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Mult Scler 2007 13: 1086 originally published online 27 April 2007
DOI: 10.1177/1352458507077622

ARTICLE *Multiple Sclerosis* 2007; **13**: 1086–1094

Anti-myelin antibodies predict the clinical outcome after a first episode suggestive of MS

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The aim of this study was to test the contribution of anti-myelin antibodies in predicting conversion from clinically isolated syndrome (CIS) to multiple sclerosis (MS) when considering either Poser's or McDonald's diagnostic criteria. Fifty-one patients with CIS and abnormal brain MRI were imaged monthly for six months and then at 12, 18, 24, 36 months. At baseline serum samples testing antibodies against myelin oligodendrocyte glycoprotein (anti-MOG) and myelin basic protein (anti-MBP) were collected. During the 36-month follow-up, 26 (51%) patients developed a relapse thus becoming clinically definite MS (CDMS) according to Poser's criteria; 46 (90.2%) patients converted to MS according to McDonald's criteria. Out of 51 patients, 28 (54.9%) had either double or single positivity for anti-myelin antibodies. Antibody status significantly predicted MS according to Poser's criteria ($P = 0.004$), but did not according to the McDonald's criteria. When compared to antibody negative patients, the risk of developing a relapse was 8.9 (95% CI: 2.7–29.8; $P < 0.001$) for anti-MBP positive (anti-MBP+) patients and 1.5 (95% CI: 0.4–5.4; $P = 0.564$) for those anti-MOG positive (anti-MOG+); double positive patients (ie, anti-MBP+/anti-MOG+) had a risk of relapse's occurrence equal to 3.4 (95% CI: 1.1–10.2; $P = 0.031$). Also, the antibody status predicted the median time span from CIS to CDMS, that was of 36 months in the anti-MOG-/anti-MBP- group, 33 months in the anti-MOG+/anti-MBP- group, 24 months in the anti-MOG+/anti-MBP+ group and 12 months in the anti-MOG-/anti-MBP+ patients ($P = 0.003$ by ANOVA). Our data support the prognostic value of anti-myelin antibodies in CIS patients at risk of CDMS, with positive patients showing shorter time interval to relapse occurrence than negative patients. *Multiple Sclerosis* 2007; **13**: 1086–1094. http://msj.sagepub.com

Key words: clinically isolated syndrome; MBP antibodies; MOG antibodies; MRI; multiple sclerosis; relapse

Introduction

Ninety percent of patients with multiple sclerosis (MS) initially presents with a clinically isolated syndrome (CIS) due to inflammatory demyelinating lesions in the optic nerve, brainstem, or spinal cord. Conversion to clinically definite MS (CDMS) [1] occurs in approximately 70% of these patients within 14 years from the first episode [2]. In the short-term, clinical conversion ranges from 38% to 45% [3,4], whereas subclinical MRI activity is detectable in more than 90% of patients [3,5]. Identifying early those predictive factors, which could contribute to clinical conversion, could be useful to anticipate the MS diagnosis and treatment [3,4].

Pathogenetic mechanisms responsible for conversion to CDMS are only partially known. They include ongoing inflammation with axonal loss [6] and subsequent cumulative disability, as well as process of epitope spreading [7,8], which may amplify inflammatory demyelination in the central nervous system (CNS). B-cell as well as T-cell responses have been widely implicated in the MS pathogenesis through an antibody-mediated demyelination

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Received 15 September 2006; accepted 30 January 2007

10.1177/1352458507077622

[9–11]. Proteins of the myelin sheath such as myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) have been identified as targets of the immune response [12–14]. It has been hypothesized that antibodies against MOG (anti-MOG) and MBP (anti-MBP) in MS might be contributing factors to disease clinical conversion and progression. Berger *et al*. reported that patients with CIS, abnormal MRI scan and positive oligoclonal bands in cerebrospinal fluid (CSF), who also were anti-MOG and anti-MBP seropositive (anti-MOG+/anti-MBP+), had further clinical episodes more frequently and earlier than those who were seronegative [15]. Therefore, the Authors suggested that the anti-myelin antibody status could provide early prediction of conversion to CDMS. By contrast, results from a different cohort of CIS patients [16] showed that the presence of serum anti-myelin antibodies did not predict conversion to MS in the short-term, either clinically or subclinically. The present study was aimed to evaluate the role of anti-myelin antibodies in predicting the long-term clinical outcome after a first demyelinating event suggestive of MS. In order to address this issue we tested the ability of anti-myelin antibodies in predicting the risk of MS according to either McDonald's [17] or Poser's criteria. We also evaluated whether anti-myelin antibodies could provide information about timing of relapse occurrence, thus being of prognostic value early during the diagnostic process.

