IgM oligoclonal bands: biomarker of targetable inflammation in PPMS

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Running head: IgM oligoclonal bands as biomarker in PPMS

Character number in the title: 67

Character number in the running head: 42

Number of words in the abstract: 150

Number of words in the body of the manuscript: 3344

Number of figures: 4 (no colour figures)

Number of tables: 5

Key words: IgM bands, IgG bands, B cell depletion, PPMS

ABSTRACT

Objective: To identify a biomarker distinguishing patients who, in spite of a primary progressive MS (PPMS) clinical course, may nonetheless benefit from immune therapy.

Methods: Presence or absence of both IgG and IgM oligoclonal bands (OCB) were blindly examined in paired 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, multicentre validation cohort.

Interpretation: 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.

INTRODUCTION

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. Indeed, the two large placebo-controlled PPMS clinical trials to date (the PROMiSe study using oral glatiramer acetate (GA)[1] and the OLYMPUS study using the anti-CD20 B cell depleting antibody rituximab)[2] failed to meet their primary end-point 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 (Gd+)-enhancing brain lesions at baseline[1,2]. While 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, in spite of a primary progressive clinical course, may nonetheless benefit from immune therapy.

Here, we studied both IgG and IgM oligoclonal bands (OCB) in the CSF of a large cohort of patients with PPMS. Using both cross-sectional samples as well as 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 presence of IgM OCB, but not of IgG OCB, is associated with a more active inflammatory disease phenotype, and that their presence may be a biomarker for the

subset of PPMS patients who, in spite of a primary progressive clinical course, may benefit from immune-directed treatments.

2. MATERIALS AND METHODS

2.1. *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 (Table 1 for Clinical/demographic features), serially recruited at four 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 Hospital Valle de Hebrón Hospital, Barcelona; and 6 at Hospital Gregorio Marañón, Madrid. We recruited an additional 23 patients (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 (Table 1) were subsequently recruited at eight international major academic hospitals, including Virgen Macarena Hospital, Sevilla (n=16); Clínico Hospital, Madrid (13); Cerrahpaşa School of Medicine, Istanbul (12), Carlos Haya Hospital, Malaga (7); Clinico Hospital, Valencia (7); Hospital Ramon y Cajal Madrid (7); St. Petersburg's Center of MS and Autoimmune Diseases, Saint Petersburg (4); and Gregorio Marañon Hospital, Madrid (1). None of the patients in the three cohorts had received immunosuppressive treatments or disease modifying therapies prior to enrolment. No 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 1,000

mg intravenous infusions, administered 2 weeks apart, every 24 weeks) and three patients received placebo infusions while being followed and sampled prospectively. In 17 of these longitudinally followed patients, paired CSF and serum samples were available for analysis at more than one 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 gadolinium contrast-enhancing (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.

2.2. *CSF analyses.*

Paired serum and CSF samples from all patients were stored at −80°C in 0.2 ml aliquots and until assessments of quantitative Ig measures as well as IgG and IgM oligoclonal band 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 nephelometer. Oligoclonal bands were analysed by isoelectric focusing (IEF) and immunoblotting using identical volumes of CSF in each lane, as previously described[7,8]. When available, 4-6 ml of fresh CSF samples were analysed 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 100 μL of PBS and divided in three equal aliquots. These aliquots were then labelled 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 (FITC) from BD Biosciences; or anti–CD5-PE from Beckman Coulter. Cells were then washed twice with PBS, 0.02% sodium azide at 4°C and analyzed on a standard FACSCanto II instrument (Beckton Dickinson, USA). 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.

2.3. *Statistical analyses*

Results were analyzed with the Prism 5.0 statistical package (GraphPad Software). We used the Mann-Whitney *U* test or the Fisher exact test for comparisons between two groups. Kruskal-Wallis or Chi-square tests were used to compare three or more groups. $p < 0.05$ was considered for assessment of statistical significance.

