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Immunoglobulin M Oligoclonal Bands: Biomarker of Targetable Inflammation in Primary Progressive Multiple Sclerosis

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Objective: To identify a biomarker distinguishing patients who, despite a primary progressive multiple sclerosis (PPMS) clinical course, may nonetheless benefit from immune therapy.
Methods: The presence or absence of both immunoglobulin (Ig) G and IgM oligoclonal bands (OCB) was blindly examined in paired cerebrospinal fluid (CSF) and serum samples from a large PPMS patient cohort, and related to clinical and imaging evidence of focal inflammatory disease activity.
Results: Using both cross-sectional samples and serial sampling in a subgroup of patients followed prospectively as part of the placebo-controlled OLYMPUS study of rituximab in PPMS, we found that the presence of CSF-restricted IgM OCB (but not of IgG OCB) is associated with an active inflammatory disease phenotype in PPMS patients. This finding was confirmed in an independent, multicenter validation cohort.
Interpretation: The presence of CSF IgM OCB may be a biomarker for a subset of PPMS patients with more active inflammatory disease, who may benefit from immune-directed treatments.

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Primary progressive multiple sclerosis (PPMS) represents one of the greatest unmet needs in the MS field. There are currently no approved therapies for patients with PPMS, and treatments that have been shown to successfully modify disease course in patients with relapsing–remitting MS (RRMS) have generally not demonstrated benefit in PPMS. The 2 large placebo-controlled PPMS clinical trials to date (the PROMiSe

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TABLE 1. Clinical and Demographic Data of Cross-Sectional, Longitudinal, and Validation Cohorts

Characteristic	Cross-Sectional Cohort, n = 80	Longitudinal Cohort, n = 23	*p*	Cross-Sectional + Longitudinal Cohorts, n = 103	Validation Cohort, n = 67	*p*[a]
Age, yr, mean ± SE	49.21 ± 1.23	51.39 ± 1.66	0.53	49.71 ± 1.02	47.17 ± 1.05	0.09
Age at diagnosis, yr, mean ± SE	41.76 ± 1.16	41.39 ± 1.97	0.80	41.67 ± 1.00	41.97 ± 1.10	0.85
Duration from diagnosis, yr, mean ± SE	7.43 ± 0.73	10.00 ± 1.64	0.11	7.80 ± 0.68	5.17 ± 0.58	0.007
Sex, male/female	38/42	15/8	0.16	53/50	28/39	0.15
EDSS score, mean ± SE	4.52 ± 0.19	4.74 ± 0.27	0.47	4.57 ± 0.16	4.18 ± 0.20	0.10
MSSS score, mean ± SE	6.88 ± 0.23	6.57 ± 0.37	0.30	6.81 ± 0.20	7.21 ± 0.24	0.17

Cross-sectional + longitudinal cohorts represent the total number of patients in the initial study.
[a]Probability value compares the validation cohort with the total number of patients in the initial study.
EDSS = Expanded Disability Status Scale; MSSS = Multiple Sclerosis Severity Scale; SE = standard error.

study using oral glatiramer acetate[1] and the OLYMPUS study using the anti-CD20 B-cell–depleting antibody rituximab[2]) failed to meet their primary endpoint of limiting progression of disability in the overall PPMS study populations.

Of interest, post hoc analyses of both the PROMiSe and OLYMPUS studies suggested that a subset of PPMS patients may nonetheless benefit from these immune-directed therapies. The subset of PPMS patients who appeared to benefit were those who were younger, exhibited more rapid progression prior to randomization, or had gadolinium contrast-enhancing (Gd^+) brain lesions at baseline.[1,2] Although this suggests that important biological heterogeneity may exist among patients clinically diagnosed with PPMS, no biological marker has been identified to date that can distinguish patients who, despite a primary progressive clinical course, may nonetheless benefit from immune therapy.

Here, we studied both immunoglobulin (Ig) G and IgM oligoclonal bands (OCB) in the cerebrospinal fluid (CSF) of a large cohort of patients with PPMS. Using both cross-sectional samples and serial sampling in a unique subgroup of patients followed prospectively as part of the placebo-controlled OLYMPUS study of rituximab in PPMS, we discovered that the presence of IgM OCB (OCMB), but not of IgG OCB (OCGB), is associated with a more active inflammatory disease phenotype, and that their presence may be a biomarker for the subset of PPMS patients who, despite a primary progressive clinical course, may benefit from immune-directed treatments.