Methods

Study design

This study was designed as a longitudinal, prospective evaluation of the natural history of CIS suggestive of MS [18]. In August 1998 we started the recruitment of patients who had suffered from either mono or multifocal CNS involvement due to a first acute neurological episode suggestive of MS in the 12 months prior to study entry. The study was approved by the local ethical committee of the University of Rome "La Sapienza" and all patients signed their written informed consent to the protocol. A baseline brain MRI scan supporting a possible diagnosis of MS according to Fazekas' criteria (ie, at least three lesions on the T2-weighted images) [19] was required as eligibility criterion. In order to reduce the likelihood of age-related non-specific abnormalities at MRI scan, only patients aged between 18 and 50 years were recruited. No steroid treatment in the two months prior to the study entry was allowed.

At the study entry patients underwent physical and neurological examinations, including blood samples, serum sample for antibody analysis, rating of disability by the Expanded Disability Status Score (EDSS) [20]; additional tests were performed where indicated in order to rule out alternative conditions mimicking MS. Neurological examinations were performed every three months and in case of relapse. A clinical relapse was defined as the appearance of new symptoms or worsening of previous symptoms/signs lasting at least 24 hours, associated with detectable changes in neurological examination performed by a neurologist blinded to the MRI and serological results. For the individual patient the study ended up when the first relapse occurred and the diagnosis of MS according to Poser's criteria was made. For the group of patients early discontinuing the study, the last MRI included in the analysis was the scheduled one before the clinical relapse occurred.

MRI protocol and analysis

The brain MRI scans were performed using a 1.5-T magnet (Philips Gyroscan NT 1.5). Transaxial proton density and T2 conventional spin-echo (CSE) images (PD and T2WI; TR = 2000 ms; TE = 20/90 ms), fast fluid-attenuated inversion-recovery (f-FLAIR) (TR = 6000 ms; TE = 150 ms) and T1-weighted CSE (T1WI; TR = 550 ms; TE = 12 ms) were acquired. Gadolinium (Gd)-enhanced T1WI scans were obtained on the transaxial plane, between five and 10 minutes after injection of 0.5 mmol/kg of gadolinium diethylenetriamine penta-acetic acid (Gd-DTPA). All transaxial scans were collected with 5-mm contiguous slices, using a 24 cm field of view and 256×256 matrix.

Patients were imaged monthly for six consecutive months and then at months 12, 18, 24, 36. For each patient all the available MRI scans were blindly examined on the hardcopy by two experienced neuroimaging readers (IP, PP) who marked and outlined on an overlaid transparency each and every detectable lesion on the post-contrast T1WI and PD sequences. For each scan, the number of Gd-enhancing and T2-hyperintense lesions was calculated. Active Scans were considered those without at least one enhancing lesion. Dissemination in space was defined according to McDonald's Criteria [17], with a threshold of at least three positive Barkhof criteria [21] and the possibility to replace a gadolinuim enhanced lesion with 9 or more T2-hyperintense lesions. Dissemination in time was defined according to the McDonald's criteria [17] (ie, either the presence of at least one Gd-enhancing lesion at month 3 or one T2-hyerintense lesion at month 6; for further scans, the presence of either one Gd-enhancing lesion or one T2-hyerintense lesion).

Anti-myelin antibody analysis

Serum samples for antibody analysis were collected at baseline by researchers unaware of the patient's clinical status and MRI findings. Blood samples were centrifuged and stored at −80°C for one month maximum. Afterwards, samples were shipped to the Neuroimmunological Research Unit (Clinical Department of Neurology, Innsbruck Medical University, Innsbruck, Austria) for analysis.

