

# Predicting Aggressive Multiple Sclerosis With Intrathecal IgM Synthesis Among Patients With a Clinically Isolated Syndrome

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## Abstract

### Objective

To determine the best method to measure intrathecal immunoglobulin (Ig) M synthesis (ITMS), a biomarker of worse prognosis in multiple sclerosis (MS). We compared the ability for predicting a poor evolution of 4 methods assessing ITMS (IgM oligoclonal bands [OCMBs], lipid-specific OCMBs [LS-OCMBs], Reibergram, and IgM index) in patients with a clinically isolated syndrome (CIS).

### Methods

Prospective study with consecutive patients performed at a referral MS center. We used unadjusted and multivariate Cox regressions for predicting a second relapse, Expanded Disability Status Scale (EDSS) scores of 4 and 6, and development of secondary progressive MS (SPMS).

### Results

A total of 193 patients were included, with a median (interquartile range) age of 31 (25–38) years and a median follow-up of 12.9 years. Among all methods, only OCMB, LS-OCMB, and Reibergram significantly identified patients at risk of some of the pre-established outcomes, being LS-OCMB the technique with the strongest associations. Adjusted hazard ratio (aHR) of LS-OCMB for predicting a second relapse was 2.50 (95% CI 1.72–3.64,  $p < 0.001$ ). The risk of reaching EDSS scores of 4 and 6 and SPMS was significantly higher among patients with LS-OCMB (aHR 2.96, 95% CI 1.54–5.71,  $p = 0.001$ ; aHR 4.96, 95% CI 2.22–11.07,  $p < 0.001$ ; and aHR 2.31, 95% CI 1.08–4.93,  $p = 0.03$ , respectively).

### Conclusions

ITMS predicts an aggressive MS at disease onset, especially when detected as LS-OCMB.

### Classification of Evidence

This study provides Class II evidence that lipid-specific IgM oligoclonal bands can predict progression from CIS to MS and a worse disease course over a follow-up of at least 2 years.

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### Class of Evidence

Criteria for rating therapeutic and diagnostic studies

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## Glossary

**aHR** = adjusted hazard ratio; **CIS** = clinically isolated syndrome; **DMT** = disease-modifying treatment; **EDSS** = Expanded Disability Status Scale; **FN** = false negative; **FP** = false positive; **HRC** = Hospital Universitario Ramón y Cajal; **Ig** = immunoglobulin; **ITMS** = intrathecal IgM synthesis; **LS-OCMB** = lipid-specific IgM oligoclonal band; **MS** = multiple sclerosis; **NPV** = negative predictive value; **OCMB** = IgM oligoclonal band; **PPV** = positive predictive value; **RRMS** = relapsing-remitting MS; **SPMS** = secondary progressive MS; **TN** = true negative; **TP** = true positive.

The pathogenesis of multiple sclerosis (MS) is characterized by a chronic immune activation; hence, a hallmark of the disease is intrathecal synthesis of immunoglobulins (Igs).<sup>1</sup> In this regard, different methods have been described to assess the intrathecal humoral immune response: quantitative (CSF/serum quotients diagrams with or without hyperbolic reference range, such as the Reibergram and Ig index)<sup>2–4</sup> and qualitative (detection of oligoclonal bands [OCBs]).<sup>1,5</sup>

Most of the Igs found in the CSF of patients with MS consist of the IgG isotype that is present in >95% of cases<sup>5</sup> and thus contribute to the diagnosis of the disease.<sup>6</sup> In contrast, intrathecal synthesis of IgM (ITMS) is present in a lower proportion of patients with MS (28%–55%),<sup>7,8</sup> and its role is mainly prognostic. As the course of MS is highly variable,<sup>9</sup> an urgent need for reliable biomarkers at the initial stage of the disease exists for accurately predicting those patients at a higher risk of a more severe evolution. ITMS has been generally related to worse outcomes throughout the disease,<sup>10–28</sup> although negative results have also been described.<sup>29–31</sup> However, reliable results can be obtained with both quantitative (IgM index and Reibergram)<sup>4,5</sup> and qualitative (IgM OCB [OCMB], including analysis for specificity to lipids—lipid-specific OCMB [LS-OCMB])<sup>5,32,33</sup> methods. Although previous data encourage the use of qualitative over quantitative analyses,<sup>34</sup> extensive discussion arose over the predictive value of each technique.<sup>13,35,36</sup>

The aim of this study was to compare 4 methods evaluating ITMS (OCMB, LS-OCMB, Reibergram, and IgM index) among patients with a clinically isolated syndrome (CIS) with respect to the capability of these methods to predict a second relapse, Expanded Disability Status Scale (EDSS) scores of 4 and 6, and development of secondary progressive MS (SPMS).

## Methods

### Study Design

A single-center, observational study with prospective collection of data was performed at the Hospital Universitario Ramón y Cajal (HRC) referral MS center, Madrid. Consecutive patients with a first typical demyelinating attack suggestive of MS (CIS), with an available MRI study at baseline and a CSF analysis were initially included. The eligibility criteria included several parameters: (1) absence of previous history of possible demyelinating events, (2) follow-up of at least 2 years, (3) CSF analysis, including intrathecal IgG and

IgM synthesis, and (4) absence of a final diagnosis different from MS.

### Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the institutional ethics board of HRC. A signed informed consent was obtained from all patients.