3. RESULTS

3.1. Cross-sectional Cohort

Of 80 patients with PPMS in the cross-sectional study, IgG OCB (OCGB) restricted to the CSF were identified in 70 (87. 5%) (Table 3; referred to as 'OCGB+') while CSF-restricted OCMB were identified in 21 (26. 2%) (Table 4; referred to as 'OCMB+'). As expected, CSF of patients with detectable IgG OCB ('OCGB+') had significantly higher IgG indices than CSF of patients lacking IgG OCB ('OCGB-') (Table 3; $p<0.0001$). However, when stratified this way based on the presence or absence of IgG OCB, no differences were noted between groups in terms of the sex distribution, age at sampling, age at MS diagnosis, disease duration, EDSS or MSSS scores (Table 3). In contrast, when the same PPMS cohort was stratified based on presence or absence of

CSF IgM OCB (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 (Table 4; p=0.001), and were also found to have higher CSF IgG indices (p=0.007) compared to the 'OCMB-' patients.

3.2. 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 oligoclonal IgG bands (OCGB+), while 8 (20.5%) had CSF oligoclonal IgM bands (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, Figure 1A) as well as significantly higher absolute B cell counts (p=0.015, Figure 1B). These differences appeared particularly pronounced for the CD5+ B cell subset (p=0.0009 for proportions and p=0.005 for absolute counts; Figure 1A and 1B). 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 presence or absence of CSF oligoclonal IgM bands (but not IgG), identifies a subset of patients with evidence of a more aggressive prior clinical course, who also exhibit a distinct CSF B-cell profile.

3.3. Baseline features of the OLYMPUS PPMS cohort

To assess for the potential association between 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 gadolinium enhancing (Gd+) brain MRI lesions at baseline (pre-treatment). We hypothesized that presence of CSF oligoclonal IgM bands 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 presence of CSF OCMB and 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 (Figure 2A; $p = 0.002$). The average number of Gd+ lesions at baseline was strikingly higher in OCMB+ versus OCMB- patients (Figure 2B; $p=0.0005$). In contrast, CSF OCGB were present in all patients (as expected, since entry into the OLYMPUS study required prior documentation of CSF-restricted oligoclonal IgG bands and/or elevated IgG-index/synthesis rate) and therefore their presence or absence at baseline did not correlate with presence of Gd+ MRI lesions. Stratification of patients based on different levels of CSF IgG synthesis (arbitrary IgG index cut-of values of 0.7, 0.9 or 1.0) also did not reveal an association between CSF IgG parameters and presence of Gd+ lesions (data not shown). Hence, prior to treatment, 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 (Figure 3).

3.4. Validation cohort

In order 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 eight major international teaching hospitals, for whom both paired CSF/serum samples were available as well as brain MRI data (both pre- and post-gadolinium infused scans) obtained close to the time of lumbar puncture (Table 1). Among these patients, 65 of 67 (97%) exhibited CSF OCGB while 33 patients exhibited OCMB. While there was no relationship between presence of OCGB (seen in almost all the patients) and Gd+ brain MRI lesions, we confirmed that the presence of OCMB clearly associated with higher number of Gd+ brain lesions ($p=0.0005$; Figure 4) in this additional PPMS population. Specifically, 13 out of the 33 IgM+ patients (39.4%) had Gd+ enhancing lesions while only two of the 34 IgM- patients (5.9%) showed these lesions (Figure 4A). The average number of Gd+ enhancing lesions was 1.09±0.35 for $OCMB^+$ patients versus 0.17±0.14 for the $OCMB^-$ patients (Figure 4B).

3.5. Longitudinal features of the OLYMPUS PPMS cohort

Since the Olympus study results[2] suggested that 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 presence of CSF OCMB in our longitudinal cohort. Though our number of OCMB+ patients is relatively small (n=5; Table 2), we noted that the two OCMB+ patients subsequently treated with placebo, continued to have additional Gd+ lesions on their follow-up brain MRIs, while the three 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's t-test).