Human recombinant MOG immunoglobulin (ie, extracellular immunoglobulin-like domain amino acids 1-125, MOG-Ig) and human myelin-derived MBP (ie, purified from human brain) IgM antibodies were analysed by Western blotting, as previously described [12,13] with minor modifications [15]. In brief, either 1 µg of recombinant MOG-Ig or 2 µg of MBP was loaded in each lane and separated in 10% Bis-Tris (NuPAGE) sodium dodecyl sulfate (SDS)-polyacrylamide gels (Novex). Separated proteins were electrotransferred to nitrocellulose membranes (Hybond-C, Amersham). The efficiency of transfer was monitored by the use of a prestained, low-range SDS-polyacrylamide gel electrophoresis standard (Bio-Rad) and by staining of the filters with Ponceau S (Sigma) after transfer. The blots were blocked with 2 percent milk powder in phosphate-buffered saline containing 0.05% Tween 20. The blots were then dried, cut into 2-mm nitrocellulose strips with a membrane cutter (Novex) and probed overnight at 4°C with diluted human serum (dilution, 1 : 500 in 2% milk powder in phosphate-buffered saline containing 0.05% Tween 20). The strips were then washed three times with phosphate buffered saline containing 0.05% Tween 20 (PBS-T) and incubated with alkaline phosphatase-conjugated anti-human IgM (dilution, 1 : 5000; JGH055043, Jackson) for one hour at room temperature. After washing, bound antibodies were detected by nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate (both, Roche Molecular Diagnostics). The strips were then washed with distilled water and dried, and two independent investigators assessed the immunoreactivity of the serum samples. A serum sample was considered to be *positive* if the immunoreactivity was equal to or greater than that of a control sample. As controls, monoclonal antibodies to MBP (MAB381, Chemicon) and MOG (8.18-C5) [22] and positive and negative human serum samples were used.

IgG antibodies to human recombinant MOG-Ig and human myelin–derived MBP were analysed by ELISA, as previously described [23]. Briefly, ELISA was performed using 96-well microtitre plates (Nunc-Immuno Maxisorp, Nunc) coated with 100 µL of either 5 mg/mL MOG-Ig or MBP solution in PBS overnight at 4°C. Plates were then blocked with 10% fetal calf serum (FCS; Invitrogen) in PBS (PBS-FCS) for one hour at room temperature and washed four times with PBS-T. Then 100 µL of the serum samples diluted 1 : 100 in PBS-FCS were added and incubated for 1 hour at room temperature with gentle shaking. Plates were washed and 100 µL of peroxidase-conjugated goat anti-human IgG (1 : 10000) secondary antibody (Dako) in PBS-FCS were added for one hour at room temperature with gentle shaking. Plates were washed, and specific antibody binding was visualized by the addition of 100 µL of tetramethylbenzidine (TMB) liquid substrate (Sigma). After 20 minutes the reaction was stopped with 50 µL 1 M H_2SO_4 and the plates were read at 492 nm. Controls wells were incubated only with secondary antibodies and all data were corrected by subtraction of these background values. All samples were analysed in duplicate and three human control sera were used to check the inter-assay variation on all ELISA plates. The inter- and intra-assay variations were $<15\%$. From 188 age and sex-matched healthy controls from Innsbruck, Austria, we determined a 'cut-off' value for anti-MOG IgG (0.6 OD units) and anti-MBP-IgG (0.4 OD units). The specificity of both assays (188 healthy controls versus 202 patiens with CDMS) was 90%, the sensitivity was 35% for MOG IgG and 21% for MBP IgG.

Statistical analysis

Continuous variables were presented using either mean ± standard deviation (SD) or median and interquartile range. When the variables were summarized by median and interquartile range, the Kruskal–Wallis rank test was used to perform multiple comparisons between groups (ie, according to the antibody status), and the Mann–Whitney test was used in the case of comparison between two independent groups. When the variables were summarized by mean ± SD, the one-way ANOVA was used to perform multiple comparisons between groups, and the *t*-test was used for comparing between two independent groups.

For comparing between groups of categorical variables Pearson's chi-square test was used. In order to assess the predictive value of different antibody patterns on the occurrence of relapse, after adjustment for potential confounding variables (ie, age, sex, disease duration, baseline EDSS score, baseline number of T1-hypointense and T2-hyperintense lesions, presence/absence of Gd-enhanced lesions on the baseline MRI scan), a Cox proportional-hazards model was used, after testing for the proportional hazard assumption. All statistical analyses were performed using SPSS-Windows version 10.0 (SPSS Inc., Chicago, USA).

Results

Baseline characteristics

Out of 60 CIS patients consecutively recruited in the study, 51 patients (31 women, 20 men) had blood samples available for testing anti-myelin antibodies. The mean time-span between the first clinical event and the study entry was 5.3 months. Table 1 summarizes demographic, clinical and MRI characteristics of this cohort of patients.