Patients and Methods

Patient Recruitment and Demographics

A total of 103 patients meeting diagnostic criteria of PPMS (revised McDonald criteria[3]) were included in the initial paired CSF/serum study. A core cross-sectional cohort comprised 80 untreated PPMS patients (see Table 1 for clinical/demographic features), serially recruited at 4 university hospital departments of neurology. Thirty-six of these patients were recruited at Ramón y Cajal Hospital, Madrid; 25 at La Fe Hospital, Valencia, 13 at Vall d'Hebron Hospital, Barcelona; and 6 at Gregorio Marañón Hospital, Madrid. We recruited an additional 23 patients (see Table 1) with confirmed PPMS (meeting both the original McDonald criteria[4] and the revised McDonald criteria[3]) participating in the blinded phase III placebo-controlled OLYMPUS trial of rituximab in PPMS.[2] As an independent validation cohort, 67 additional PPMS patients (see Table 1) were subsequently recruited at 8 international major academic hospitals, including Virgen Macarena Hospital, Seville (n = 16); Clinico Hospital, Madrid (n = 13); Cerrahpaşa School of Medicine, Istanbul (n = 12), Carlos Haya Hospital, Málaga (n = 7); Clinico Hospital, Valencia (n = 7); Ramon y Cajal Hospital, Madrid (n = 7); St Petersburg Center for Multiple Sclerosis and Autoimmune Diseases, St Petersburg (n = 4); and Gregorio Marañon Hospital, Madrid (n = 1). None of the patients in the 3 cohorts had received immunosuppressive treatments or disease-modifying therapies prior to enrollment. No magnetic resonance imaging (MRI) data were available in the cross-sectional cohort. A summary of paired CSF/serum samples available from patients in the OLYMPUS clinical trial cohort is shown in Table 2. Twenty of these 23 patients received rituximab (two 1,000mg intravenous infusions, administered 2 weeks apart, every 24 weeks), and 3 patients received placebo infusions while being followed and sampled prospectively. In 17 of these longitudinally followed patients, paired CSF and serum

TABLE 2. Samples Available from Patients Participating in the Longitudinal OLYMPUS Trial of Rituximab in Primary Progressive Multiple Sclerosis

Patient	OCMB	OCGB	Treatment	Timing of CSF Sampling
1	Neg	Pos	Rituximab	Baseline
2	Neg	Pos	Rituximab	47 weeks
3	Neg	Pos	Rituximab	47 weeks
4	Neg	Pos	Rituximab	47 weeks
5	Neg	Pos	Rituximab	Baseline
6	Neg	Pos	Rituximab	47 weeks
7	Neg	Pos	Placebo	47 weeks
8	Neg	Pos	Rituximab	Baseline
9	Neg	Pos	Rituximab	Baseline
10	Neg	Pos	Rituximab	47 weeks
11	Neg	Pos	Rituximab	Baseline
12	Neg	Pos	Rituximab	Baseline
13	Neg	Pos	Rituximab	23 weeks
14	Neg	Pos	Rituximab	23 weeks
15	Neg	Pos	Rituximab	23 weeks
16	Neg	Pos	Rituximab	23 weeks
17	Neg	Pos	Rituximab	Baseline
18	Neg	Pos	Rituximab	Baseline
19	Pos	Pos	Placebo	47 weeks
20	Pos	Pos	Placebo	47 weeks
21	Pos	Pos	Rituximab	47 weeks
22	Pos	Pos	Rituximab	Baseline
23	Pos	Pos	Rituximab	23 weeks

CSF = cerebrospinal fluid; Neg = negative; OCGB = immunoglobulin G oligoclonal band status; OCMB = immunoglobulin M oligoclonal band status; Pos = positive.

samples were available for analysis at >1 time point. Baseline measures included disability assessments using the Expanded Disability Status Scale (EDSS)[5] and the Multiple Sclerosis Severity Scale (MSSS),[6] as well as brain MRI measures of the volume of T2 hyperintense lesion burden and the number of T1 Gd^+ lesions. New brain MRI T2 lesions were assessed at weeks 6, 48, and 96 of follow-up, and the number of Gd^+ lesions was measured at 96 weeks of follow-up. All CSF and serum analyses were blinded to neurological and imaging evaluations. Participants in both cross-sectional and longitudinal cohorts were recruited following informed consent and based on study protocols that were approved by each institutional ethics committee.

CSF Analyses

Paired serum and CSF samples from all patients were stored at −80°C in 0.2ml aliquots until assessment of quantitative Ig measures as well as IgG and OCMB studies. For determination of IgG and IgM indices, levels of IgG, IgM, and albumin were quantified in paired serum and CSF samples using a Siemens (Erlangen, Germany) nephelometer. Oligoclonal bands were analyzed by isoelectric focusing and immunoblotting using identical volumes of CSF in each lane, as previously described.[7,8] When available, 4 to 6ml of fresh CSF samples were analyzed for immune cell subsets using flow cytometry and a standardized protocol. Briefly, CSF was centrifuged at 500 × *g* for 15 minutes, and the cellular pellet was washed with phosphate-buffered saline (PBS), then resuspended in 100μl of PBS and divided into 3 equal aliquots. These aliquots were then labeled for 30 minutes at 4°C with optimized concentrations of combinations of the following monoclonal antibodies or isotype controls: mouse isotypes IgG1-PE, IgG1-PerCP-Cy5.5, IgG1-APC, anti–CD19-PerCP-Cy5.5, and anti–CD45-fluorescein isothiocyanate from BD Biosciences (Franklin