DISSCUSION

Approximately 10-15% of all patients with MS suffer a primary progressive MS (PPMS) course, with unremitting progression of disability from onset[9]. In 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 or not PPMS is indeed 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 two 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 presence of active focal inflammatory disease, as typically observed in patients with relapsing remitting MS (RRMS), may represent a viable target for immune-therapy. Identifying a biological marker that may 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 oligoclonal bands (OCB) that have been extensively studied in patients with RRMS, might be associated with distinct PPMS phenotypes. In RRMS, both IgG and IgM OCB have been considered as potential

biomarkers of disease activity and treatment response[12-15]. IgG OCB 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, IgM OCB, described in approximately 40% of RRMS patients, appear to correlate better with a number of parameters of inflammatory disease activity. Presence of CSF IgM OCB 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, presence of CSF IgM OCB has been associated with a more substantial T2 lesion load, a greater number of new gadolinium-enhancing 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 harbour CSF-restricted IgG OCB over time[18] while only a subgroup of these patients exhibit CSF-restricted IgM OCB[25,26]. However, the relationship between intrathecal IgG and IgM OCB, 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 presence or absence of CSF IgM OCB (but not of CSF IgG OCB), 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 CNS inflammation. Presence of gadolinium-enhancing brain lesions was almost exclusively seen in the subset (approximately 20-25%) of

PPMS patients harbouring CSF IgM OCB, a finding we subsequently confirmed in an independent multi-center 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 required documentation of abnormal CSF IgG measures and indeed all patients in this cohort exhibited CSF-restricted IgG OCB, indicating that presence or absence of IgG OCB could not be a marker of presence or absence of gadolinium-enhancing lesions. Essentially all gadolinium-enhancing lesions were seen in the 22% of PPMS patients who prior to treatment exhibited CSF IgM OCB. Within this subset of patients with IgM OCB at baseline, placebo-treated patients continued to experience new gadolinium-enhancing brain lesions and accumulation of new T2 lesions over time. In contrast, the small number of rituximab-treated patients who had baseline CSF IgM OCB, experienced essentially no new brain MRI activity in follow-up. Our main observation points to the measurement of CSF IgM OCB as a potential biomarker of the subset of PPMS patients with more active focal inflammatory disease activity. Together, our findings raise the possibility that presence of CSF IgM OCB 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 presence of IgM OCB 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 IgM OCB may directly contribute to CNS injury. Another possibility is that the development of CSF-restricted IgM OCB is a consequence of a more active inflammatory response within the CNS. In this regard, the subset of RRMS patients with CSF IgM OCB were previously noted to exhibit higher CSF levels of TNF-alpha as well as the chemokine CXCL13, during relapses[28]. TNF-alpha 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 harbouring IgM OCB, and which we find here significantly enriched also in the subset of PPMS patients exhibiting IgM OCB. 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 IL-10 expressing CD5+ B Cells that have the potential to downregulate (or acquiesce) inflammatory responses including CNS autoimmunity[33,34].

The observation that presence of CSF restricted IgM OCB is associated with gadolinium-enhancing 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. While 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 vs IgG measures when assessed one year following natalizumab initiation[15], we also note more recent work assessing longer-term effects of natalizumab on CSF which demonstrated significant decreases in CSF IgG indices, including a small but significant proportion of patients in whom IgG OCB had essentially disappeared[35]. Whether or not IgM OCB 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 presence of CSF-restricted IgM OCB should be further investigated as a potential biomarker of the subset of PPMS patients who, in spite of a primary progressive clinical course, may nonetheless benefit from immune-directed treatment. Future longitudinal studies assessing multiple CSF parameters (including OCMB, OCGB, IgG/M-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.

ACKNOWLEDGEMENTS

This work was supported by grants SAF/ 2012-34670 FIS/PI12/00239 and RD12/0032/0005 from the Spanish Ministry of Economy and Competitiveness (LV) and the Canadian Institutes of Health Research and the Research Foundation of the Multiple Sclerosis Society of Canada (ABO). Funders did not have any role in study design; collection, analysis, and interpretation of data; writing the report; and the decision to submit the report for publication.

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FIGURE LEGENDS

Figure 1. Proportions and absolute counts of CSF B cells in patients with PPMS, stratified based on presence or absence of CSF-restricted IgM OCB. 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 presence or absence of CSF oligoclonal IgG bands.