The CSF analysis was needed for diagnostic purpose only in 19 out of 51 patients (37.25%): 14 were positive and five were negative for oligoclonal bands (OBs).

Clinical and MRI follow-up findings

During the 36-month follow-up, 26 (51%) patients developed a relapse thus becoming CDMS; mean interval to relapse occurrence was 14.3 ± 9.9 months (range 1–33). Patients converting to MS according to McDonald's criteria were 46 (90.2%); mean time to diagnosis according to McDonald's criteria was 7.5 ± 7.3months (range 3–36).

Figure 1 shows the cumulative number of patients who converted to MS according to either McDonald's or Poser's criteria at different time points (ie, 3, 6, 12, 18, 36 months). Baseline demographic, clinical and MRI characteristics did not differ between patients satisfying either set of diagnostic criteria (data not shown).

Table 1 Baseline demographic, clinical and MRI characteristics of CIS patients ($n = 51$)

Variables	
Women (%)	31 (60.8)
Mean years of age (SD)	30.2 (7.0)
Mean number of months from the first episode (SD)	5.3 (3.4)
Types of presentation [no. patients (%)]	
Optic neuritis	17 (33.3)
Brainstem-cerebellar syndrome	10 (19.6)
Myelitis	11 (21.6)
Multifocal	13 (25.5)
Brain MRI scan	
*No. T2-hyperintense lesions**	17 (12–38)
*No. T1-hypointense lesions**	4 (3–10)
*No. Gd-enhancing lesions**	0 (0–2)
No. patients with Gd-enhancing lesions (%)	21 (41.2)
*No. patients with DIS** (%)*	45 (88.2)
Clinical disability [no. patients (%)]	
EDSS = 0	8 (15.7)
EDSS = 1	17 (33.3)
EDSS = 1.5	14 (27.5)
EDSS ⩾ 2.0	12 (23.5)
Mean EDSS (range)	1.3 (0–3.5)

*Median value and interquartile range.
**Dissemination in space according to Barkhof's criteria.

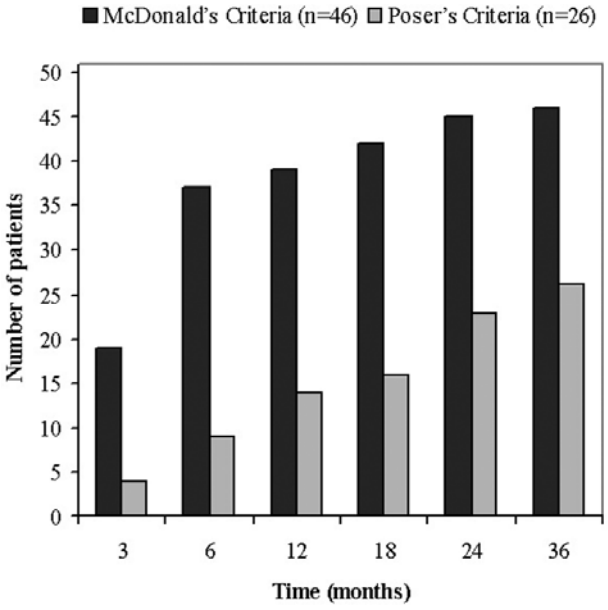

Figure 1 Cumulative number of patients converting to MS according to either McDonald's or Poser's criteria during the study period.

The only significant predictor of fulfilling McDonald's criteria was the presence of a baseline active scan (HR = 3.1, 95% CI: 1.4–7.0; $P = 0.005$).

Patients with a McDonald diagnosis of MS at month 3 developed a relapse during the follow-up more often than patients McDonald negative (79% versus 34.4%, $P = 0.002$). The risk of developing CDMS was significantly higher in those patients who were McDonald positive at month 3 (OR = 7.2, 95% CI: 1.8–27.8; $P < 0.001$). Furthermore, McDonald positive patients at month 3 developed a diagnosis of MS according to Poser's criteria in a shorter time interval than McDonald negative patients (seven versus 36 months; $P = 0.0002$ by Mann–Whitney *U*-test). Figure 2 shows the difference in the cumulative probability of remaining relapse-free in McDonald positive *versus* McDonald negative patients at month 3 assessed by the Log-Rank test ($P = 0.00038$).