TABLE 3. Clinical, Demographic, and Laboratory Features of Cross-Sectional Primary Progressive Multiple Sclerosis Patient Cohort, Classified according to Presence or Absence of Cerebrospinal Fluid OCGB

Characteristic	$OCGB^+$, n = 70	$OCGB^-$, n = 10	*p*
Age, yr, mean ± SE	48.88 ± 3.45	51.40 ± 1.32	0.40
Age at diagnosis, yr, mean ± SE	41.31 ± 1.23	44.78 ± 3.52	0.27
Disease duration, yr, mean ± SE	7.31 ± 0.82	6.08 ± 0.76	0.67
Sex, male/female	32/38	6/4	0.50
EDSS score, mean ± SE	4.51 ± 0.21	4.60 ± 0.44	0.92
MSSS score, mean ± SE	6.85 ± 0.26	7.07 ± 0.47	0.99
Cells, mean ± SE	3.02 ± 0.51	2.57 ± 1.39	0.46
IgM index, mean ± SE	0.13 ± 0.02	0.09 ± 0.02	0.47
IgG index, mean ± SE	0.98 ± 0.08	0.52 ± 0.03	<0.0001

EDSS = Expanded Disability Status Scale; Ig = immunoglobulin; MSSS = Multiple Sclerosis Severity Scale; OCGB = IgG oligoclonal bands; $OCGB^+$ = patients with OCGB; $OCGB^-$ = patients lacking OCGB; SE = standard error.

Lakes, NJ); or anti–CD5-PE from Beckman Coulter (Fullerton, CA). Cells were then washed twice with PBS and 0.02% sodium azide at 4°C and analyzed on a standard FACSCanto II instrument (Becton Dickinson, Franklin Lakes, NJ). A minimum of 500 events were collected for analysis of each staining. The percentages of cells that stained positively for specific antigens were recorded for each sample.

Statistical Analyses

Results were analyzed with the Prism 5.0 statistical package (GraphPad Software, San Diego, CA). We used the Mann–Whitney *U* test or the Fisher exact test for comparisons between 2 groups. Kruskal–Wallis or chi-square tests were used to compare ≥3 groups; $p < 0.05$ was considered statistically significant.

Results

Cross-Sectional Cohort

Of 80 patients with PPMS in the cross-sectional study, OCGB restricted to the CSF were identified in 70 (87. 5%; Table 3; referred to as $OCGB^+$), whereas CSF-restricted OCMB were identified in 21 (26. 2%; Table 4; referred to as $OCMB^+$). As expected, CSF of patients with detectable OCGB ($OCGB^+$) had significantly higher IgG indices than CSF of patients lacking OCGB ($OCGB^-$; see Table 3; $p < 0.0001$). However, when

TABLE 4. Clinical, Demographic, and Laboratory Features of Cross-Sectional Primary Progressive Multiple Sclerosis Patient Cohort, Classified according to Presence or Absence of Cerebrospinal Fluid OCMB

Characteristic	$OCMB^+$, n = 21	$OCMB^-$, n = 59	*p*
Age, yr, mean ± SE	45.32 ± 2.89	50.48 ± 1.3	0.12
Age at diagnosis, yr, mean ± SE	39.77 ± 2.44	42.41 ± 1.32	0.31
Disease duration, yr, mean ± SE	5.17 ± 1.09	7.84 ± 0.89	0.063
Sex, male/female	13/8	25/34	0.14
EDSS score, mean ± SE	4.95 ± 0.35	4.37 ± 0.23	0.17
MSSS score, mean ± SE	8.06 ± 0.24	6.47 ± 0.29	0.003
Cells, mean ± SE	2.63 ± 1.12	3.05 ± 0.52	0.54
IgM index, mean ± SE	0.24 ± 0.07	0.09 ± 0.01	0.001
IgG index, mean ± SE	1.22 ± 0.05	0.81 ± 0.05	0.007

EDSS = Expanded Disability Status Scale; Ig = immunoglobulin; MSSS = Multiple Sclerosis Severity Scale; OCMB = IgM oligoclonal bands; $OCMB^+$ = patients with OCMB; $OCMB^-$ = patients lacking OCMB; SE = standard error.

stratified this way based on the presence or absence of OCGB, no differences were noted between groups in terms of the sex distribution, age at sampling, age at MS diagnosis, disease duration, or EDSS or MSSS scores (see Table 3). In contrast, when the same PPMS cohort was stratified based on the presence or absence of CSF OCMB (see Table 4), patients who were $OCMB^+$ had on average significantly higher MSSS scores ($p = 0.003$), consistent with a more aggressive disease course prior to the cross-sectional evaluation. Patients in the $OCMB^+$ group exhibited, as expected, higher CSF IgM indices as compared to the $OCMB^-$ patients (see Table 4; $p = 0.001$), and were also found to have higher CSF IgG indices ($p = 0.007$) compared to the $OCMB^-$ patients.

