Urinary Myelin Basic Protein–like Material in Patients With Multiple Sclerosis During Interferon Beta-1b Treatment

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Objectives: To determine levels of urinary myelin basic protein–like material (MBPLM) in patients with multiple sclerosis (MS) openly treated with interferon beta-1b and to correlate these with clinical changes.

Background: Levels of urinary MBPLM correlate with the presence of the progressive phase of MS and with the disease burden detected on T2-weighted, cranial magnetic resonance imaging. Measurement of urinary MBPLM level may be a feasible test for monitoring or predicting response to therapeutic measures.

Design and Methods: In a prospective study at one site, 166 patients with MS (131 with relapsing-remitting [RR] and 35 with secondary progressive [SP] disease) were treated for a minimum of 1 year and up to 3 years with interferon beta-1b and underwent assessment for neurologic disability (Expanded Disability Status Scale and Scripps Neurological Rating Scale) and change in disease subtype. Urine samples were obtained at 1219 of 1378 clinic visits, and urinary MBPLM level was determined and related to creatinine level to adjust for renal function.

Results: Statistical analysis using the general linear models procedure confirmed previous findings that the level of urinary MBPLM related to urinary creatinine level (MBPLM/creatinine) was higher \((P \leq 0.001)\) in patients with SP than RR MS. Of the 131 patients with RR MS, SP disease developed in 13 during the observation period. Compared with those in the RR group, the RR to SP group had a higher level \((P \leq 0.001)\) of urinary MBPLM and did not differ from the SP group.

Conclusions: The level of urinary MBPLM is higher in SP MS than RR MS but not in RR MS that converts to SP MS. Level of urinary MBPLM may permit the examination of treatment tested to prevent RR disease from becoming progressive.

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The approval by the Food and Drug Administration in the United States of 3 agents—interferon beta-1b, interferon beta-1a, and glatiramer acetate—for treating relapsing-remitting (RR) and secondary progressive (SP) multiple sclerosis (MS) and the improvements in the conduct of clinical trials—notably a more practical composite clinical scoring system and advances in analysis of cranial magnetic resonance imaging (MRI) findings—have raised expectations and quickened the pace for improved treatment for MS. To make therapeutic trials for new agents shorter and more affordable, especially for patients with primary progressive or SP MS, surrogate markers are urgently needed.

A surrogate marker may be defined as a nonclinical assessment that may predict ultimate clinical change. A surrogate marker for disease activity, ie, relapses and remissions, is available or seems near in findings on serial cranial MRI with gadolinium enhancement and in the level of myelin basic protein–like material (MBPLM) in cerebrospinal fluid (CSF). For the progressive phase of disease, whether in primary progressive or SP MS, surrogate markers are yet to be defined. Findings on cranial MRI or magnetic resonance spectroscopy are not definitive in reflecting change in disability.

Although MBPLM level in CSF is usually normal in primary progressive and SP MS, urinary MBPLM level is elevated in SP MS. Furthermore, in the pivotal trial of interferon beta-1b for RR MS, urinary MBPLM level was elevated in patients with RR MS that converted to SP disease and was directly correlated with the lesion number and burden of disease on results of T2-weighted, serial cranial MRI. As an extension of this earlier observation of urinary MBPLM level being a pos-
MATERIALS AND METHODS

SPECIMENS

A total of 1219 daily random urine specimens were collected during scheduled ambulatory clinic visits from 166 patients with RR and SP MS who were treated with interferon beta-1b, 250 µg subcutaneously on alternate days, for at least 1 year from November 1, 1993, through December 31, 1996. Patients with SP MS had relapses in the 2 years before initiation of treatment.15 Urine specimens were collected at 3-month intervals during the first 12 months of treatment and every 6 months afterwards. At least 3 urine specimens had to be available during a 1-year period for the patient to be included in the study.

PATIENT GROUP

Ages of the patients ranged from 17 to 61 years, with 138 female and 28 male patients. All patients had relapsing MS, i.e., RR MS or SP MS with superimposed relapses. At the time of each clinic visit, patients were assigned a score based on the Expanded Disability Status Scale (EDSS)16 and the Scripps Neurological Rating Scale (SNR) findings.17 All scores were given without knowledge of the urinary MBPLM level. At the initial visit, EDSS scores of the study group ranged from 0 to 7.5 (Figure 1). Of the group, 89.8% were white and 10.2% were African American. The patients with any systemic diseases, such as systemic lupus erythematosus, that might cause central nervous system damage similar to that of MS were omitted from the study. The patients in whom interferon beta-1b therapy had to be stopped for more than 1 month during the course of the study were also excluded. Following these guidelines, the subtypes18 of MS were RR in 118 patients (RR group), SP in 35 (SP group), and RR that converted to SP during the 1- to 3-year course of treatment in 13 patients (RR to SP group). The determination of clinical change at each clinic visit and during the course of the study was based on (1) a persistent change of 1.0 in the EDSS score16 with a score of less than 5.5 and a change of 0.3 with a score of 5.5 to 6.5, or (2) a decrease in the SNR score by 7 points or more.17