IgM antibody status and development of MS

Twenty-three out of 51 patients (45.1%) were seronegative to anti-MOG and anti-MBP IgM antibodies, whereas 28 (54.9%) patients showed a positive test: 13 (25.5%) patients were seropositive to both anti-MOG and anti-MBP IgM antibodies, 17 (13.7%) were seropositive only to anti-MBP and

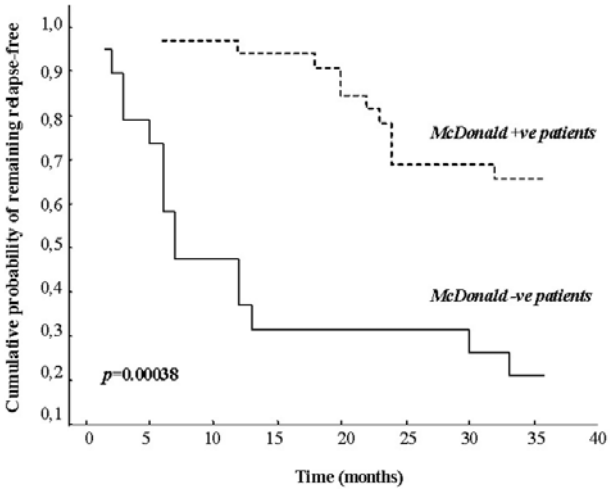

Figure 2 Cumulative probability of remaining relapse-free during the study period in patients *with* (*McDonald+ve*) and in patients *without* (*McDonald-ve*) a diagnosis of MS according to McDonald's criteria at month 3 by the Log-Rank test ($P = 0.00038$).

eight (15.7%) were seropositive to anti-MOG IgM antibodies.

Baseline demographic and clinical features of the patients according to the antibody status are reported in Table 2. The anti-MBP+ IgM group had a significantly higher proportion of women. Anti-MOG+ IgM patients were significantly younger and had shorter disease duration than anti-MOG− patients. Comparisons of the baseline characteristics did not show any other significant between-group difference.

Table 3 reports the number of patients developing MS on the basis of their baseline antibody status (IgM). There was no significant between-group difference in the proportion of patients developing a McDonald diagnosis of MS ($P = 0.496$). By contrast, there was a significant difference in fulfilling the Poser's criteria of MS between the groups of patients seropositive for at least one anti-myelin antibody type (ie, patients with either double or single positivity to anti-MOG and anti-MBP) and seronegative patients ($P = 0.027$).

Cox regression analyses showed that antibody status did not predict the conversion to MS according to the McDonald's criteria ($P = 0.207$), as expected on the basis of univariate analysis. By contrast, the antibody status significantly predicted a diagnosis of CDMS ($P = 0.004$).

Figure 3 shows the cumulative risk for a Poser-based diagnosis of MS according to the antibody status. When compared to seronegative patients, the risk of developing a relapse was 8.9 (95% CI: 2.7–29.8; $P < 0.001$) for anti-MBP+ patients and 1.5 (95% CI: 0.4–5.4; $P = 0.564$) for anti-MOG+ patients; anti-MBP+/anti-MOG+ patients had a risk of relapse occurrence equal to 3.4 (95% CI: 1.1–10.2; $P = 0.031$). Also, antibody status was able to predict the timing of relapse occurrence with a median time to relapse equal to 36 months in the anti-MOG−/anti-MBP− group, 33 months in the anti-MOG+/anti-MBP− group, 24 months in the anti-MOG+/anti-MBP+ group and

Table 2 Baseline demographic, clinical and MRI characteristics of CIS patients according to the antibody status