CSF B-Cell Measures

We next considered the profiles of CSF B cells and B-cell subsets in PPMS patients, stratified according to the presence or absence of either OCGB or OCMB. CSF immune cell analysis was possible in samples from 39 patients. Of these, 34 (87.2%) had CSF OCGB ($OCGB^+$), whereas 8 (20.5%) had CSF OCMB ($OCMB^+$), similar to the overall cohort. Compared to patients lacking OCMB ($OCMB^-$), CSF of patients who were $OCMB^+$ exhibited significantly higher proportions of B cells ($p = 0.001$, Fig 1A) as well as significantly higher absolute B-cell counts ($p = 0.015$, see Fig 1). These differences appeared particularly pronounced for the $CD5^+$ B-cell subset ($p = 0.0009$ for proportions and $p = 0.005$ for absolute counts; see Fig 1A, B). In contrast, no differences were seen in CSF B cells when comparing PPMS patients stratified based on $OCGB^+$ versus $OCGB^-$ status (data not shown). Together, these results suggest that among patients clinically diagnosed with PPMS, stratification based on the presence or absence of CSF OCMB (but not OCGB) identifies a subset of patients with evidence of a more aggressive prior clinical course, who also exhibit a distinct CSF B-cell profile.

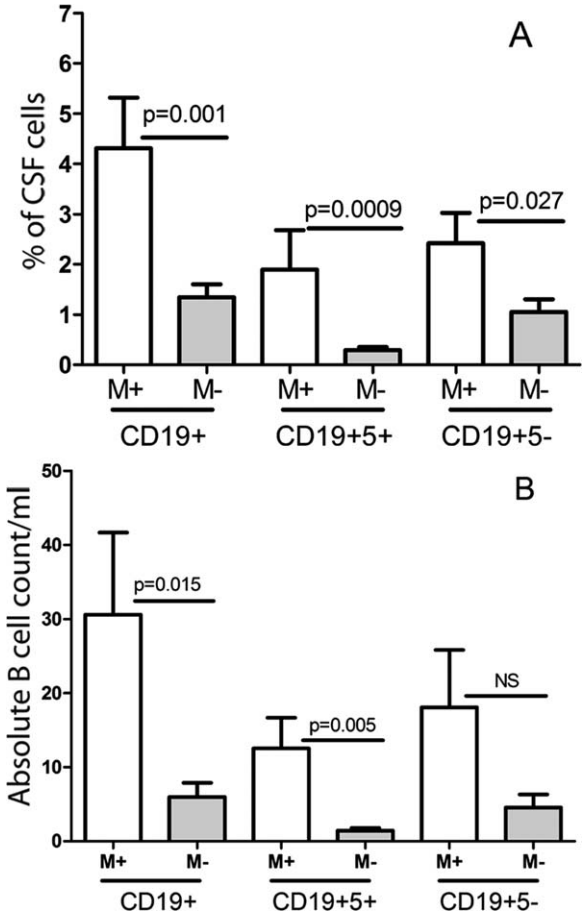

FIGURE 1: Proportions and absolute counts of cerebrospinal fluid (CSF) B cells in patients with primary progressive multiple sclerosis (PPMS), stratified based on the presence or absence of CSF-restricted immunoglobulin M oligoclonal bands (OCMB). PPMS patients exhibiting OCMB restricted to CSF (M^+), when compared with PPMS patients lacking CSF OCMB (M^-), show high percentages (A) and absolute cell counts (B) of CSF B cells ($CD19^+$), particularly of the $CD5^+$ B-cell subset. The same was not seen when patients were stratified based on the presence or absence of CSF oligoclonal immunoglobulin G bands. NS = not significant.

Baseline Features of the OLYMPUS PPMS Cohort

To assess for the potential association between the presence of OCMB and more objective measures of inflammatory disease activity, we had the opportunity to examine an additional cohort of 23 PPMS patients participating in the OLYMPUS trial of rituximab in PPMS,[2] where Hawker et al reported that 24.5% of patients exhibited Gd^+ brain MRI lesions at baseline (pretreatment). We hypothesized that the presence of CSF OCMB may identify these patients with active (though subclinical) inflammatory lesions. Among these 23 PPMS patients, 22% exhibited CSF OCMB. We observed a striking association between the presence of CSF OCMB and the presence of Gd^+ brain lesions at baseline. The majority (80%) of $OCMB^+$ patients had baseline Gd^+ lesions, whereas only a small minority (5%) of patients lacking OCMB had Gd^+ brain lesions (Fig 2A; $p = 0.002$). The average number of Gd^+ lesions at baseline was strikingly higher in $OCMB^+$ versus $OCMB^-$ patients (see Fig 2B; $p = 0.0005$). In contrast, CSF OCGB were present in all patients (as expected, because entry into the OLYMPUS study required prior documentation of CSF-restricted OCGB and/or elevated IgG-index/synthesis rate), and therefore their presence or absence at baseline did not correlate with the presence of Gd^+ MRI lesions. Stratification of patients based on