Figure 2. Gadolinium-enhancing (Gd+) brain lesions are present almost exclusively in the subset of OLYMPUS PPMS patients with CSF IgM OCB. We studied the association of IgM status and the presence of Gd+ enhancing lesions in the OLYMPUS cohort (n=23). (A) Baseline Gd+ lesions were present in a significantly higher proportion (80%) of patients with CSF IgM OCB (M+), compared to only a small minority (5%) of patients lacking CSF IgM OCB (M-). (B) The average number of Gd-enhancing lesions at baseline was significantly higher in patients with CSF IgM OCB (M+) as compared to patients lacking CSF IgM OCB (M-). In contrast, CSF IgG OCB were present in all patients, and hence their presence did not correlate with Gd+ lesions.

Figure 3. MS severity scale scores (MSSS) in cohorts of PPMS patients stratified based on presence or absence of CSF IgM OCB. Patients with oligoclonal IgM bands (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 presence or absence of CSF oligoclonal IgG bands.

Figure 4. Gadolinium-enhancing (Gd+) lesions are present almost exclusively in the Validation cohort PPMS patients harbouring CSF IgM OCB. We studied the association of IgM status and the presence of Gd+ enhancing lesions in the Validation cohort (n=67). (A) The majority of Gd+ brain lesions were noted in patients with CSF IgM OCB (M+). Gd+ lesions were present in a significantly higher proportion (39.4%) of patients with CSF IgM OCB (M+), compared to only (5.9%) of patients lacking CSF IgM OCB (M-). (B) The average number of Gd-enhancing lesions was significantly higher in patients with CSF IgM OCB (M+) as compared to patients lacking CSF IgM OCB (M-).

Table 1. Clinical and demographic data of Cross-sectional, Longitudinal and Validation Cohorts

	Cross-sectional Cohort n=80	Longitudinal Cohort n=23	p	*Cross Sectional + Longitudinal Cohorts n=103	Validation Cohort n=67	**p
Age (Years, 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 (Years, 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 (Years, 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.

** p-value compares the Validation Cohort with the total number of patients in the initial study.

SE: Standard error.

Table 2. Samples available from patients participating in the longitudinal Olympus trial of rituximab in PPMS.

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

22	Pos	Pos	Rituximab	Baseline
23	Pos	Pos	Rituximab	23W

OCMB: Oligoclonal IgM band status OCGB: OligoclonalIgG band status; Neg: Negative; Pos: Positive; W: Weeks.

Table 3. Clinical, demographic and laboratory features of cross-sectional PPMS patient cohort, classified according to presence or absence of CSF IgG OCB (OCGB).

	Patients with IgG OCB (OCGB+) n=70	Patients lacking IgG OCB (OCGB-) n=10	p
Age (Years, Mean ± SE)	48.88±3.45	51.40±1.32	0.40
Age at diagnosis (Years, Mean ± SE)	41.31±1.23	44.78±3.52	0.27
Disease duration (Years, 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

OCGB: Oligoclonal IgG bands; SE: Standard error.

Table 4. Clinical, demographic and laboratory features of cross-sectional PPMS patient cohort, classified according to presence or absence of CSF IgM OCB (OCMB).