Variables	Anti-MOG− anti-MBP− $n = 23$ (45.1%)	Anti-MOG+ anti-MBP+ $n = 13$ (25.5%)	Anti-MOG+ anti-MBP− $n = 8$ (15.7%)	Anti-MOG− anti-MBP+ $n = 7$ (13.7%)	*P*-value
Women, no. (%)	11 (47.8)	11 (84.6)	3 (37.5)	6 (85.7)	0.039
Years of age*	32 (25–35)	27 (25–33)	26 (22.5–28)	32 (28–42)	0.044
Months from the first episode*	4 (2–5)	6 (5–11)	3 (1–5)	5 (3–10)	0.011
Types of presentation (%)					
Optic neuritis	8 (34.8)	4 (30.8)	4 (50.0)	1 (14.3)	
Brainstem-cerebellar syndrome	4 (17.4)	3 (23.1)	2 (25.0)	1 (14.3)	
Myelitis	3 (13.0)	3 (23.1)	1 (12.5)	4 (57.1)	
Multifocal	8 (34.7)	3 (23.1)	1 (12.5)	1 (14.3)	0.464
Brain MRI at baseline					
*No. T2-hyperintense lesions**	15 (9–23)	23 (12–51)	16.5 (11.5–38.5)	29 (21–82)	0.069
*No. T1-hypointense lesions**	4 (2–9)	9 (3–12)	3 (2.5–7.5)	4 (4–9)	0.486
*No. Gd-enhancing lesions**	0 (0–2)	0 (0-0)	0 (0–1.5)	1 (0–5)	0.281
No. patients with active scan	10 (43.5)	3 (23.1)	3 (37.5)	5 (71.4)	0.212
*No. patients with DIS***	19 (82.6)	12 (92.3)	7 (87.5)	7 (100.0)	0.605
Clinical disability [no. patients (%)]					
EDSS = 0	3 (13.0)	3 (23.1)	1 (12.5)	1 (14.3)	
EDSS = 1	8 (34.8)	3 (23.1)	3 (37.5)	3 (42.9)	
EDSS = 1.5	8 (34.8)	2 (15.4)	3 (37.5)	1 (14.3)	
EDSS ⩾ 2	4 (17.4)	5 (38.5)	1 (12.5)	2 (28.5)	0.864
*EDSS score**	1.5 (1–1.5)	1.5 (1–2)	1.25 (1–1.5)	1 (1–2.5)	0.983

*Median value and interquartile range.
**Dissemination in space according to Barkhof's criteria.

Table 3 Conversion to MS according to the baseline antibody status

Conversion to MS	Anti-MOG+ anti-MBP+ n = 13	Anti-MOG+ anti-MBP− *n* = 8	Anti-MOG− anti-MBP+ *n* = 7	Anti−MOG− anti-MBP− *n* = 23	*P-value*
McDonald's criteria *n* = 46 (%)	13 (100)	6 (75)	7 (100)	20 (87)	0.496
Poser's criteria *n* = 26 (%)	7 (54)	4 (50)	7 (100)	8 (35)	0.027

12 months in anti-MOG−/anti-MBP+ patients ($P = 0.003$ by ANOVA).

IgG antibody status and development of MS

Thirty-nine out of 51 patients (76.5%) were seronegative to anti-MOG and anti-MBP IgG antibodies, whereas 12 (23.5%) patients showed a positive test: one (1.9%) patient was seropositive to both anti-MOG and anti-MBP IgG antibodies, nine patients (17.6%) were seropositive only to anti-MBP and two patients (3.9%) were seropositive to anti-MOG IgG antibodies.

The IgG status did not significantly influence the risk of developing CDMS (eg, MOG IgG: HR = 1.54, 95% CI: 0.44–5.27; $P = 0.495$; MBP IgG: HR = 0.98, 95% CI: not estimated due to the very small sample size; $P = 0.978$).

Discussion

Results from this cohort confirm previous findings from the placebo arms of the two major clinical trials in CIS [3,5], reporting similar values of conversion to MS. In our study 51% patients over 36 months and 45% patients over 24 months developed a relapse thus becoming CDMS according to Poser's criteria. Similar percentage of conversion was observed in the placebo arm of the ETOMS study (ie, 45%) whereas in the CHAMPS trial the frequency of conversion over two years was slightly lower (ie, 38%). Results from our study showed a conversion to MS according to McDonald's criteria in 88% of patients at two years and 91% of patients at three years, with similar rates of conversion as in the ETOMS and CHAMPS trials over a two-year follow-up [5,24]. However, previous natural history studies in CIS reported a frequency of patients developing MS