### Data Collection

Patients attending our MS Unit starting in June 1996 who agreed to participate were prospectively collected after providing signed informed consent. Participants fulfilling the inclusion criteria were recruited until December 2017, and the follow-up period was until July 05, 2020. Variables collected included demographic, clinical, radiologic, and CSF data. Details of disease-modifying treatments (DMTs) that were administered during disease evolution with dates of onset and discontinuation were also recorded. DMTs were classified into 2 groups for analytical purposes: (1) all interferon- $\beta$  formulations, glatiramer acetate, teriflunomide, dimethyl fumarate, fingolimod, azathioprine, and methotrexate and (2) natalizumab, alemtuzumab, ocrelizumab, rituximab, mitoxantrone, and cyclophosphamide. Only treatments maintained for a period of  $\geq 3$  months were considered.

The end points that were assessed in the present study consisted of 3 parameters: (1) development of a second relapse, (2) reaching an irreversible 6-month confirmed EDSS score of 4 or 6, and (3) conversion to SPMS.

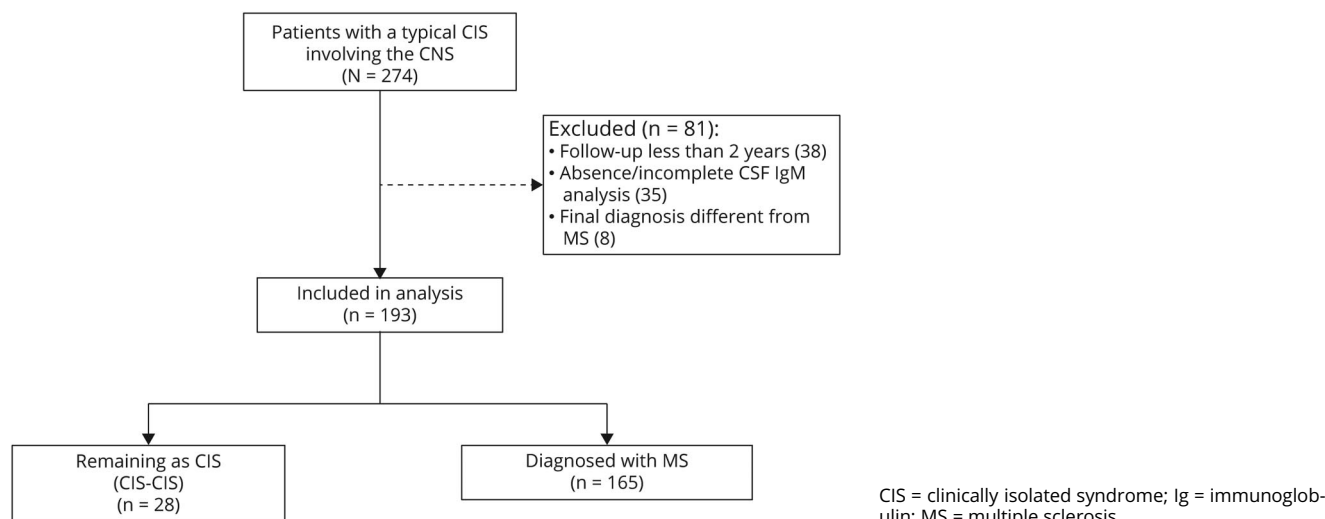
### Clinical Definitions

A relapse was defined as a single clinical episode of patient-reported symptoms with objective findings reflecting a demyelinating event involving the CNS with a duration of at least 24 hours (in the absence of fever or infection).<sup>37</sup> The diagnosis of MS was established according to 2017 McDonald criteria.<sup>6</sup> Scores of 4 and 6 based on the EDSS were only considered if they were irreversible and 6-month confirmed. Finally, we used the recent criteria proposed for the diagnosis of SPMS.<sup>38</sup>

### Procedures

MRI scans were performed on a 0.5 or 1.5-T magnet with a slice thickness varying from 2 to 5 mm. Images were obtained in the axial plane, using the following pulse sequences: T1-weighted conventional spin-echo, spin-echo proton-density weighted, T2-weighted spin-echo, and/or fluid-attenuated inversion recovery sequence. Lumbar punctures were performed

**Figure 1** Flowchart of Participants



by trained neurologists in nontreated patients or in those at least 3 months after the last corticosteroid dose.

### CSF Analysis

Intrathecal IgM synthesis was calculated using 2 quantitative and 2 qualitative methods within a month after sample collection. Samples were stored at  $-80^{\circ}\text{C}$  until assayed. Serum and CSF IgG, IgM, and albumin were quantified by nephelometry using a BN ProSpec nephelometer (Siemens Healthcare Diagnostics, Marburg, Germany). A plot of CSF/serum quotients with hyperbolic function provided the IgM Reibergram. A Reibergram<sup>4</sup>  $>0\%$  and an IgM index value  $>0.1$  as previously reported<sup>14,28,34</sup> were considered hereinafter as increased. OCMB and LS-OCMB were studied in serum and CSF via isoelectric focusing and immunoblotting as previously described.<sup>13</sup> A patient was considered to have OCMB when  $\geq 2$  IgM bands were detected in the CSF but not in the paired serum sample. Whenever OCMB additionally recognized CNS lipids, LS-OCMB was reported as positive.

