ORIGINAL ARTICLE

Antimyelin Antibodies as a Predictor of Clinically Definite Multiple Sclerosis after a First Demyelinating Event

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BACKGROUND

Most patients with multiple sclerosis initially present with a clinically isolated syndrome. Despite the fact that clinically definite multiple sclerosis will develop in up to 80 percent of these patients, the course of the disease is unpredictable at its onset and requires long-term observation or repeated magnetic resonance imaging (MRI). We investigated whether the presence of serum antibodies against myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) in patients with a clinically isolated syndrome predicts the interval to conversion to clinically definite multiple sclerosis.

METHODS

A total of 103 patients with a clinically isolated syndrome, positive findings on cerebral MRI, and oligoclonal bands in the cerebrospinal fluid were studied. At base line, serum samples were collected to test for anti-MOG and anti-MBP antibodies with Western blot analysis, and the lesions detected by cerebral MRI were quantified. Neurologic examinations for relapse or disease progression (defined as conversion to clinically definite multiple sclerosis) were performed at base line and subsequently every three months.

RESULTS

Patients with anti-MOG and anti-MBP antibodies had relapses more often and earlier than patients without these antibodies. Only 9 of 39 antibody-seronegative patients (23 percent) had a relapse, and the mean (±SD) time to relapse was 45.1±13.7 months. In contrast, 21 of 22 patients (95 percent) with antibodies against both MOG and MBP had a relapse within a mean of 7.5±4.4 months, and 35 of 42 patients (83 percent) with only anti-MOG antibodies had a relapse within 14.6±9.6 months ($P<0.001$ for both comparisons with antibody-seronegative patients). The adjusted hazard ratio for the development of clinically definite multiple sclerosis was 76.5 (95 percent confidence interval, 20.6 to 284.6) among the patients who were seropositive for both antibodies and 31.6 (95 percent confidence interval, 9.5 to 104.5) among the patients who were seropositive only for anti-MOG antibodies, as compared with the seronegative patients.

CONCLUSIONS

Analysis of antibodies against MOG and MBP in patients with a clinically isolated syndrome is a rapid, inexpensive, and precise method for the prediction of early conversion to clinically definite multiple sclerosis. This finding may be important for the counseling and care of patients with a first demyelinating event suggestive of multiple sclerosis.

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Multiple sclerosis is the most common disabling neurologic disease in young adults.[1] Ninety percent of patients with multiple sclerosis initially present with a clinically isolated syndrome due to an inflammatory demyelinating lesion in the optic nerve, brain stem, or spinal cord.[2] Thirty percent of these patients with a clinically isolated syndrome will have progression to clinically definite multiple sclerosis within 12 months after presentation.[2-4] The course of disease in multiple sclerosis is highly variable and hard to predict, ranging from a benign course to a classic relapsing–remitting, chronic progressive, or rare fulminant course, despite several clinical,[5-8] immunologic,[9] and radiologic[2,4,10] investigations to identify risk factors for disease progression. In patients presenting with a first demyelinating event, the individual prognosis and prediction of clinically definite multiple sclerosis would be of value for counseling and management.[11] Patients at high risk for rapid progression to clinically definite multiple sclerosis could be offered disease-modifying treatments, which have recently been shown to be beneficial in early multiple sclerosis.[12,13]

The pathogenetic mechanisms responsible for conversion from a clinically isolated syndrome to clinically definite multiple sclerosis are still unknown but may include ongoing axonal loss,[14] with subsequent cumulative disability, and the process of epitope spreading,[15,16] which may amplify inflammatory demyelination in the central nervous system. After the initial inflammatory demyelinating event, further epitope spreading may prime autoreactive cellular and humoral immune responses to target additional myelin and nonmyelin antigens or epitopes.[15-18] Thus, amplification of the autoimmune process could account for disease progression in multiple sclerosis.[19] Another possible mechanism is antibody-mediated demyelination.[20-22] It is known that antibodies against myelin basic protein (MBP) are present in early multiple sclerosis.[23,24] Another potential target antigen for autoreactive antibodies might be myelin oligodendrocyte glycoprotein (MOG), which is specific to the central nervous system and is located exclusively on the surface of myelin sheaths and oligodendrocytes.[25] Antibodies against MOG cause demyelination in vitro[26] and in animal models of multiple sclerosis[27,28] and have been found in active lesions in patients with multiple sclerosis.[29] A substantial subgroup of patients with multiple sclerosis mount a persistent autoantibody response to the extracellular immunoglobulin domain of MOG[30] with a predominance of anti-MOG IgM antibodies.[23,24]

In this study, we examined whether the presence of serum anti-MOG and anti-MBP antibodies predicts conversion to clinically definite multiple sclerosis among patients who have a clinically isolated syndrome and findings on magnetic resonance imaging (MRI) and cerebrospinal fluid analysis that are suggestive of multiple sclerosis.

