administrative, technical, or material support: Villar, Lünemann, Correale, Held, Bittner, Pérez-Miralles, Galimberti, Llufriu, Hoepner, Martínez-Yélamos, Hegen, Téllez, Khalil, Oechtering, Pérez-Sempere, Martínez-Rodríguez, Strijbis, Eichau, Gutierrez, Veiga González, Ferrer, Bachhuber, Midaglia, Chan, Paul, Lycke, Zipp, Hemmer, Kuhle, Comabella.
Supervision: Lünemann, García-Merino, Pérez-Sempere, Martínez-Rodríguez, Eichau, Costa-Frossard, Paul, Rovira, Hemmer, Comabella.

Conflict of Interest Disclosures: Dr Villar reported personal fees and grants from Roche Pharma, Novartis, Bristol Myers Squibb, and Merck and personal fees from Biogen and Neuraxpharm outside the submitted work. Dr Lünemann reported personal fees from AbbVie, Alexion, Adivo, Argenx, Biogen, Merck, Moderna, Novartis, Roche, Sanofi, Takeda, CSL Behring, UCB Pharma, Amgen, and Janssen-Cilag and grants from Moderna, Alexion, Argenx, and Roche outside the submitted work. Dr Correale reported personal fees from Merck Argentina and Roche Argentina grants and personal fees from Novartis Argentina and Biogen outside the submitted work. Dr Schwab reported grants from DFG, Roche, and Biogen outside the submitted work. Dr Bittner reported grants from the Hermann and Lilly Schilling Foundation and the Deutsche Forschungsgemeinschaft during the conduct of the study; personal fees from Biogen, Bristol Myers Squibb, Hexal, Merck Healthcare, Novartis, Roche, Sanofi, and Teva and grants from Novartis outside the submitted work. Dr Troiano reported personal fees from Sandoz, Roche, Novartis, Merck, Alexion, and Biogen outside the submitted work. Dr Furlan reported personal fees from Sanofi, Novartis, Novartis, Bristol Myers Squibb, Biogen, Neuraxpharma, Alexion, and Roche and grants from Merck and Roche outside the submitted work. Dr Tumani reported personal fees from Alexion, Biogen, Bristol Myers Squibb, Celgene, Janssen Cilag, Merck, Roche, Siemens, Teva, Viatris, Hexal, and Horizon, nonfinancial support from Bayer, Fresenius, Biotechne, Fujirebio, and Quanterix, and grants from Genzyme Sanofi, Novartis, and Chemische Fabrik Karl Bucher outside the submitted work. Dr Pérez-Miralles reported personal fees from Sanofi, Neuraxpharm, Bristol Myers Squibb, Janssen, and Roche outside the submitted work. Dr Rosenstein reported personal fees from Sanofi, Biogen, Novartis, and Merck outside the submitted work. Dr Thouvenot reported grants from Public Health Research Consortium National, Agence Nationale de la Recherche, Novartis, Biogen, France SEP, and the EDMUS Foundation, personal fees from Biogen,