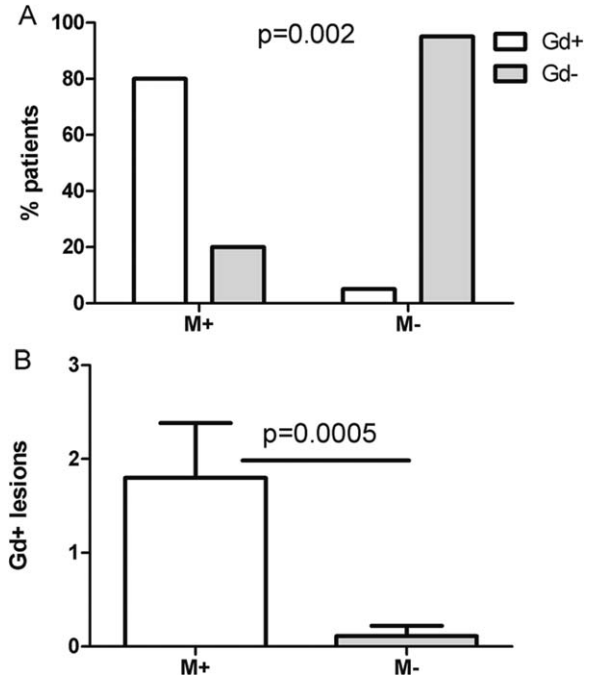

FIGURE 2: Gadolinium-enhancing (Gd^+) brain lesions are present almost exclusively in the subset of OLYMPUS primary progressive multiple sclerosis patients with cerebrospinal fluid (CSF) immunoglobulin M (IgM) oligoclonal bands (OCMB). We studied the association of IgM status and the presence of Gd^+ lesions in the OLYMPUS cohort (n = 23). (A) Baseline Gd^+ lesions were present in a significantly higher proportion (80%) of patients with CSF OCMB (M^+), compared to only a small minority (5%) of patients lacking CSF OCMB (M^-). (B) The average number of Gd^+ lesions at baseline was significantly higher in patients with CSF OCMB (M^+) as compared to patients lacking CSF OCMB (M^-). In contrast, CSF immunoglobulin G oligoclonal bands were present in all patients, and hence their presence did not correlate with Gd^+ lesions.

different levels of CSF IgG synthesis (arbitrary IgG index cutoff values of 0.7, 0.9, or 1.0) also did not reveal an association between CSF IgG parameters and the presence of Gd^+ lesions (data not shown). Hence, prior to treatment, the presence of CSF OCMB (but not of CSF OCGB) distinguished PPMS patients with Gd^+ brain MRI lesions. Perhaps also noteworthy is the observation that $OCMB^+$ patients in this relatively small OLYMPUS cohort also exhibited a trend for higher MSSS scores (7.75 ± 0.49) compared to $OCMB^-$ patients (6.24 ± 0.42; $p = 0.09$), consistent with our finding in the larger cross-sectional cohort described above, and in the validation cohort described below (Fig 3; Table 5).

Validation Cohort

To confirm our core finding of CSF OCMB as a putative biomarker of the subset of PPMS patients with active focal inflammatory (Gd^+) brain MRI lesions, we recruited 67 additional well-characterized PPMS patients from 8 major international teaching hospitals, for whom paired CSF/serum samples were available as well as brain MRI data (both before and after gadolinium-infused scans) obtained close to the time of lumbar puncture (see Table 1). Among these patients, 65 of 67 (97%) exhibited CSF OCGB, whereas 33 patients exhibited OCMB. Although there was no relationship between the presence of OCGB (seen in almost all the patients) and Gd^+ brain MRI lesions, we confirmed that the presence of OCMB was clearly associated with a higher number of Gd^+ brain lesions ($p = 0.0005$; Fig 4) in this additional PPMS population. Specifically, 13 of the 33 IgM^+ patients (39.4%) had Gd^+ enhancing lesions, whereas only 2 of the 34 IgM^- patients (5.9%) showed these lesions (see Fig 4A). The average number of Gd^+ lesions was 1.09 ± 0.35 for $OCMB^+$ patients versus 0.17 ± 0.14 for the $OCMB^-$ patients (see Fig 4B).

Longitudinal Features of the OLYMPUS PPMS Cohort

Because the OLYMPUS study results[2] suggested that the presence of baseline Gd^+ lesions may be associated with a beneficial therapeutic response to B-cell depletion, we explored whether we could detect an association between serial MRI measures of disease activity and the presence of CSF OCMB in our longitudinal cohort. Although our number of $OCMB^+$ patients is relatively small (n = 5; see Table 2), we noted that the 2 $OCMB^+$ patients