PATIENTS

Patients with a first acute neurologic event suggestive of multiple sclerosis were enrolled in the study after their written informed consent to the protocol, as approved by the institutional review board, had been obtained. All the patients underwent cerebral MRI (T_2-weighted and gadolinium-enhanced, T_1-weighted scanning) within two weeks after the onset of the initial neurologic symptom. Patients were excluded from the study if they did not have typical disseminated white-matter lesions according to Fazekas and colleagues' criteria.[31] In addition, initial diagnostic examination of the cerebrospinal fluid was performed in all the patients, and those whose cerebrospinal fluid did not show oligoclonal bands were excluded. Patients were also excluded from the study if they had a history of any kind of previous neurologic symptoms or signs; clinical, laboratory, MRI, or cerebrospinal fluid findings suggestive of any diagnosis other than multiple sclerosis[32]; or primary progressive multiple sclerosis (diagnosed before month 12).

All the patients were treated initially with 1000 mg of intravenous methylprednisolone for three to five consecutive days. The follow-up period of clinical monitoring for further relapses or disease progression lasted at least 12 months. Each patient underwent neurologic examination at base line and subsequently on a scheduled basis every three months by neurologists who were unaware of the antibody status of the patient. Neurologic examinations included repeated interviews and assessments for symptoms suggestive of relapse or disease progression. If a relapse was suspected, it had to be confirmed by one of the neurologists as consisting of the onset of a new neurologic symptom or the exacerbation of a previous symptom for at least 48 hours and at least four weeks after the initial demyelinating event. Exacerbations due to exogenous triggers (e.g., drugs, heat, fever, or infection) were judged

not to be relapses. If a relapse was confirmed, the patient was given the diagnosis of clinically definite multiple sclerosis.

ANTIBODY ANALYSIS

Serum samples for antibody analysis were collected at base line, before methylprednisolone treatment. The people performing the antibody analyses were unaware of the patient's clinical status and MRI findings. Human recombinant MOG immunoglobulin and human myelin–derived MBP were prepared as previously described.[23] Anti-MOG and anti-MBP antibodies were analyzed by Western blotting, also as previously described[23,24] but with minor modifications, as follows. In brief, either 1 μg of recombinant MOG immunoglobulin or 2 μg of MBP was loaded in each lane and separated in 10 percent 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 percent 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 percent milk powder in phosphate-buffered saline containing 0.05 percent Tween 20). The strips were then washed three times with phosphate-buffered saline containing 0.05 percent Tween 20 and incubated with alkaline phosphatase–conjugated antihuman 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 the immunoreactivity of the serum samples was assessed by two independent investigators. 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)[25] and positive and negative human serum samples were used. Monoclonal anti-MOG antibody 8.18-C5 and positive human serum samples are available on request to interested parties who wish to replicate our results.

STATISTICAL ANALYSIS

The characteristics of the patients and their disease were compared among the groups according to antibody status with the use of one-way analysis of variance, chi-square testing, or Kruskal–Wallis testing. The cumulative risk of the development of clinically definite multiple sclerosis was calculated for each group according to the Kaplan–Meier method, and differences between the groups were evaluated in a univariate analysis with the log-rank test. The Cox proportional-hazards model was used to assess the predictive value of anti-MOG and anti-MBP antibodies in a multivariable analysis, with adjustment for potential confounding variables. The final model included age, sex, the duration of disease, the IgG index (calculated according to the formula [cerebrospinal fluid IgG÷serum IgG]÷[cerebrospinal fluid albumin÷serum albumin], with a usual upper limit of 0.65),[33] and the number of lesions on MRI. The relative risk of the development of clinically definite multiple sclerosis is expressed as a hazard ratio and 95 percent confidence interval. After Bonferroni's correction for three comparisons, P values of less than 0.017 were considered to indicate statistical significance.