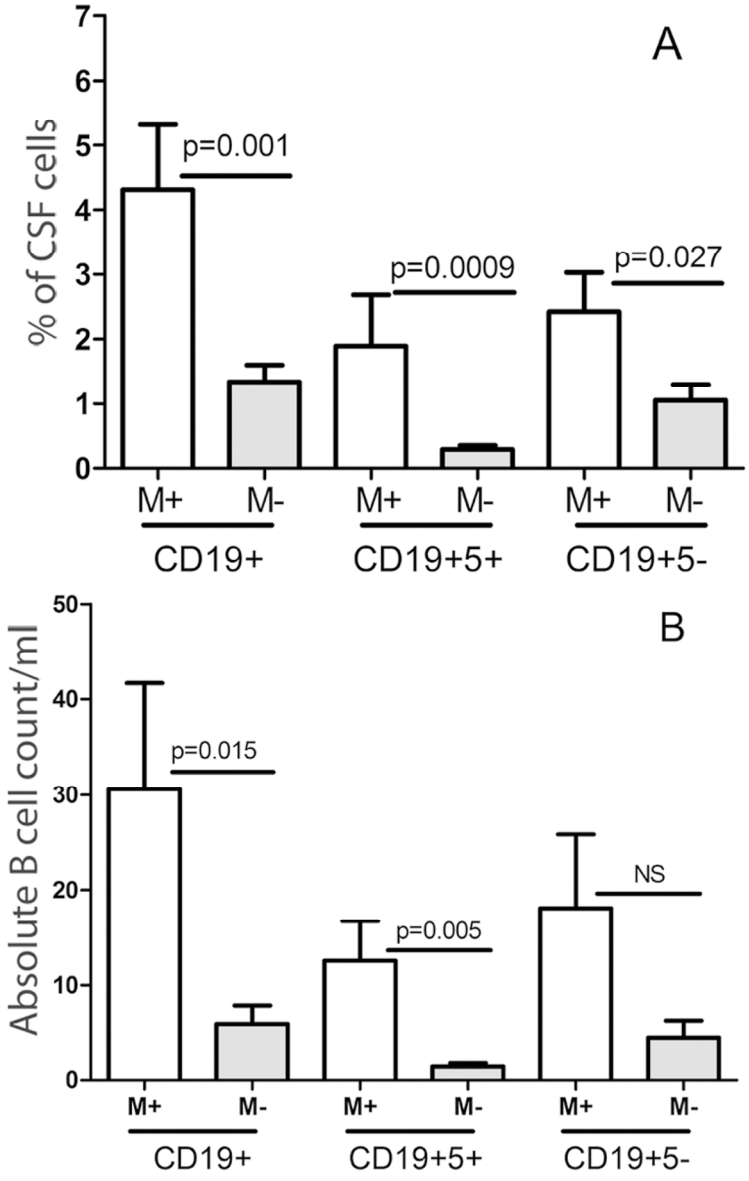
	Patients with IgM OCB (OCMB+) n=21	Patients lacking IgM OCB (OCMB-) n=59	p
Age (Years, Mean ± SE)	45.32±2.89	50.48±1.3	0.12
Age at diagnosis (Years, Mean ± SE)	39.77±2.44	42.41±1.32	0.31
Disease duration (Years, Mean ± SE)	5.17±1.09	7.84±0.89	0.063
Sex (Male/Female)	13/8	25/34	0.14
EDSS score (Mean ± 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

OCB: Oligoclonal bands; OCMB: Oligoclonal IgM bands; SE: Standard error

Table 5. Clinical, demographic and laboratory features of validation cohort patients classified according to presence or absence of CSF IgM OCB (OCMB).

	Patients with IgM OCB (OCMB+) n=33	Patients lacking IgM OCB (OCMB-) n=34	p
Age (Years, Mean ± SE)	45.08±1.37	49.20±1.51	0.09
Age at diagnosis (Years, Mean ± SE)	41.09±1.40	42.81±1.70	0.61
Disease duration (Years, 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