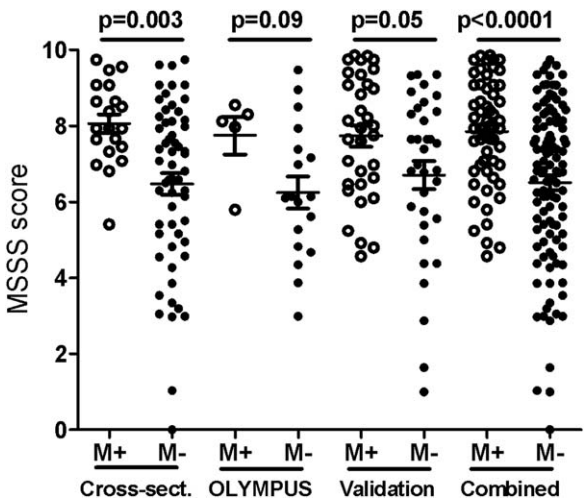

FIGURE 3: Multiple Sclerosis Severity Scale (MSSS) scores in cohorts of primary progressive multiple sclerosis patients stratified based on presence or absence of cerebrospinal fluid (CSF) immunoglobulin M oligoclonal bands (OCMB). Patients with OCMB (M^+) showed higher average MSSS values than those lacking these antibodies (M^-). Data were similar in the cross-sectional (n = 80), longitudinal (OLYMPUS, n = 23), and validation (n = 67) cohorts, and in the combination of all (Combined, n = 170). The same was not seen when patients were stratified based on the presence or absence of CSF immunoglobulin G oligoclonal bands.

TABLE 5. Clinical, Demographic, and Laboratory Features of Validation Cohort Patients Classified according to Presence or Absence of Cerebrospinal Fluid OCMB

Characteristic	$OCMB^+$, n = 33	$OCMB^-$, n = 34	*p*
Age, yr, mean ± SE	45.08 ± 1.37	49.20 ± 1.51	0.09
Age at diagnosis, yr, mean ± SE	41.09 ± 1.40	42.81 ± 1.70	0.61
Disease duration, yr, mean ± SE	3.98 ± 0.62	6.32 ± 0.94	0.054
Sex, male/female	14/19	14/20	1.0
EDSS score, mean ± SE	4.33 ± 0.29	4.03 ± 0.27	0.50
MSSS score, mean ± SE	7.73 ± 0.28	6.71 ± 0.37	0.051
IgM index, mean ± SE	0.18 ± 0.03	0.09 ± 0.01	0.0003
IgG index, mean ± SE	1.18 ± 0.15	0.81 ± 0.07	0.015

EDSS = Expanded Disability Status Scale; Ig = immunoglobulin; MSSS = Multiple Sclerosis Severity Scale; OCMB = IgM oligoclonal bands; $OCMB^+$ = patients with OCMB; $OCMB^-$ = patients lacking OCMB; SE = standard error.

subsequently treated with placebo continued to have additional Gd^+ lesions on their follow-up brain MRIs, whereas the 3 $OCMB^+$ patients treated with rituximab exhibited no Gd^+ lesions on multiple follow-up MRIs (mean cumulative number of Gd^+ lesions 3 vs 0, respectively). This observation was reinforced when considering the number of newly developing T2 brain lesions in these $OCMB^+$ patients; in serial imaging at weeks 6, 48, and 96, the mean numbers of new T2 lesions were 1.5, 4.5, and 9.0, respectively, in the placebo-treated patients, versus 0.33, 0, and 0 lesions at the same time points in the rituximab-treated patients (a mean cumulative number of lesions of 15 vs 0.33; $p = 0.04$; Student *t* test).

Discussion

Approximately 10 to 15% of all patients with MS suffer a PPMS course, with unremitting progression of disability from onset.[9] In the absence of approved disease-modifying treatment, and following several unsuccessful clinical trial programs in PPMS, many in the community have been left with a view that immune-directed therapy has no role in this form of MS, with others questioning whether PPMS is part of the MS disease spectrum or a separate disease entity.[10,11] We were intrigued by the suggestion from post hoc analyses of the 2 large PPMS clinical trials,[1,2] including the more recent OLYMPUS trial of anti-CD20 B-cell depletion with rituximab, that important heterogeneity may exist within patients who clinically exhibit a PPMS course, in particular that a subset of PPMS patients exists in whom the presence of active focal inflammatory disease, as typically observed in patients with RRMS, may represent a viable target for immunotherapy. Identifying a biological marker that may