A total of 119 patients were consecutively identified as potential study participants between March 1994 and March 2001. Nine of these patients were initially excluded because oligoclonal bands were not detected in their cerebrospinal fluid. Seven additional patients were excluded because of primary progressive multiple sclerosis. None of the remaining 103 patients received any disease-modifying treatment between the time of enrollment in the study and the diagnosis of clinically definite multiple sclerosis, if such a diagnosis was made. Ninety-six patients (93 percent) were clinically monitored on a scheduled basis every three months; for only seven patients was there one six-month interval between scheduled clinical visits. None of the 103 patients were lost to follow-up during the study period.

Seventy-three of the 103 patients were female and 30 were male; their mean age at the onset of disease was 32.0 years (range, 13 to 54), and as of February 28, 2002, the mean follow-up period was 50.9 months (range, 12 to 96). The characteristics of the patients and their disease are shown in Table 1. The types of symptoms seen at presentation were distributed similarly among the groups of patients. Antibody status was not associated with the type of

Table 1. Characteristics of the 103 Patients and Their Disease, According to Antibody Status.*

Variable	Negative for Anti-MOG and Anti-MBP	Positive for Anti-MOG and Negative for Anti-MBP	Positive for Anti-MOG and Anti-MBP
No. of patients	39	42	22
Female sex — no. (%)	24 (62)	30 (71)	19 (86)
Age — yr	32.5±10.4	30.6±10.8	33.3±10.0
Type of symptoms at presentation — no. (%)			
Sensory	14 (36)	17 (40)	9 (41)
Optic (optic neuritis)	14 (36)	14 (33)	7 (32)
Motor	2 (5)	2 (5)	1 (5)
Brain stem	6 (15)	7 (17)	4 (18)
Cerebellar	3 (8)	2 (5)	1 (5)
Follow-up — mo	51.8±17.4	52.5±25.5	46.4±26.5
IgG index†	0.4±0.4	1.1±0.6	1.0±0.6
No. of lesions on T_2-weighted MRI			
Mean	4.5±2.4	5.6±2.4	6.0±2.6
Range	2–14	2–17	2–16
No. of lesions on T_1-weighted, gadolinium-enhanced MRI			
Mean	0.8±0.8	1.3±1.2	1.5±1.3‡
Range	0–2	0–4	0–4

* Plus–minus values are means ±SD. The characteristics were compared by one-way analysis of variance, chi-square testing, or Kruskal–Wallis testing. After Bonferroni's correction for the three comparisons, P values of less than 0.017 were considered to indicate statistical significance. Because of rounding, not all percentages total 100. MOG denotes myelin oligodendrocyte glycoprotein, and MBP myelin basic protein.

† The IgG index is calculated according to the formula (cerebrospinal fluid IgG ÷ serum IgG) ÷ (cerebrospinal fluid albumin ÷ serum albumin) and has a usual upper limit of 0.65.

‡ P=0.008 for the comparison with the patients who were negative for anti-MOG and anti-MBP antibodies.

clinical syndrome or the clinical outcome. Twenty-two patients (21 percent) were seropositive for both anti-MOG and anti-MBP antibodies, 42 patients (41 percent) were seropositive only for anti-MOG antibodies, and 39 patients (38 percent) were seronegative for both anti-MOG and anti-MBP antibodies.

Table 2 shows the number of patients with a relapse and the mean relapse-free period according to the antibody status. Only 9 of the 39 patients who were seronegative for anti-MOG and anti-MBP antibodies (23 percent) had had a relapse of multiple sclerosis as of February 28, 2002. Eight of these patients were seropositive for anti-MOG antibodies at the time of their relapse. In contrast, 35 of the 42 patients (83 percent) with antibodies against MOG (but not against MBP) and 21 of the 22 patients (95 percent) with antibodies against both MOG and MBP had a relapse during the study period (P<0.001 for both comparisons with the patients who had neither antibody).

Of the patients who had a relapse during the study period, those who were seronegative for anti-MOG and anti-MBP antibodies had their first relapse after a mean (±SD) interval of 45.1±13.7 months. In contrast, patients who initially were seropositive for both anti-MOG and anti-MBP antibodies had their first relapse after only 7.5±4.4 months (P<0.001 for the comparison with the seronegative patients). For patients with anti-MOG antibodies but not anti-MBP antibodies, the mean time to relapse was 14.6±9.6 months (P<0.001 for the comparison with the seronegative patients). Of the nine patients excluded from the study because of the absence of oligoclonal bands in the cerebrospinal fluid, only one was seropositive for anti-MOG antibodies. This patient had his first relapse after 16 months.