### Classification of Evidence

Our primary research question was to compare the prognostic value of 4 methods assessing ITMS to predict the risk of a second relapse and a worse disease course in patients with a CIS. The classification of evidence assigned to this question is Class II.

### Statistical Analysis

Continuous variables were reported as mean  $\pm$  SD or median with range or interquartile range (IQR) and were evaluated with the Wilcoxon rank-sum test. Categorical variables were described using absolute and relative frequencies and analyzed with a  $\chi^2$  or Fisher exact test when appropriate. The kappa statistic was used for the between-methods agreement analysis.

We performed Cox proportional hazard regressions to estimate the adjusted hazard ratios (aHRs) along with 95% CI as measures of association between test results and end points. Adjustments were made for potential confounding factors (sex, age at CIS, topography of CIS, disease duration at the time of lumbar puncture, and treatments received  $>3$  months before outcome assessment). Time to second relapse and disability end points (EDSS scores 4 and 6 and SPMS development) were compared using Kaplan-Meier curves and a log-rank test. Patients who did not reach SPMS or with final EDSS scores of  $<4$  during follow-up were considered as censored at the time of last clinical assessment.

The following indices were calculated along with corresponding 95% CI for all end points:

- Sensitivity:  $(\text{TP}/[\text{TP} + \text{FN}]) \times 100$
- Specificity:  $(\text{TN}/[\text{TN} + \text{FP}]) \times 100$
- Positive predictive value (PPV):  $(\text{TP}/[\text{TP} + \text{FP}]) \times 100$
- Negative predictive value (NPV):  $(\text{TN}/[\text{TN} + \text{FN}]) \times 100$

True positives (TPs) were considered those test positive cases (with ITMS) reaching the end point of interest (conversion to relapsing-remitting MS [RRMS]/SPMS or reaching EDSS scores of 4 or 6) during follow-up, and false positives (FPs) were considered those test positive cases that did not. Patients with a negative test result (without ITMS) but presenting with the end points were considered false negatives (FNs), whereas those remaining as CIS or with EDSS scores  $<4$  during follow-up were considered as true negatives (TNs). For the between-methods comparisons of sensitivity and specificity, we applied the McNemar test.

**Table 1** Patient Characteristics

	Patients with CIS (n = 193)
Age, y, median (IQR)	31 (25–38)
Women, n (%)	130 (67.4)
<b>CIS type</b>	
Optic nerve	47 (24)
Brainstem	51 (26)
Spinal cord	73 (37.2)
Cerebral hemisphere	16 (8.2)
Multifocal	5 (2.6)
Paroxysmal symptoms	4 (2)
Time of follow-up, y, median (IQR)	12.9 (6.1–18.2)
EDSS score at first relapse, median (range)	2 (1–6)
Baseline EDSS score after the first relapse, median (range)	1 (0–3.5)
<b>T2 lesions at baseline, n (%)</b>	
0	12 (6.4)
1–3	34 (18.1)
4–9	48 (25.5)
10–50	80 (42.6)
>50	14 (7.5)
Gadolinium-enhancing lesions, median (range)	0 (0–15)
Time to lumbar puncture, mo, median (IQR)	5.38 (1.08–21.1)
CSF IgG OCB, n (%)	156 (80.8)
CSF IgG index, median (IQR)	0.8 (0.60–1.07)
<b>DMT at the time of CIS, n (%)</b>	
First line <sup>a</sup>	30 (15.5)
Second line <sup>b</sup>	3 (1.6)
Time to first treatment, y, median (IQR)	2.07 (0.90–4.72)

Abbreviations: CIS = clinically isolated syndrome; DMT = disease-modifying treatment; EDSS = Expanded Disability Status Scale; Ig = immunoglobulin; IQR = interquartile range; OCB = oligoclonal band.

<sup>a</sup>First line: subcutaneous or IM interferon- $\beta$ , glatiramer acetate, teriflunomide, dimethyl fumarate, fingolimod, azathioprine, and methotrexate.

<sup>b</sup>Second line: natalizumab, alemtuzumab, ocrelizumab, rituximab, mitoxantrone, and cyclophosphamide.

All analyses were conducted using Stata 14 (StataCorp, College Station, TX). All tests were 2 tailed, with  $p < 0.05$  as the level of statistical significance.

## Data Availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

# Results

## Patient Characteristics

Two hundred seventy-four patients with a typical CIS involving the CNS were initially included. We excluded participants with a follow-up of  $<2$  years ( $n = 38$ ), with absence or incomplete CSF IgM analysis ( $n = 35$ ), and with a final diagnosis different from MS ( $n = 8$ ) as shown in Figure 1, representing a 29.6% of dropouts. A total of 193 patients were included in the analyses, 130 (67.4%) women with a median (IQR) age at CIS of 31 (25–38) years. Patients were followed up for a median (IQR) of 12.9 (6.1–18.2) years. Table 1 outlines the baseline characteristics of all patients. During the course of their disease, most patients received at least 1 DMT (eTable 1, [links.lww.com/NXI/A514](https://links.lww.com/NXI/A514)).

## Intrathecal IgM Synthesis

Seventy-two (37.3%) patients had  $\geq 2$  OCMBs and were considered positive, whereas 53 (27.5%) had also LS-OCMBs and 32 (16.6%) showed a positive Reibergram. An index  $>0.1$  was observed in 81 (42%) patients. The between-methods agreement analysis using the kappa statistic is shown in eTable 2 ([links.lww.com/NXI/A514](https://links.lww.com/NXI/A514)). As expected, agreement was highest between OCMB and LS-OCMB (substantial agreement,  $\kappa = 0.77$ ), followed by IgM index and Reibergram, which was moderate ( $\kappa = 0.41$ ).