Merck, Novartis, Roche, and Sanofi outside the submitted work. Dr Llufriu reported grants from Instituto de Salud Carlos III, the Agency for Management of University and Research Grants, and Bristol Myers Squibb and personal fees from Biogen, Novartis, Merck, Bristol Myers Squibb, Johnson & Johnson, and Sanofi outside the submitted work. Dr Khoury reported personal fees from Biogen outside the submitted work. Dr Hoepner reported grants from Bristol Myers Squibb, Biogen, ChiesiAI, Swiss MS Society, and the Sitem Insel Support Fund outside the submitted work and speaker/advisor honorary from Merck, Novartis, Roche, Biogen, Alexion, Sanofi, Janssen, Bristol Myers Squibb, Teva/Meph, and Almirall. Dr Martínez-Yélamos reported support for attending congress and scientific meetings from Biogen, Bristol Myers Squibb, Janssen, Merck, Neuraxpharm, Novartis, Roche and Sandoz; the institution where he works (Hospital Universitari de Bellvitge/ Institut d'Investigació Biomèdica de Bellvitge) has received and destined exclusively to support the research of the unit, fees for advisory council, collaborations, sponsorships, donations, and advice from Almirall, Bayer, Biogen, Bristol Myers Squibb, Celgene, Genzyme, Horizon/ Amgen, Janssen, Kern Pharma, Lilly, Merck, Neuraxpharm, Novartis, Roche, Sandoz, and Sanofi. Dr Hegen reported personal fees from Biogen, Bristol Myers Squibb, Novartis, Roche, Sanofi-Genzyme, Amgen, Janssen, Merck, and Teva and grants from Novartis outside the submitted work. Dr Oechtering reported grants from Swiss MS Society, Roche, and Novartis outside the submitted work. Dr Killestein reported consulting fees paid to their institution from F Hoffmann-La Roche, Biogen, Merck, Novartis, Sanofi, Immunic, and Sandoz, outside the submitted work. Dr Eichau reported personal fees from Novartis, Sanofi, Biogen, Bristol Meyers Squibb, Roche, Almirall, and Neuraxpharm outside the submitted work. Dr Colombo reported personal fees from Roche, Merck, Sanofi, Novartis, Biogen, Bristol Myers Squibb, Janssen, and Alexion outside the submitted work. Dr Zettl reported personal fees from Almirall, Alexion, Bayer, Biogen, Bristol Myers Squibb, Roche, Norvartis, Merck, and Janssen during the conduct of the study. Dr Falk reported grants from German Research Foundation, and Roche during the conduct of the study. Dr Quiroga-Varela reported grants from the Fundación Francisco Soria y Melguizo, Merck, Novartis, and Horizon Therapeutics. Dr Costa-Frossard reported receiving speaker fees and travel support and/or serving on advisory boards for Biogen, Sanofi, Merck, Bayer, Novartis, Roche, Teva, Celgene, Ipsen, Biopas, Bristol Myers Squibb, Janssen, and Almirall. Dr Monreal reported personal fees from Biogen, Merck, Novartis, Roche, Almirall, Johnson & Johnson, Bristol Myers Squibb, Sanofi, Neuraxpharma, and PSI outside the submitted work. Dr Chan reported speaker's/board honoraria for hospital research funds from Alexion, Horizon, Merck KGaA, Novartis, Janssen, Bristol Myers Squibb, Roche, Sanofi Genzyme, Teva, and CSL Behring and grants from CSL Behring, Biogen, Novartis, UCB, the Swiss National Foundation, and the European Union outside the submitted work. Dr Paul reported consulting/lecture fees from Roche, F. Hoffmann-La Roche, Alexion, Merck KGaA, Medscape, UCB, BioNTech, Bristol Myers Squibb, Hexal AG, Novartis, Teva, Horizon Therapeutics, and Sanofi, lecture fees from MICE Service GmbH, Janssen-Cilag, Chugai Pharma, FU Conform, Ethos, Biogen, VINDICO Medical Education, and Touch Independent Medical Education, travel expenses from the University Süd-Dänemark, other from Deutsche Multiple Sklerose Gesellschaft, the University Oxford, the International Multiple Sclerosis Cognition Society, Janssen, Massachusetts General Hospital, and Universitätsmedizin Göttingen, and consulting relationships with Sandoz and Ridgeline Discovery outside the submitted work. Dr Rovira reported personal fees from Novartis, Bristol Myers Squibb, Roche, Biogen, Sanofi, Bayer, and Merck outside the submitted work. Dr Tintore reported personal fees from Almirall, Bayer Schering Pharma, Biogen, Genzyme, Immunic Therapeutics, Janssen, Merck-Serono, Novartis, Roche, Sanofi-Aventis, Viela Bio, and Teva during the conduct of the study and personal fees from Parexel, UCB Biopharma, and the Relapse Adjudication Committee outside the submitted work. Dr Lycke reported personal fees for travel, lecture honoraria, and service on scientific advisory boards Biogen, Sanofi, Merck, and Sandoz and grants from Sanofi outside the submitted work. Dr Hemmer reported grants from the German Research Foundation, Bundesministerium für Bildung und Forschung, and the European Commission during the conduct of the study; personal fees from Novartis, Roche, Sandoz, Polpharma, GLG Consulting, and AllergyCare, and grants from Polpharma and Roche outside the submitted work. Dr Kuhle reported grants from the Swiss MS Society, the Swiss National Research Foundation, Progressive MS Alliance, Immunic, Merck, Neurogenesis, Novartis, Quanterix, Roche, and Stata DX outside the submitted work. Dr Montalban reported payment to their institution from AbbVie, Actelion, Alexion, Biogen, Bristol Myers Squibb/Celgene, EMD Serono, Genzyme, Hoffmann-La Roche, Immunic, Janssen Medday, Medscape, Merck, Mylan, Nervgen, Neuraxpharm, Novartis, PeerVoice, Samsung-Biosys, Sandoz, Sanofi-Genzyme, Teva Pharmaceutical, TG Therapeutics, and Excemed outside the submitted work. No other disclosures were reported.

Funding/Support: This work was supported by a grant from the National Multiple Sclerosis Society (RG-2110-38567).

Role of the Funder/Sponsor: The funder had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. The funder of the study had no role in study design, data collection, data analysis, data interpretation, writing of the manuscript, or the decision to submit the manuscript for publication.

Data Sharing Statement: See Supplement 2.

Additional Contributions: We acknowledge the patients and the Biobank HUB-ICO-IDIBELL (PT20/00171) integrated in the ISCIII Biobanks and Biomodels Platform and Xarxa Banc de Tumors de Catalunya for their collaboration. The authors also thank the Hospital Universitario Puerta de Hierro Majadahonda/Instituto de Investigación Sanitaria Puerta de Hierro-Segovia de Arana Biobank (Carlos III Health Institute Biobanks and Biomodels Platform) for the human specimens used in this study.

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