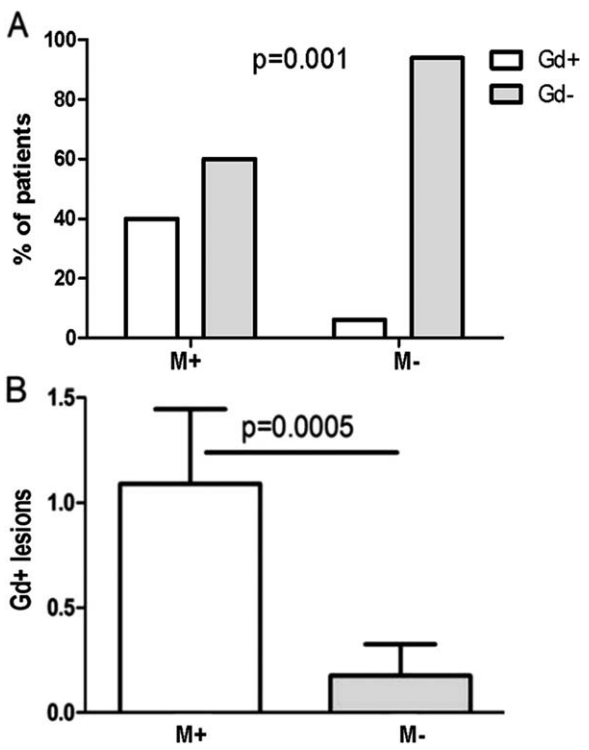

FIGURE 4: Gadolinium-enhancing (Gd^+) lesions are present almost exclusively in the validation cohort of primary progressive multiple sclerosis (PPMS) patients harboring cerebrospinal fluid (CSF) immunoglobulin M (IgM) oligoclonal bands (OCMB). We studied the association of IgM status and the presence of Gd^+ lesions in the validation cohort (n = 67). (A) The majority of Gd^+ brain lesions were noted in patients with CSF OCMB (M^+). Gd^+ lesions were present in a significantly higher proportion (39.4%) of patients with CSF OCMB (M^+), compared to only (5.9%) of patients lacking CSF OCMB (M^-). (B) The average number of Gd^+ lesions was significantly higher in patients with CSF OCMB (M^+) as compared to patients lacking CSF OCMB (M^-).

distinguish these patients would be of considerable interest, as this could guide effective treatment decisions for at least a subset of patients diagnosed with PPMS.

We considered whether the inflammatory profile of CSF, and in particular the presence and type of CSF-restricted OCB that have been extensively studied in patients with RRMS, might be associated with distinct PPMS phenotypes. In RRMS, both IgG and OCMB have been considered as potential biomarkers of disease activity and treatment response.[12–15] OCGB are found in the great majority of RRMS patients,[16–18] which may in part explain why their presence or absence has not been consistently correlated with clinical or radiological measures of disease activity.[12,14,19] In contrast, OCMB, described in approximately 40% of RRMS patients, appear to correlate better with a number of parameters of inflammatory disease activity. The presence of CSF OCMB in patients with clinically isolated syndromes has been associated with earlier evidence of new disease activity conferring the diagnosis of clinically definite RRMS.[20] In cohorts of patients with established RRMS, the presence of CSF OCMB has been associated with a more substantial T2 lesion load, a greater number of new Gd^+ lesions, and a higher relapse rate,[13,21] as well as with increased rates of brain atrophy,[22] earlier conversion to secondary progressive MS, and more rapid worsening of neurological disability.[23,24] Like RRMS patients, most PPMS patients are eventually found to harbor CSF-restricted OCGB over time,[18] whereas only a subgroup of these patients exhibit CSF-restricted OCMB.[25,26] However, the relationship between intrathecal IgG and OCMB, and measures of inflammatory disease activity or disease outcome, has not been addressed in PPMS.

Our study reveals that stratification of PPMS patients based on the presence or absence of CSF OCMB (but not of CSF OCGB) identifies a subset of PPMS patients who experienced a more aggressive prior clinical course, exhibit an increased number and distinct profile of CSF B cells, and are substantially more likely to manifest with imaging evidence of active central nervous system (CNS) inflammation. The presence of Gd^+ brain lesions was almost exclusively seen in the subset (approximately 20–25%) of PPMS patients harboring CSF OCMB, a finding we subsequently confirmed in an independent multicenter validation cohort.

Our unique access to a limited collection of CSF samples in a prospectively followed cohort of the OLYMPUS trial of rituximab in PPMS also enabled us to explore the effect of B-cell depletion on the relationship between baseline CSF OCB and disease activity. Inclusion criteria into the OLYMPUS study included documentation of abnormal CSF IgG measures, and all patients in this cohort exhibited CSF-restricted OCGB, indicating that the presence or absence of OCGB could not be a marker of the presence or absence of Gd^+ lesions. Essentially all Gd^+ lesions were seen in the 22% of PPMS patients who prior to treatment exhibited CSF OCMB. Within this subset of patients with OCMB at baseline, placebo-treated patients continued to experience new Gd^+ brain lesions and accumulation of new T2 lesions over time. In contrast, the small number of rituximab-treated patients who had baseline CSF OCMB experienced essentially no new brain MRI activity in follow-up. Our main observation points to the measurement of CSF OCMB as a potential biomarker of the subset of PPMS patients with more active focal inflammatory disease activity. Together, our findings raise the possibility that the presence of CSF OCMB identifies a subset of PPMS patients who may benefit from immune-targeted therapy that is effective in patients with relapsing forms of MS.