OCB: Oligoclonal bands; OCMB: Oligoclonal IgM bands; SE: Standard error



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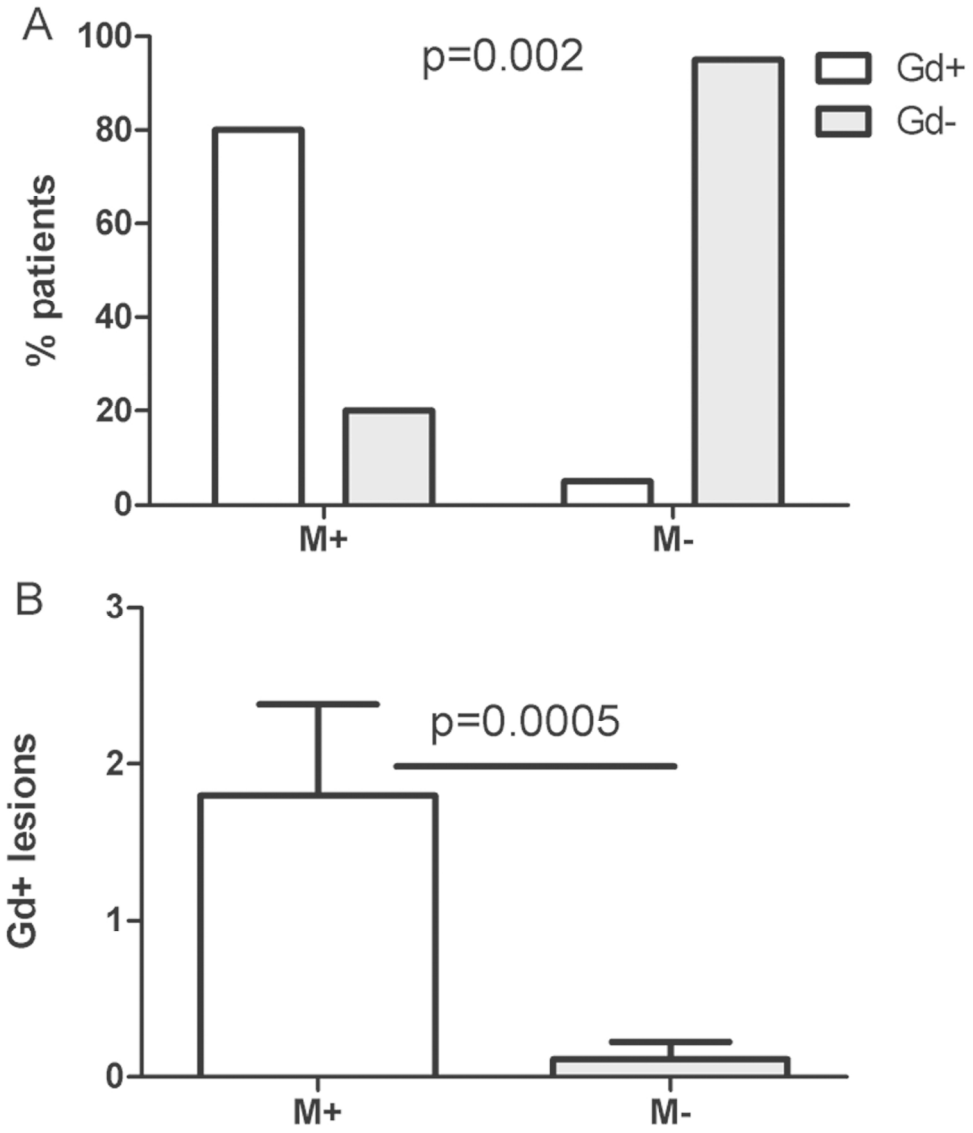

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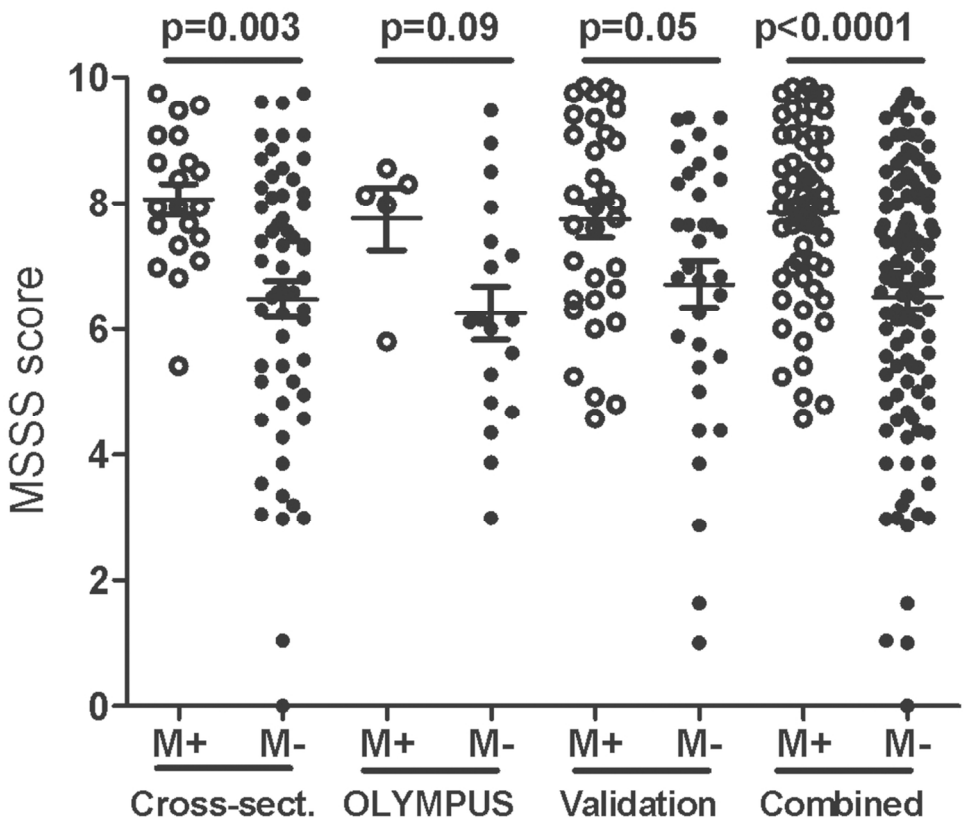