## Second Relapse

One hundred forty-nine (77.2%) patients experienced a second relapse during follow-up. Overall, the risk was 40.4%, 53.9%, and 72.4% after 12, 24, and 60 months, respectively. Neither Reibergram nor IgM index  $>0.1$  identified patients experiencing a subsequent relapse. Conversely, both OCMB and LS-OCMB were significantly associated with a higher risk of a second relapse at a shorter time (aHR 2.11, 95% CI 1.51–2.96,  $p < 0.001$ ; and aHR 2.50, 95% CI 1.72–3.64,  $p < 0.001$ , respectively) (Table 2). After 12 months, the risk was 66% among patients with LS-OCMB compared with 30.7% among patients without LS-OCMB, increasing to 83.7% and 68.1%, respectively, after 5 years, as shown in Figure 2. The Kaplan-Meier curves of all methods are shown in Figure 2.

## Disability End Points

Forty-one patients (21.2%) reached an EDSS score of 4. After 10 and 15 years, the Kaplan-Meier estimate of cumulative incidence was 14.2% and 24.8%, respectively. The risk of the EDSS score of 4 after 10 and 15 years was 26.3% and 40.7%, respectively, among patients with LS-OCMB compared with 9% and 18%, respectively, among patients without LS-OCMB (aHR 2.96, 95% CI 1.54–5.71;  $p = 0.001$ ). Both Reibergram and OCMB showed a trend toward a higher risk of reaching an EDSS score of 4 (aHR 2.02, 95% CI 0.96–4.23,  $p = 0.064$ , and aHR 1.75, 95% CI 0.94–3.28,  $p = 0.08$ , respectively) (Table 2). Conversely,

**Table 2** Unadjusted and Multivariable Cox Regression Models for Predicting the Risk of a Second Relapse, Reaching EDSS Scores of 4 and 6, and Development of SPMS

	Unadjusted			Multivariable model <sup>a</sup>		
	HR	95% CI	<i>p</i> Value	HR	95% CI	<i>p</i> Value
<b>Second relapse</b>						
<b>OCMB</b>	1.75	1.26–2.43	<i>0.001</i>	2.11	1.51–2.96	<i>&lt;0.001</i>
<b>LS-OCMB</b>	1.99	1.40–2.84	<i>&lt;0.001</i>	2.50	1.72–3.64	<i>&lt;0.001</i>
<b>Reibergram</b>	0.69	0.43–1.10	0.12	0.70	0.43–1.13	0.14
<b>IgM index (&gt;0.1)</b>	0.96	0.69–1.34	0.82	0.99	0.71–1.38	0.94
<b>EDSS score 4</b>						
<b>OCMB</b>	1.94	1.05–3.58	<i>0.035</i>	1.75	0.94–3.28	0.08
<b>LS-OCMB</b>	2.83	1.53–5.23	<i>0.001</i>	2.96	1.54–5.71	<i>0.001</i>
<b>Reibergram</b>	2.10	1.02–4.29	<i>0.043</i>	2.02	0.96–4.23	0.06
<b>IgM index (&gt;0.1)</b>	1.58	0.85–2.94	0.15	1.45	0.77–2.73	0.25
<b>EDSS score 6</b>						
<b>OCMB</b>	2.44	1.16–5.12	<i>0.018</i>	2.42	1.12–5.20	<i>0.02</i>
<b>LS-OCMB</b>	3.95	1.88–8.28	<i>&lt;0.001</i>	4.96	2.22–11.07	<i>&lt;0.001</i>
<b>Reibergram</b>	2.15	0.91–5.06	0.08	2.29	0.95–5.52	0.065
<b>IgM index (&gt;0.1)</b>	1.67	0.80–3.49	0.17	1.56	0.73–3.33	0.25
<b>SPMS</b>						
<b>OCMB</b>	1.81	0.90–3.64	0.09	1.52	0.74–3.15	0.26
<b>LS-OCMB</b>	2.45	1.22–4.91	<i>0.01</i>	2.31	1.09–4.93	<i>0.03</i>
<b>Reibergram</b>	2.39	1.06–5.36	<i>0.035</i>	2.33	1.01–5.36	<i>0.048</i>
<b>IgM index (&gt;0.1)</b>	1.43	0.71–2.91	0.32	1.28	0.61–2.68	0.51

Abbreviations: CIS = clinically isolated syndrome; EDSS = Expanded Disability Status Scale; HR = hazard ratio; Ig = immunoglobulin; IF = intrathecal fraction; LS-OCMB = lipid-specific IgM oligoclonal band; OCMB = IgM oligoclonal band; SPMS = secondary progressive multiple sclerosis.

<sup>a</sup> Cox proportional hazard regression, adjusted by sex, age at CIS, topography of CIS, disease duration at the time of lumbar puncture, and disease-modifying treatments as time-dependent covariates.

Italics indicate differences are statistically significant ( $p < 0.05$ ).

IgM index >0.1 was not associated with a higher risk of this outcome. The cumulative incidence of disability end point curves according to LS-OCMB and Reibergram results are detailed in Figure 3, whereas the Kaplan-Meier curves of OCMB and IgM index are shown in Figure 4.