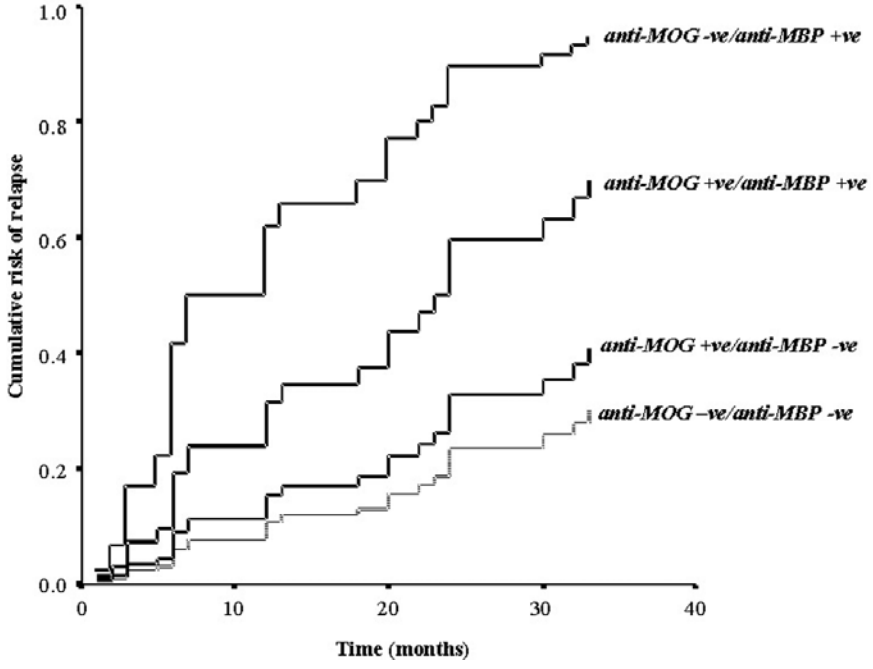

Figure 3 Cumulative risk of relapse occurrence according to the baseline antibody status. When compared to antibody negative patients (*anti-MOG-ve/anti-MBP-ve*), the risk of developing a relapse was 8.9 (95% CI: 2.7–29.8; $P < 0.001$) in the anti-MPB+ group (*anti-MOG-ve/anti-MBP+ve*) and 1.5 (95% CI: 0.4–5.4; $P = 0.564$) in the anti-MOG+ group (*anti-MOG+ve/anti-MBP-ve*); anti-MOG+/anti-MBP+ patients (*anti-MOG+ve/anti-MBP+ve*) had a risk of relapse occurrence equal to 3.4 (95% CI: 1.1–10.2; $P = 0.031$).

according to McDonald's criteria lower than that described in this study over the same time period [25–27]. Between study differences in the patients' inclusion criteria and characteristics could account for this discrepancy. Previous natural history studies on CIS [25–27] allowed for patients with normal MRI scan whereas this study included CIS patients who had at least three lesions detectable on T2-weighted images. This difference could explain the high frequency of patients (88.2%) fulfilling the McDonald's criteria for dissemination in space at baseline, whereas in previous studies dissemination in space at baseline was found in a low percentage of patients ranging from 36% to 42%.

Our study also confirms previous findings suggesting the high sensitivity of the McDonald's criteria in anticipating the diagnosis of MS soon after the first demyelinating event due to their ability in detecting sub-clinical disease activity [25,26]. It also reveals the ability of the McDonald's criteria in identifying those patients who are at high risk of developing a relapse, thus becoming CDMS according to Poser's criteria. A diagnosis of CDMS during the follow-up was observed in 79% of those patients who became McDonald positive at month 3, whereas only 34% of McDonald negative patients showed a relapse during the follow-up. On the same line, Tintoré *et al.* [26] reported that 80% of patients receiving a McDonald diagnosis of MS at one year presented a new relapse within the subsequent two years.

In our study the presence of MRI disease activity at baseline was the only significant predictor for developing MS according to McDonald's criteria. In the placebo arm of the CHAMPS trial [5] Gd-enhancement at baseline was associated with a combined outcome defined as the development of either CDMS or at least two enlarging T2 lesions on the brain MRI scan at six, 12 and 18 months.

The most relevant point arising from this study is the prognostic value of the anti-myelin antibody status assessed at the very beginning of the disease. Anti-MBP IgM positivity was significantly associated with the occurrence of a clinical relapse during the follow-up. Berger *et al*. [15] have already reported that the combined presence of serum IgM antibodies against MOG and MBP in patients with CIS was predictive of conversion to CDMS. However, they did not analyse the individual contribution of each anti-myelin antibody type in predicting the occurrence of relapse. In our cohort anti-MBP+/anti-MOG− patients had higher risk of developing a relapse (8.9 versus 3.4) and shorter median time to relapse occurrence (12 months *versus* 24 months) than anti-MBP+/anti-MOG+ patients. The different anti-MOG and anti-MBP predictive power for diagnosing MS could reflect different disease stages. Comparing the baseline demographic and clinical characteristics across groups with different anti-myelin antibody status, we found that patients anti-MOG+ and anti-MBP− were younger and had shorter disease duration. According to previous studies on anti-MOG and anti-MBP antibodies in MS as well as in other neurological diseases, anti-MOG IgG antibodies response appears to be established early in the course of the disease, afterwards showing a similar percentage across different stages; by contrast anti-MBP response appears to accumulate over time [12,13]. Also, anti-MOG and anti-MBP antibodies could be associated with different tissue damage patterns. In a recent study on CIS patients, Kuhle *et al*. reported a significantly higher number of T2-hyperintense lesions in anti-MBP+ [28]. Our study showed a similar trend in anti-MBP+ patients, suggesting that the presence of anti-MBP antibodies could be associated with a more advanced stage of the disease process.