MEASUREMENTS IN URINE

Urine samples were stored frozen at −20°C or colder until analyzed. Levels of urinary MBPLM were quantitated using a double antibody radioimmunoassay in which rabbit serum R110 was used as first antibody; human myelin basic protein (MBP) peptide 69-89, as the radioligand; and human MBP peptide 83-89, as the assay standard.14 The characteristics and validation of this radioimmunoassay have been described elsewhere.14 Creatinine concentration in urine was determined14 as a means for relating urinary MBPLM level to varying renal function.

STATISTICS

All statistical analyses were performed using the Statistical Analysis System (SAS Version 6.12; SAS Inc, Cary, NC). Pearson correlation coefficients were used for overall correlations between variables. General linear models procedures were used to compare urinary MBPLM values among diagnosis subgroups and with respect to clinical status. Moving average techniques were used for smoothing and curve fitting to study urinary changes during the course of treatment. Data are reported as mean ± SEM, which was derived from our general linear models procedures. A P value of <.05 was considered significant.

RESULTS

OVERALL COMPARISONS AMONG SUBGROUPS

When all collected urine specimens were analyzed, the RR group had significantly lower (P<.001) values of urinary MBPLM (131.9 ± 10.4 ng/mL) than did the SP (210.4 ± 14.2 ng/mL) or the RR to SP group (214.2 ± 19.7 ng/mL) during the course of the study. This significance was retained for urinary MBPLM levels related to urinary creatinine levels (MBPLM/creatinine level) (Table 1). There was no significant difference between individuals who began the study with SP MS with superimposed relapses compared with those in the RR to SP group (P = .86).

COMPARISON WITH RESPECT TO TIME

Levels of MBPLM, whether expressed as nanograms per milliliter urine or per milligram creatinine, showed no significant difference over time during the course of the treatment with interferon beta-1b (P=.58). However, the mean values of MBPLM for patients in the SP or the RR to SP group were significantly higher (P<.001) than in those in the RR group, whether expressed as urinary MBPLM (data not shown) or as MBPLM/creatinine level (Figure 2). The greater variation in the values of MBPLM in the latter part of the study (Figure 2) is due to the smaller number of patients observed for the full 3 years.

CORRELATION OF CHANGES IN URINARY MBPLM LEVELS AND CLINICAL STATUS

As observed previously, urinary MBPLM and MBPLM/creatinine levels correlated significantly (P<.001) overall in a direct fashion with the EDSS score and indirectly with SNR scores.14 For comparison of changes in clinical status during the course of this treatment, individuals underwent assessment at baseline and then again whether they were better, the same, or worse at each follow-up visit.

Expanded Disability Status Scale

The initial values of MBPLM/creatinine showed a significant difference among diagnosis groups (P = .03). The SP and the RR to SP groups had significantly higher
(\(P = .04\) and \(P = .046\), respectively) MBPLM/creatinine values than the RR group. Among patient visits when the EDSS score was improved from baseline, no significant difference was noted among the patient groups \((P = .26)\). However, the mean MBPLM/creatinine level in the RR to SP group was still elevated compared with that of the other groups. Small sample size probably affected this comparison and precluded it from reaching a significant level. Among visits when no significant change in EDSS score was noted, MBPLM/creatinine level was significantly different among patient groups \((P < .001)\). The RR group had significantly lower levels than those in the RR to SP \((P < .001)\) and the SP groups \((P < .001)\). The RR to SP group did not differ significantly from the SP group \((P = .22)\). At visits when the EDSS score was worse than at baseline, MBPLM/creatinine level was significantly different among diagnosis groups \((P < .001)\). Scores in the RR group were significantly lower than those in the RR to SP \((P = .04)\) and the SP groups \((P < .001)\). The RR to SP group did not differ significantly from the SP group \((P = .06)\). The comparisons using urinary MBPLM level gave the same results as those using MBPLM/creatinine level except for the initial group at the time of assessment before beginning treatment with interferon beta-1b, where the RR group was not significantly different from the SP group \((P = .08)\).