The need for an assisted device to walk, that is an EDSS score of 6, was observed in 15% of patients (8.9%, 18.1%, and 25.9% after 10, 15, and 20 years, respectively). LS-OCMB showed the most accurate prediction of the risk of an EDSS score of 6 (aHR 4.96, 95% CI 2.22–11.07;  $p < 0.001$ ) (Table 2), as 17.9%, 36.2%, and 49.8% of patients with LS-OCMB reached this end point after 10, 15, and 20 years, respectively. In contrast, only 5.1%, 10%, and 14.9% of patients without LS-OCMB showed progression to EDSS of 6 after the same periods, respectively. The presence of OCMB, even without a lipid specificity, was also associated with a significant higher risk, but this method provided a lower prediction (aHR 2.42,

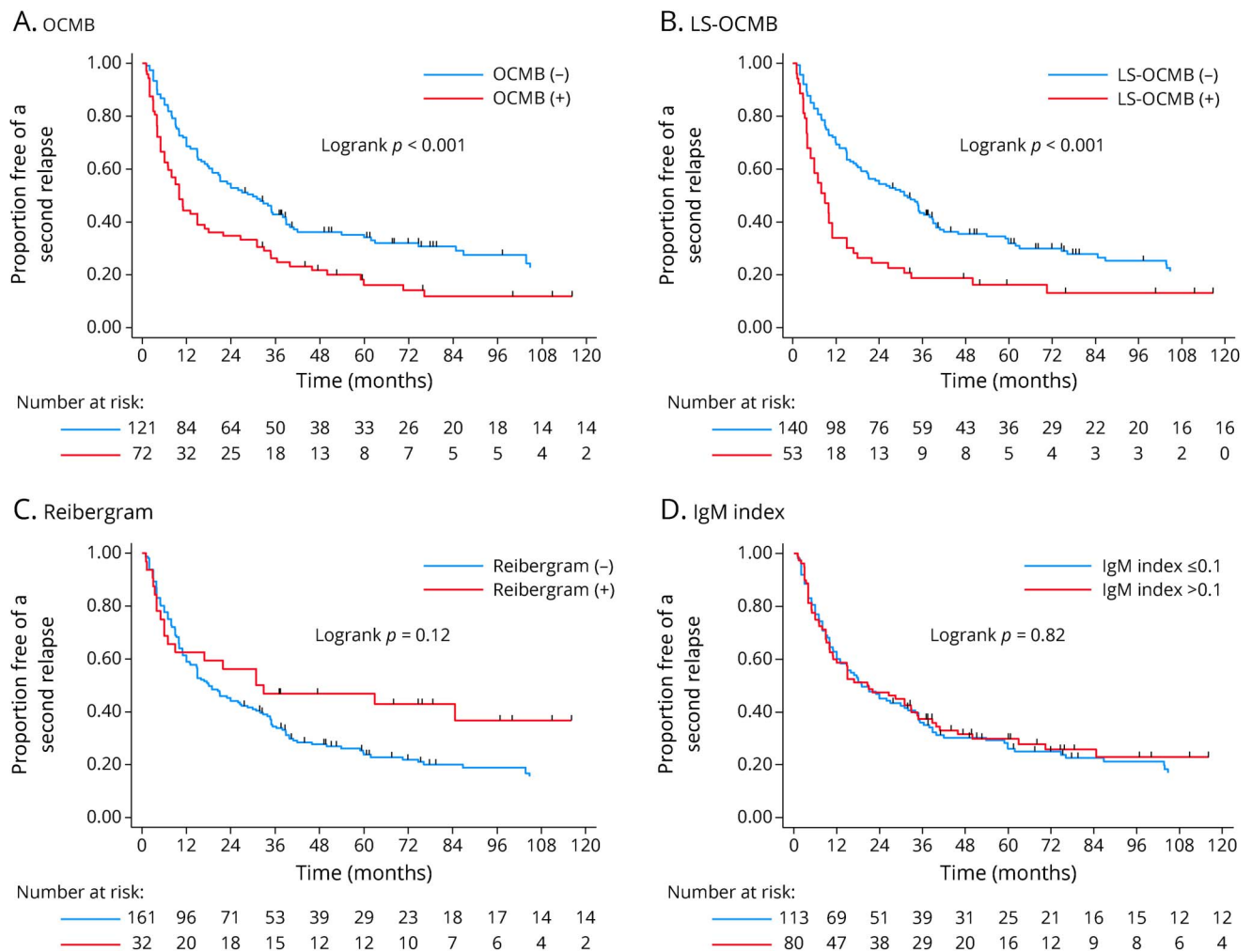
95% CI 1.13–5.20;  $p = 0.02$ ). On the other side, Reibergram and index were related to a not significant higher risk ( $p = 0.065$  and  $p = 0.25$ , respectively) (Table 2, Figures 3 and 4).

The development of SPMS was observed in 17.3% and 30.9% of patients after 15 and 20 years, respectively. The cumulative incidence was significantly different between those patients with LS-OCMB and those without (24.6% and 51% vs 14.2% and 20.4% after 15 and 20 years, respectively) (aHR 2.31, 95% CI 1.08–4.93;  $p = 0.03$ ). The Reibergram also predicted the conversion to SPMS (aHR 2.33, 95% CI 1.01–5.36;  $p = 0.048$ ), unlike OCMB and IgM index (Table 2, Figures 3 and 4).