Our study confirms that the antibody status significantly predicts the second clinical episode within three years after the first demyelinating event. The study by Lim *et al*. [16] showed that the presence of anti-myelin antibodies did not predict conversion to MS according to either the McDonald's or the Poser's criteria within one year after CIS. Between-study differences could account for these discrepancies. Our cohort included only patients with abnormal MRI, whereas Lim *et al*. included patients with normal MRI scan and restricted the study to patients who had experienced an isolated optic neuritis. By contrast, in our cohort, whose clinical characteristics matched those of the Berger's study, either mono- or multifocal CNS involvement was allowed, and disseminated white-matter lesions on the baseline MRI scan were required as inclusion criteria. However, a crucial point accounting for these conflicting findings is the length of the follow-up. The one-year follow-up in the Lim's study makes a diagnosis of CDMS unlikely in the majority of patients, limiting the possibility of exploring the predictive value of anti-myelin antibodies in the medium/long-term, which is, by contrast, a valuable point of the present study.

Our study also showed that the presence of anti-myelin antibodies can predict the timing of relapse occurrence, with positive patients developing the second episode earlier than negative patients. These data are in agreement with the ones by Berger *et al*. [15] and the recent findings by Rauer *et al*. [29].

However, in our CIS cohort the anti-myelin antibody status was not able to predict the conversion to MS according to McDonald's criteria. Therefore, we can speculate that there might be a lag between the development of brain lesions on MRI, which allows us making an early diagnosis of MS, and the effect of anti-myelin immune response, possibly delayed in time.

Our study suggests that anti-myelin antibodies, particularly anti-MBP, could be related to demyelination, either as a direct pathogenic factor or as expression of an immune response attitude. When anti-MBP antibodies are present, the chance of having a relapse is higher possibly because they express a more extensive, aggressive pattern of tissue damage, or because they merely coexist with an advanced stage of disease. Clarifying the relationship between antibodies and patterns of tissue damage, as well as a longitudinally evaluation of changes in the antibody status might help in understanding the contribution of anti-myelin antibodies to the further development of MS. More recent unpublished reports [30,31] suggest no predictive value of antimyelin antibodies in MS. In the BENEFIT study [30] Kappos *et al*. reported data from 462 CIS patients with at least two MRI silent lesions, showing that the presence of anti-myelin antibodies was not associated with higher risk of relapse occurrence or shorter time to the second attack. Some negative results were found by Pelayo *et al*. [31] who studied 114 CIS patients with abnormal baseline MRI. Due to the multicentre trial design, a large number of centres contributed to the BENEFIT data collection, thus introducing a source of heterogeneity in the final CIS cohort. Although single-centre studies may also harbour some limitations, they have the advantage of applying standard operating procedures to all the data collection procedures and analyses. Moreover, in the BENEFIT study the follow-up period was of only 24 months. Finally, other considerations such as different genetic backgrounds have also to be taken into account. For example, differences between the Barcelona cohort and other CIS cohorts in the percentage of abnormal baseline MRI in patients with optic neuritis have been reported and Barcelona patients seem also to have a milder disability after five years. It is important to note that these controversial results were all obtained using the same anti-myelin antibody detection method, eg, immunoblotting, suggesting that discrepancies may primarily reflect different cohorts' characteristics rather than methodological issues.

The anti-myelin antibody status along with MRI disease activity measures might be a promising source of diagnostic/prognostic information in MS. However, further investigations on anti-myelin antibodies are mandatory, as the current knowledge on their role in the context of the MS pathophysiology and clinical evolution at an individual level does not yet allow to use them as a surrogate marker of disease activity in MS.

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