One might speculate on how the presence of OCMB may relate to underlying MS disease mechanisms. On one hand, intrathecal synthesis of oligoclonal IgM against myelin lipids has been shown to predict an aggressive disease course in MS,[20] and more recently IgM antibodies have been found to target oligodendrocytes and axons within MS lesions,[27] raising the possibility that at least some of the IgM that comprises CSF OCMB may directly contribute to CNS injury. Another possibility is that the development of CSF-restricted OCMB is a consequence of a more active inflammatory response within the CNS. In this regard, the subset of RRMS patients with CSF OCMB were previously noted to exhibit higher CSF levels of TNFα as well as the chemokine CXCL13 during relapses.[28] TNFα is known to induce CXCL13 secretion by macrophages,[29] and CXCL13 in turn has been implicated in the recruitment of B cells into the CNS.[30,31] Of note, CXCL13 plays a particularly important role in trafficking and homing of $CD5^+$ B cells,[30] a subset of B cells previously shown to be enriched in the CSF of RRMS patients harboring OCMB, and which we find here significantly enriched also in the subset of PPMS patients exhibiting OCMB. It is interesting to speculate on the potential significance of this finding, given the very different functions previously ascribed to CD5-expressing B cells. Earlier work identified $CD5^+$ B cells as requiring less T-cell help for activation and expansion, and as a potential source of low-affinity "natural" antibodies, including autoantibodies.[32] More recent work has identified a small subset of interleukin 10–expressing $CD5^+$ B cells that have the potential to downregulate (or acquiesce) inflammatory responses including CNS autoimmunity.[33,34]

The observation that the presence of CSF-restricted OCMB is associated with Gd^+ brain lesions is consistent with the possibility that IgM-producing cells in the CSF of PPMS patients may be more dependent on renewal from the periphery than IgG-producing cells. Although this would be in keeping with prior observations in patients with RRMS showing that treatment with natalizumab (which effectively limits trafficking of immune cells, including B cells, into the CNS) resulted in more rapid and profound decreases in CSF IgM versus IgG measures when assessed 1 year following natalizumab initiation,[15] we also note more recent work assessing longer-term effects of natalizumab on CSF that demonstrated significant decreases in CSF IgG indices, including a small but significant proportion of patients in whom OCGB had essentially disappeared.[35] Whether OCMB are themselves pathogenic or represent mere markers of active CNS inflammation, our findings indicate that their presence in the CSF identifies a subset of PPMS patients with a more aggressive clinical course and a high degree of focal inflammatory disease activity of the type that characterizes relapsing MS disease activity. We propose that the presence of CSF-restricted OCMB should be further investigated as a potential biomarker of the subset of PPMS patients who, despite a primary progressive clinical course, may nonetheless benefit from immune-directed treatment. Future longitudinal studies assessing multiple CSF parameters (including OCMB, OCGB, IgG/IgM indices, and CSF IgG/IgM amounts) in well-characterized cohorts will further elucidate the relationship between intrathecal immunoglobulin parameters, CNS inflammation, and treatment responsiveness in MS.

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Potential Conflicts of Interest

L.M.V.: consultancy, Biogen Idec; grants/grants pending, Merck Serono, Biogen Idec, Teva, Genzyme; speaking fees, Merck Serono, Biogen Idec, Teva, Genzyme, Binding Site, Novartis, Bayer. B.C.: board membership, consultancy, speaking fees, Biogen, Novartis, Sanofi, Merck Serono, Bayer, Almirall, Genzyme, Teva. M.C.: consultancy, Bayer Schering Pharma, Biogen Idec, Merck Serono, Teva Pharmaceuticals; grants/grants pending, Bayer Schering Pharma, Merck Serono, Teva Pharmaceuticals, Novartis, Genzyme. G.I.: consultancy, Biogen Idec, Merck Serono, Sanofi, Teva, Novartis, Genzyme, Almirall. R.A.: grants/grants pending, Merck Serono, Biogen Idec, Novartis, Teva, Sanofi Aventis, Genzyme, Bayer. S.V.L.: consultancy, employment, Laboratory Diagnostics in MS. X.M.: speaking fees, consultancy, travel expenses, Almirall, Bayer, Biogen Idec, EMD, Merck, Genentech, Geneuro, Genzyme, Neurotec, Novartis, Roche, Sanofi-Genzyme, Teva. O.F.: board membership, consultancy, speaking fees, Bayer Schering, Biogen Idec, Merck Serono, Teva, Novartis, Sanofi; grants/grants pending, Bayer Schering, Biogen Idec, Merck Serono, Teva, Novartis, Sanofi, Actelion, Almirall. R.A.-L.: grants/grants pending, Merck Serono, Biogen Idec, Novartis. D.M.: employment, Genentech. A.S.: consultancy, Novartis, Teva; grants/grants pending, Scientific and Technological Research Council of Turkey; paid educational presentations, Serono Symposia International Foundation, Gen Pharmaceuticals of Turkey; travel expenses, Merck Serono, Teva-Aventis, Gen Pharmaceuticals of Turkey. E.E.: board membership, consultancy, grants/grants pending, Biogen Idec, Novartis, Sanofi-Aventis, Genzyme, Pharmstandart, R-Pharm, Pharmsyntez, Genfa Medica, Takeda, Generium. J.C.A.-C.: board membership, Biogen, Novartis, Sanofi, Merck Serono, Bayer, Roche, Genzyme, Teva; speaking fees, Biogen Idec, Merck, Bayer, Sanofi, Teva, Genzyme, Almirall, Merck Serono, Novartis.

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