We further performed analyses of sensitivity and specificity for all methods to test their accuracy for predicting disability outcomes. As shown in eFigure 1 ([links.lww.com/NXI/A514](https://links.lww.com/NXI/A514)), LS-OCMB showed the highest performance with a



**Figure 2** Time to a Second Relapse With All Methods Assessing ITMS



Kaplan-Meier survival curves and results from the log-rank test for the survival-free probability of a second relapse with (A) OCMB, (B) LS-OCMB, (C) Reibergram, and (D) IgM index. Ig = immunoglobulin; ITMS = intrathecal IgM synthesis; OCMB = IgM oligoclonal band; LS-OCMB = lipid-specific IgM oligoclonal band.

moderate sensitivity and a high specificity. Although Reibergram slightly improved specificity, differences were not significant except for SPMS ( $p = 0.035$ ). Conversely, LS-OCMB provided a 2-fold higher sensitivity for reaching EDSS scores of 4 and 6 and SPMS ( $p < 0.01$  for all comparisons) (eFigure 1). Neither OCMB nor IgM index improved sensitivity compared with LS-OCMB, and specificity was significantly lower ( $p < 0.001$ ). The PPV and NPV of all methods are shown in eTable 3.

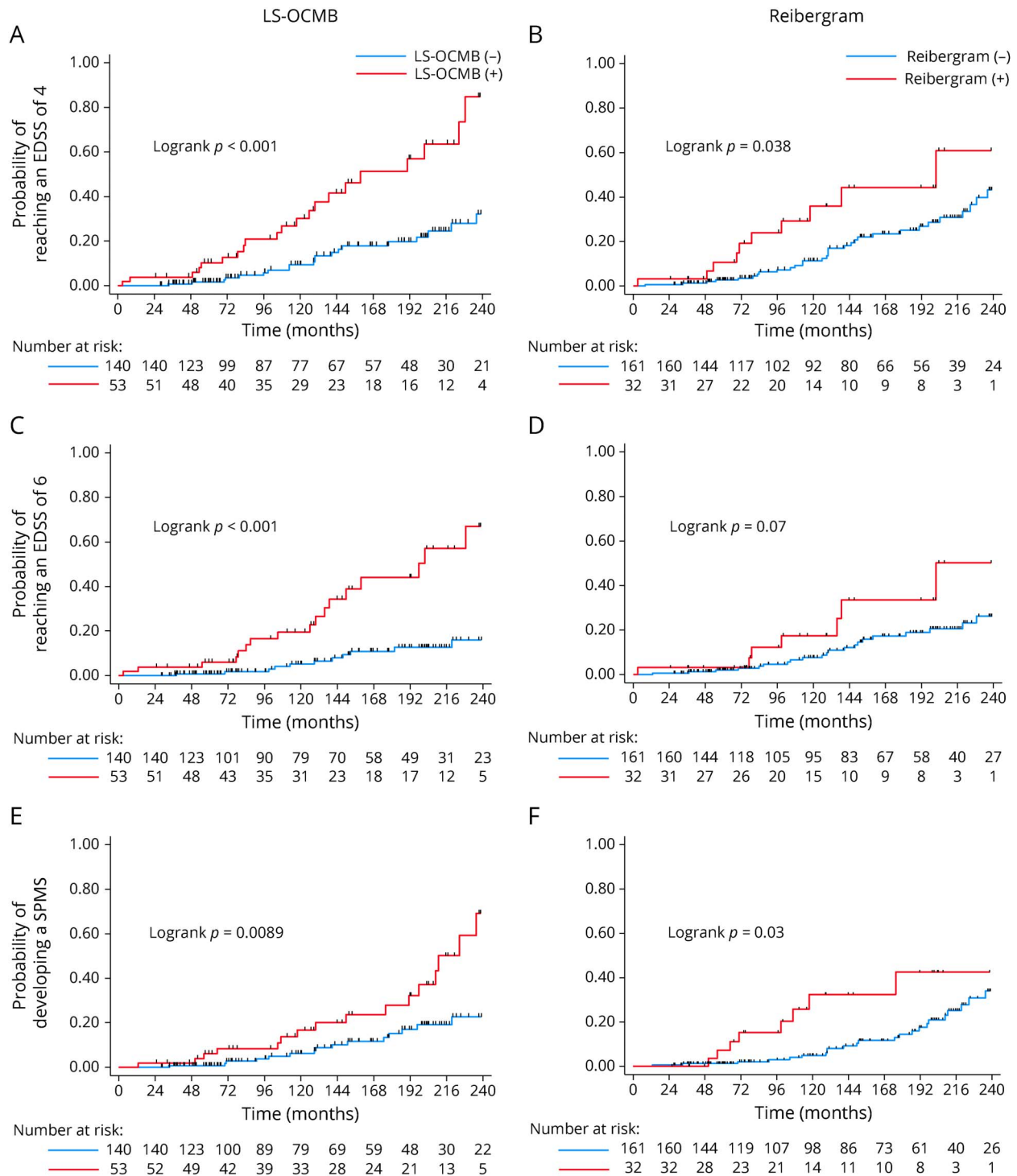
### Sensitivity Analyses

To evaluate the robustness of the results, we performed the following sensitivity analysis restricting the analysis to several groups of patients: (1) patients with clinically definite MS ( $n = 149$ ) (eTable 4, [links.lww.com/NXI/A514](https://links.lww.com/NXI/A514)), (2) patients with a 2017 McDonald RRMS ( $n = 165$ ) (eTable 5), and (3) patients with at least 10 years of follow-up ( $n = 120$ ) (eTable 6). All analyses yielded similar results as seen with the main one.