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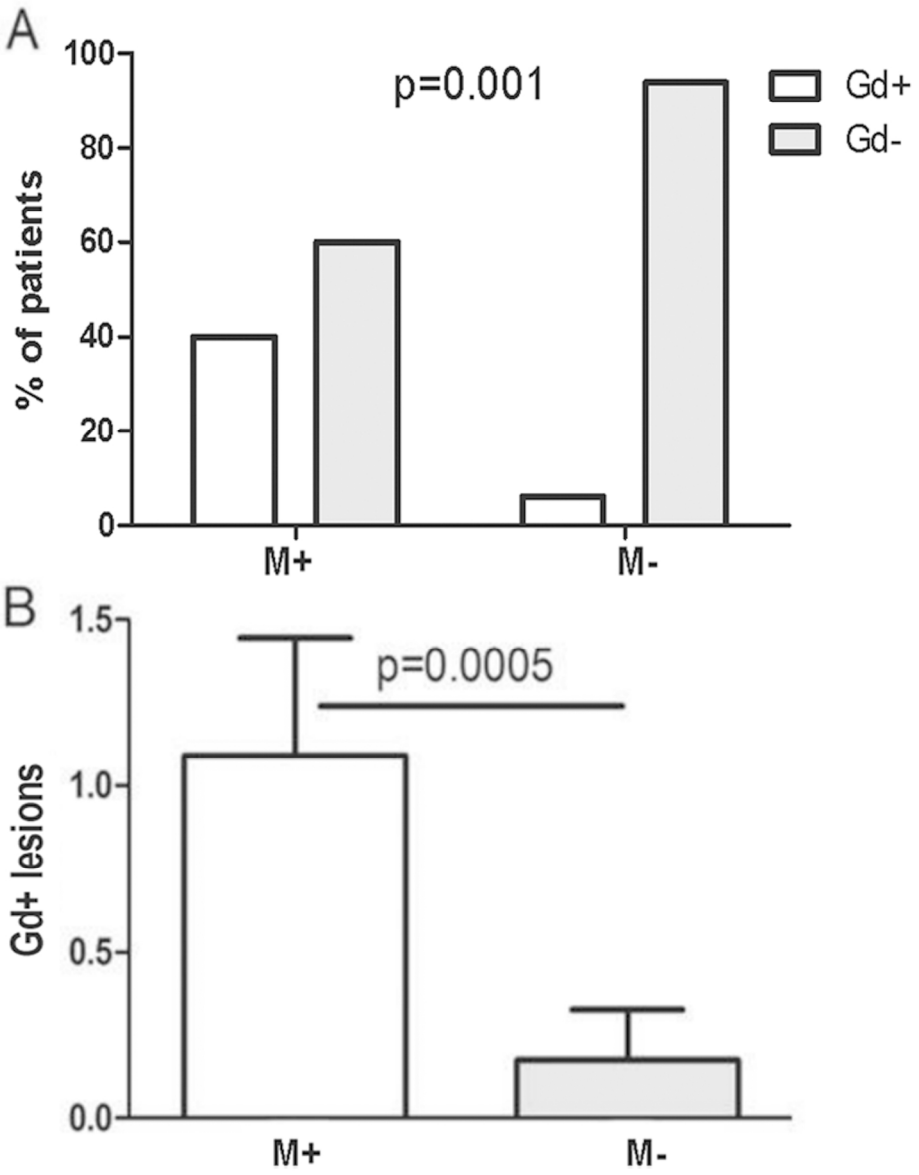

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