When the data were examined for correlation between changes in clinical function based on EDSS score and measurement of urinary MBPLM/creatinine level within the diagnosis group (means shown in Table 2), several statistically significant relationships emerged. First, patients with SP MS that clinically deteriorated showed a higher \((P = .02)\) level of urinary MBPLM/creatinine than those from baseline and those with SP MS that clinically improved \((P = .048)\) (Table 2). Second, patients with RR MS that improved had higher values than those at the initial visit \((P < .001)\) and compared with patients with RR MS that had clinically stabilized \((P = .002)\) or clinically worsened \((P = .001)\) (Table 2). However, in the RR MS group, those whose MS was the same or worse showed no significant difference from the initial values. In the RR to SP group, urinary MBPLM/creatinine level did not differ significantly from that at the initial visit regardless of clinical outcome.

![Figure 1. Distribution of initial Expanded Disability Status Scale (EDSS) scores from 166 patients with multiple sclerosis treated with interferon beta-1b.](https://archneur.jamanetwork.com/)

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>No. of Patients</th>
<th>Urinary MBPLM, ng/mL</th>
<th>MBPLM/Creatinine†</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR</td>
<td>118</td>
<td>131.9 ± 10.4</td>
<td>159.5 ± 7.1</td>
</tr>
<tr>
<td>RR to SP</td>
<td>13</td>
<td>214.2 ± 19.7</td>
<td>208.2 ± 13.3</td>
</tr>
<tr>
<td>SP</td>
<td>35</td>
<td>210.4 ± 14.2</td>
<td>208.0 ± 9.6</td>
</tr>
</tbody>
</table>

*MBPLM indicates myelin basic protein–like material; RR, relapsing-remitting multiple sclerosis (MS); SP, secondary progressive MS; and RR to SP, MS that changes from RR to SP disease. For both measures, RR group is significantly different from RR to SP and SP groups at \(P < .001\); RR to SP and SP groups are not significantly different \((P = .86)\).

†Measured as nanograms of MBPLM per milligram of urinary creatinine.

![Figure 2. Levels of myelin basic protein–like material (MBPLM) in relationship to urinary creatinine (MBPLM/creatinine) during the course of treatment with interferon beta-1b. Patients who began with secondary progressive (SP) disease or in whom relapsing-remitting (RR) disease became SP during the course of treatment (progressive group) had a higher mean MBPLM/creatinine value than those patients who continued to manifest RR disease (RR group) \((P < .001)\).](https://archneur.jamanetwork.com/)

### Scripps Neurological Rating Scale

The results (data not shown) for initial values of MBPLM/creatinine are the same as those in the EDSS comparison. As with the EDSS measure among patient visits when the SNR score was improved from baseline, no significant difference was noted \((P = .07)\). Among visits when no significant SNR score change was noted, MBPLM/creatinine level was significantly different among groups \((P < .001)\). The SNR scores in the RR group were significantly lower than those of the RR to SP \((P = .009)\) and the SP groups \((P < .001)\). The RR to SP group did not differ significantly from the SP group \((P = .42)\). At visits when the SNR score was significantly worse than at baseline, MBPLM/creatinine level was significantly different among diagnosis groups \((P = .01)\). Scores in the RR group were significantly lower than in the RR to SP \((P = .02)\) and the SP groups \((P = .009)\). Scores in the RR to SP group did not differ significantly from those of the SP group \((P = .91)\). In general, the comparisons using urinary MBPLM level gave the same results as those using MBPLM/creatinine level, except in patients whose MS worsened according to the SNR score, the RR vs RR to SP group comparison did not reach significance \((P = .09)\).
Table 2. Correlation of MBPLM/Creatinine and Change in EDSS Score in Patient Groups*

<table>
<thead>
<tr>
<th></th>
<th>Initial (n = 152)</th>
<th>Better (n = 190)</th>
<th>Same (n = 609)</th>
<th>Worse (n = 268)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBPLM/Creatinine</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>RR</td>
<td>117.7 ± 8.7</td>
<td>183.0 ± 16.5</td>
<td>167.7 ± 11.7</td>
<td>148.9 ± 18.3</td>
</tr>
<tr>
<td>RR to SP</td>
<td>172.9 ± 26.1</td>
<td>236.4 ± 46.9</td>
<td>242.0 ± 22.8</td>
<td>197.7 ± 25.1</td>
</tr>
<tr>
<td>SP</td>
<td>155.1 ± 15.7</td>
<td>139.8 ± 40.5</td>
<td>213.9 ± 15.0</td>
<td>244.6 ± 21.3</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>.03</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