### Discussion

The recognition and validation of reliable and reproducible biomarkers to predict the evolution of patients with MS is a main field of investigation. The monoclonal antibodies approved for use in treating MS provided considerable improvements in terms of efficacy<sup>39,40</sup> and improved the prognosis of patients, especially when initiated at an early stage.<sup>41–44</sup> However, serious or life-changing adverse events are more frequent with these DMTs, and thus, treatment decisions have gained complexity because benefits must be thoroughly balanced with risks.<sup>45</sup> The availability of a test accurately recognizing patients at high risk of disability at disease onset and therefore who are candidates for these highly effective DMTs may tip the balance in favor of prescribing them at an early stage. In most cases, CSF analysis will be performed only once, but CSF parameters that remain steady throughout the disease with a prognostic value might be of a great value. The intrathecal

**Figure 3** Time to EDSS Scores of 4 and 6 and Conversion to Secondary Progressive Multiple Sclerosis With LS-OCMB and Reibergram

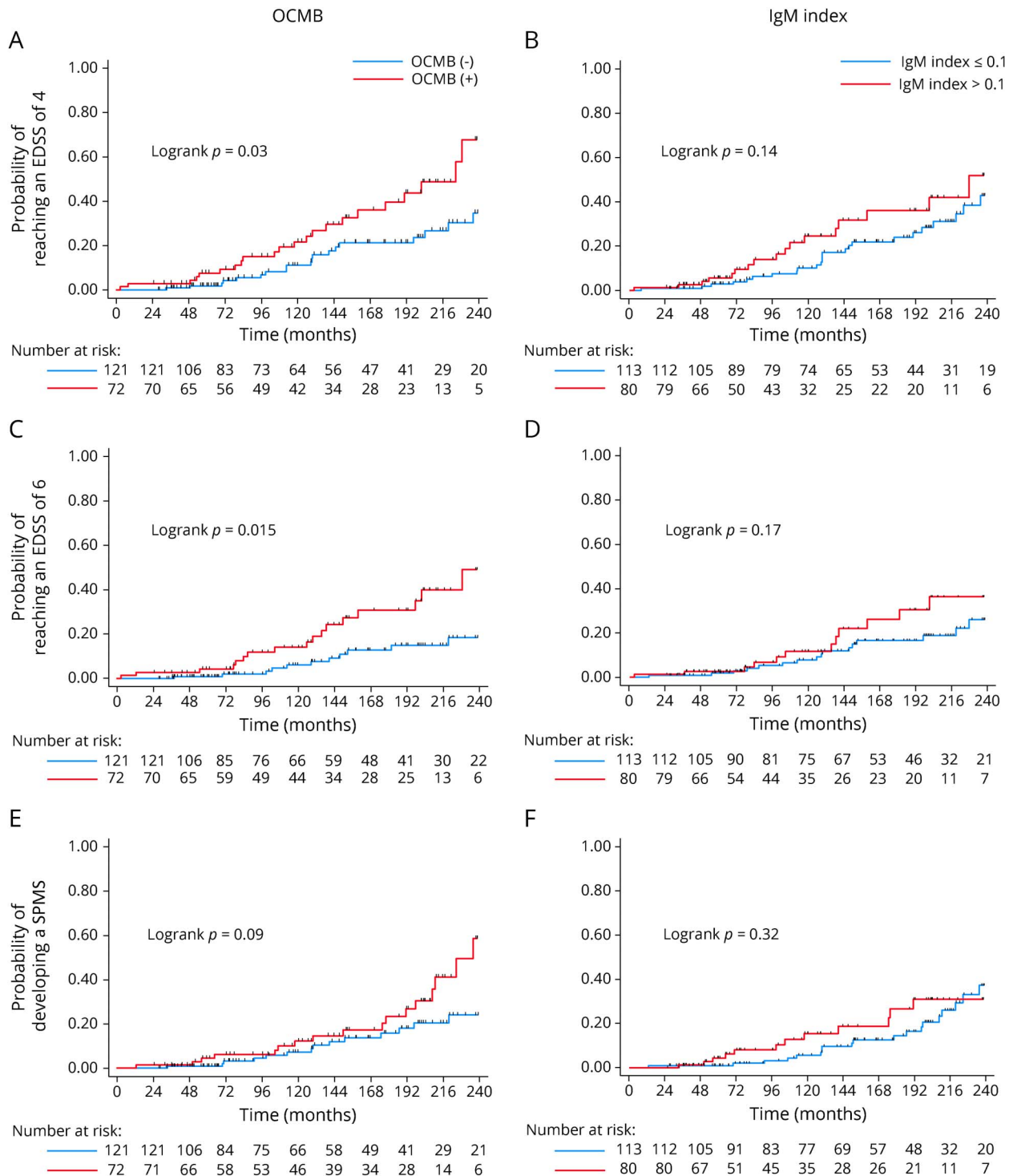


Cumulative incidence and results from the log-rank test for (A, B) EDSS score 4, (C, D) EDSS score 6, and (E, F) development of SPMS with (A, C, E) LS-OCMB and (B, D, F) Reibergram. EDSS = Expanded Disability Status Scale; Ig = immunoglobulin; LS-OCMB = lipid-specific IgM oligoclonal band; SPMS = secondary progressive multiple sclerosis.