Figure 1 shows that the risk of clinically definite multiple sclerosis was significantly lower among the patients who were seronegative for both anti-MOG and anti-MBP antibodies than among those who were seropositive only for anti-MOG antibodies or for both anti-MOG and anti-MBP antibodies (P<0.001 for both comparisons, by the log-rank test). The multivariate analysis (Table 3) revealed that seropositivity for anti-MOG but not anti-MBP antibodies was associated with a risk of clinically definite multiple sclerosis that was 32 times the risk associated with seronegativity for both antibodies (adjusted hazard ratio, 31.6; 95 percent confidence interval, 9.5 to 104.5; P<0.001). Seropositivity for both anti-MOG and anti-MBP antibodies was associated with a risk that was 76 times the risk associated with seronegativity (adjusted hazard ratio, 76.5; 95 percent confidence interval, 20.6 to 284.6; P<0.001).

One of us, who had no access to the patients' clinical information or information about their antibody status, assessed and counted the number of lesions seen on T_2-weighted and T_1-weighted, gadolinium-enhanced MRI. As expected, patients with both anti-MOG and anti-MBP antibodies had higher mean numbers of lesions on T_2-weighted and T_1-weighted, gadolinium-enhanced MRI than did

patients who were seronegative for the antibodies (Table 1). However, the number of lesions varied widely among the individual patients, from 2 to 17 lesions on T_2-weighted MRI and from 0 to 4 lesions on T_1-weighted, gadolinium-enhanced MRI. In adjusted analyses, the number of lesions seen on MRI was not independently associated with the risk of relapse (Table 3).

DISCUSSION

Patients with a clinically isolated syndrome in whom neurologic symptoms, MRI examinations, and analyses of cerebrospinal fluid suggest that a given neurologic event is a first demyelinating event due to multiple sclerosis face an uncertain future. Previous studies have focused on such patients either to unravel the pathogenesis and dynamics of the progression of disease or to evaluate the benefit of early treatment.[2,5-9,12,13] The importance of MRI in early multiple sclerosis has been demonstrated.[2-4,10] Studies have shown that the number of lesions on T_2-weighted MRI is associated with more than an 80 percent risk of conversion to multiple sclerosis within 10 years, that the number of lesions on initial contrast-enhanced MRI is associated with the development of multiple sclerosis within 3 years, and that increases in the volume of lesions seen on MRI correlate with the degree of long-term disability due to multiple sclerosis.[2,4,10] Consequently, these MRI findings in early multiple sclerosis have added important information to new diagnostic guidelines in multiple sclerosis.[3,4]

In general, however, no clinical, radiologic, or immunologic variable allows precise prediction of the interval between presentation with a clinically isolated syndrome suggestive of multiple sclerosis and the presence of clinically definite multiple sclerosis. The uncertainty of prediction and prognosis causes uncertainty in individual counseling and management.

In this study, we were able to show that in individual patients with a clinically isolated syndrome, the initial detection of serum antibodies against MOG and MBP predicts early conversion to clinically definite multiple sclerosis, whereas the absence of these antibodies suggests that the patient will remain disease-free for several years. We found that 83 percent of the patients who were seropositive only for anti-MOG antibodies and 95 percent of those who were seropositive for both anti-MOG and anti-MBP had a first relapse during the mean follow-up period of 52 months, whereas 77 percent of the patients who were seronegative for both anti-MOG and anti-MBP antibodies remained relapse-free during that period.

Our results are consistent with data from previous long-term follow-up studies in some interesting ways. First, 30 to 40 percent of patients with multiple sclerosis are classified as having a relative-

Table 2. Number of Relapses and Length of Relapse-free Period, According to Antibody Status.*

Variable	Negative for Anti-MOG and Anti-MBP (N=39)	Positive for Anti-MOG and Negative for Anti-MBP (N=42)	Positive for Anti-MOG and Anti-MBP (N=22)
Patients with a relapse during the study period — no. (%)	9 (23)	35 (83)†	21 (95)†
Time to relapse among patients who had a relapse — mo	45.1±13.7	14.6±9.6†	7.5±4.4†‡

* Plus–minus values are means ±SD. The characteristics were compared by one-way analysis of variance, chi-square testing, or Kruskal–Wallis testing. After Bonferroni's correction for the three comparisons, P values of less than 0.017 were considered to indicate statistical significance. MOG denotes myelin oligodendrocyte glycoprotein, and MBP myelin basic protein.
† P<0.001 for the comparison with the patients who were negative for anti-MOG and anti-MBP antibodies.
‡ P=0.002 for the comparison with the patients who were positive for anti-MOG antibodies and negative for anti-MBP antibodies.