For comparison among the 3 patient groups
<table>
<thead>
<tr>
<th>Individual group comparisons</th>
<th>p</th>
<th>p</th>
<th>p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR vs RR-SP</td>
<td>.046</td>
<td>.26</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>RR vs SP</td>
<td>.04</td>
<td>.27</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>RR-SP vs SP</td>
<td>.56</td>
<td>.10</td>
<td>.04</td>
<td>.06</td>
</tr>
</tbody>
</table>

*EDSS indicates Expanded Disability Status Scale. Other abbreviations are defined in the first footnote to Table 1. Change in EDSS score indicates changed MS status.
†Measured as nanograms of MBPLM per milligram of urinary creatinine.

COMMENT

These findings confirm and extend previously reported observations of a link between an elevation of urinary MBPLM level and the presence of SP MS. Those patients in the RR to SP group had higher values than those in the RR group. As noted before in a randomized trial of interferon beta-1b and placebo, urinary MBPLM level is elevated before the clinical change in RR to SP disease is appreciated. Our study indicates that the same change in urinary MBPLM level occurs before subtype transition in patients treated with interferon beta-1b.

Subtyping of MS rests solely on clinical features, so that urinary MBPLM level may serve to select a subpopulation of patients with RR MS who are at risk for early progression. If such a subpopulation in the RR MS group could be identified, stratification of patients for clinical trials can be more accurately performed. Predictive information about future changes or therapeutic responses was previously observed in measurement of CSF MBPLM levels that, when elevated in patients with worsening MS, was associated with a better response to glucocorticoid therapy.

Myelin basic protein–like material, which reacts with antibodies to MBP but has not been validated by biochemical or chemical characterization to be MBP or a peptide thereof, is dissimilar in the CSF and urine, where it has different clinical implications. In CSF, MBPLM has a molecular weight of less than 1000; reacts as a noncryptic, or accessible, MBP epitope; and correlates clearly and in a nonspecific fashion with acute central nervous system myelin damage. Urinary MBPLM has a molecular weight of less than 1000; reacts as a cryptic, or buried, MBP epitope; and has a more complex relationship with clinical conditions. The level of urinary MBPLM is not a marker of acute central nervous system disease or relapses of MS. Rather, it correlates with the presence of progression of disease or failure of remission.

The elevation of urinary MBPLM levels in SP MS as published previously and in our report, its elevation in children aged 2 to 8 years, and its direct correlation with cranial T2-weighted MRI findings of burden of disease have been the bases for postulating that urinary MBPLM is a result of normal turnover of MBP that is increased when MBP is synthesized but not incorporated into the central nervous system myelin sheath at the termination of myelogenesis or failed remyelination.

Because of the presumed variation in renal clearance of MBPLM, a means for relating MBPLM to renal function has been sought. Although creatinine may not be perfect for this role, no better measure currently exists. Conclusions reached about correlations between urinary MBPLM levels and clinical changes existed regardless of the use of creatinine as a factor in data expression.

The design of our study did not permit a determination of an effect of interferon beta-1b. Patients with SP MS that remained the same or worsened showed a further increase in urinary MBPLM levels. This suggests that a positive therapeutic effect of interferon beta-1b may be shown by a stabilization or lowering of the level of urinary MBPLM, whereas a rise implies treatment failure.

The clinical correlation of urinary MBPLM level with SP MS represents group data and cannot yet be used in observing individual patients. Although this is currently a limitation in its clinical use, similar problems exist for using cranial MRI. In fact, it remains unclear which MRI technique or analysis is best for correlating with progression. Dark holes on T1-weighted MRI findings, atrophy, or certain other methods have their advocates. The decrease in N-acetylaspartate level detected by magnetic resonance spectroscopy may hold potential.

The only other urine measure reported to correlate with clinical events in MS is neopterin level, which rises as disease activity increases and relapses occur. Known to be induced by infections and interferon, an increase in urine neopterin level cannot be used to monitor change in patients receiving interferon beta.

As previously shown for patients receiving interferon beta-1b or placebo, our study confirms the observation that the measurement of MBPLM levels reveals significant differences that delineate 2 populations of patients with MS even when treated with interferon beta-1b. The further refinement of the clinical application of urinary MBPLM will be most advanced by correlative studies with serial MRI or a precise chemical characterization of MBPLM with subsequent improvement in its analysis.
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REFERENCES