synthesis of IgM has been proposed as a prognostic factor for nearly 30 years, but methodological problems have been proposed<sup>35,46</sup> against a general incorporation of routine CSF analysis. IgM is present in CSF at a clearly lower

concentration than IgG and has a higher molecular weight due to its pentameric structure. Thus, a proper storage is crucial to measure accurately ITMS. In this context, we performed this study following a large cohort of patients

**Figure 4** Time to EDSS Scores of 4 and 6 and Conversion to Secondary Progressive Multiple Sclerosis With OCMB and IgM Index



Cumulative incidence with the log-rank test results for (A, B) EDSS score 4, (C, D) EDSS score 6, and (E, F) development of SPMS with (A, C, E) OCMB and (B, D, F) IgM index. EDSS = Expanded Disability Status Scale; Ig = immunoglobulin; OCMB = IgM oligoclonal band.

with a CIS for a median of 12.9 years. We compared the predictive value of 4 methods detecting ITMS in their ability at the disease onset to detect patients at risk of a poor disease evolution.

LS-OCMB significantly identified patients with a CIS with a higher risk of a second relapse, reaching EDSS scores of 4 and 6 and converting to SPMS at an earlier stage in both unadjusted and multivariate analyses. The risk was at least



2-fold higher for all outcomes and was mainly evident for the risk of the EDSS score of 6, increased almost 5 times. This is especially relevant because despite 80% of patients with MS received at least 1 DMT before reaching an EDSS score of 3 (16.4% also a highly effective DMT), the cumulative incidence of the EDSS score of 6 after 20 years reached almost 50% among patients with LS-OCMB compared with less than 15% among patients without LS-OCMB. On the other side, although the Reibergram and OCMB could also serve as valid tools, they only identified patients at risk of some of the end points and to a lesser degree. Conversely, IgM index showed a poor value in predicting all outcomes. All sensitivity analyses performed reinforced these findings.

Further analysis of the diagnostic accuracy of all methods showed that both LS-OCMB and Reibergram had a high specificity for predicting disability milestones, in contrast to OCMB and IgM index. However, LS-OCMB provided a 2-fold significantly higher sensitivity than Reibergram, even if these results are probably underestimated by the effect of DMTs (especially for LS-OCMB), impossible to adjust in this case.

These results are consistent with the several previous studies that have associated ITMS (especially LS-OCMB) with a more aggressive course of MS in terms of disease activity and progression.<sup>10–28</sup> However, it remained unknown whether the different methods provided similar accuracy. In this study, LS-OCMB showed a greater value than Reibergram, and this difference might probably be explained by the already reported lower sensitivity of Reibergram compared with OCMB.<sup>47,48</sup> A positive Reibergram has strongly been associated with worse outcomes,<sup>21,26–28</sup> but their higher percentage of false-negative results compared with OCMB may diminish its utility.<sup>48</sup> Compared with OCMB, LS-OCMB may identify more accurately those patients at a higher risk of early disability by the fact that OCMB might be due to transient immune activation while LS-OCMB a sustained IgM response associated with CD5<sup>+</sup> B cells.<sup>17</sup> Regarding the IgM index, our negative results are in line with other studies<sup>18</sup> related to the high number of FP,<sup>34</sup> although conclusions cannot be accurately drawn as positive associations have been also described.<sup>24,28</sup>

For this reason, ITMS, when assessed by LS-OCMB, appears to be a good biomarker to identify patients who would be suitable to receive highly effective DMTs in an early stage.

Limitations were found in this study. The use of several DMTs on patients from this cohort could have affected the risk of subsequent disability, probably causing underestimation of the prognostic accuracy. This finding may be especially relevant for LS-OCMB as our group was using LS-OCMB as a biomarker of worse prognosis, and highly effective DMTs had been administered at earlier stages and

more frequently (eTable 1, [links.lww.com/NXI/A514](https://links.lww.com/NXI/A514)) in these patients. However, the percentage of patients treated with highly effective DMTs before reaching the disability outcomes was less than 20%, and Cox regression models were also adjusted by DMTs. Second, analyses were not adjusted by prognostic MRI markers such as T2 lesion load or T1 contrast-enhancing lesions, and thus, potential bias could have been introduced. Notwithstanding, future studies taking these variables into account are warranted. Third, the follow-up was variable across participants, and disability end points were generally achieved after a long period. However, a percentage of patients followed for <5 years were represented by only 16.1% of all the patients, and the sensitivity analysis restricted to patients with >10 years of follow-up yielded similar results.

Among patients with a typical CIS, the demonstration of ITMS by LS-OCMB accurately predicted a second relapse, the development of early disability, and conversion to SPMS. Thus, LS-OCMB, together with clinical and radiologic biomarkers, could help with the selection of patients at a higher risk of progression who would be potential candidates for receiving highly effective DMTs in an early stage.

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<b>Paulette Walo-Delgado, MD</b>	Hospital Universitario Ramón y Cajal, Madrid, Spain	Major role in acquisition of data and revised the manuscript for intellectual content
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