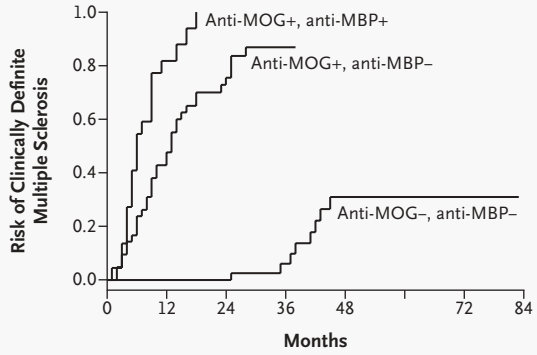

Figure 1. Kaplan–Meier Estimates of the Risk of Clinically Definite Multiple Sclerosis, According to Antibody Status.

P<0.001 for the comparison between the patients who were seronegative for antibodies against both myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) and the patients who were seropositive only for anti-MOG antibodies or for both anti-MOG and anti-MBP antibodies. Plus signs denote seropositive, and minus signs seronegative.

Table 3. Hazard Ratios for the Development of Clinically Definite Multiple Sclerosis.*

Variable	Hazard Ratio (95% CI)	P Value
Antibody status		
Negative for anti-MOG, negative for anti-MBP	1.00	
Positive for anti-MOG, negative for anti-MBP	31.6 (9.5–104.5)	<0.001
Positive for anti-MOG, positive for anti-MBP	76.5 (20.6–284.6)	<0.001
Female sex	0.93 (0.50–1.72)	0.81
Age at onset of disease (per year)	1.02 (0.99–1.04)	0.29
Duration of disease (per month)	1.01 (1.00–1.02)	0.07
IgG index (per unit)†	0.99 (0.67–1.45)	0.94
No. of lesions on T_2-weighted MRI (per lesion)	1.10 (0.98–1.23)	0.11
No. of lesions on T_1-weighted, gadolinium-enhanced MRI (per lesion)	1.18 (0.92–1.51)	0.18

* Hazard ratios were calculated with the final Cox proportional-hazards model. MOG denotes myelin oligodendrocyte glycoprotein, MBP myelin basic protein, and MRI magnetic resonance imaging.

† The IgG index is calculated according to the formula (cerebrospinal fluid IgG ÷ serum IgG) ÷ (cerebrospinal fluid albumin ÷ serum albumin) and has a usual upper limit of 0.65.

ly benign course of disease.[5] In our study, 38 percent of the participants were seronegative for anti-MOG and anti-MBP antibodies. Antibody status may therefore identify, at the onset of disease, the patients who are likely to have a relatively benign course of disease. Second, in a recent trial of early treatment, patients in the placebo group had conversion to clinically definite multiple sclerosis within a mean period of 8.4 months.[13] In the current study, patients who were seropositive for both anti-MOG and anti-MBP antibodies had clinically definite multiple sclerosis within a mean of 7.5 months.

Although, in general, antibodies against myelin are neither a specific nor a diagnostic feature of multiple sclerosis, it seems that specific demyelinating antibodies are involved in the immunopathogenesis of the disorder in at least a subgroup of patients.[35,36] We would like to emphasize that we cannot prove whether the measured antimyelin antibodies in our patients are antibodies with demyelinating capacity or whether they represent an epiphenomenon of myelin destruction. However, seropositivity for anti-MOG and anti-MBP antibodies reflects the presence of active disease, which is corroborated by higher mean numbers of lesions on T_2-weighted and T_1-weighted, gadolinium-enhanced MRI in this group of patients, with subsequent early conversion to clinically definite multiple sclerosis. Thus, our data support the concept that antigen or epitope spreading in an early phase of disease correlates with the progression of disease.[16,19]

In our adjusted analyses, seropositivity for anti-MOG or both anti-MOG and anti-MBP antibodies, but not the number of lesions seen on MRI, was associated with an increased risk of relapse of multiple sclerosis. Thus, our study demonstrates that analysis of these antibodies, which is simple to perform and less expensive than MRI, can be used to estimate the individual risk of an early first relapse and therefore of clinically definite multiple sclerosis. The predictive value of antimyelin antibodies may be important for counseling purposes or for early treatment to prevent the disease from progressing.[12,13] However, for antibody-seronegative patients, who have a chance of remaining relapse-free for several years after the initial demyelinating event, immunomodulatory therapy might be postponed until necessary.

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