Aim. To determine the frequency and clinical and laboratory features of patients with multiple sclerosis characterized by uncommon cerebrospinal findings, ie, negative oligoclonal band or increased number of mononuclear cells in cerebrospinal fluid.

Methods. The retrospective analysis included medical records of 233 patients (158 women and 75 men) admitted to the Department of Neurology, Ljubljana Medical Center, between January 1, 1990, and December 31, 1999 and discharged with the diagnosis of multiple sclerosis. We determined clinical features and cerebrospinal fluid parameters of patients with oligoclonal band-negative multiple sclerosis and ≤15 mononuclear cells/mm³ in cerebrospinal fluid and compared them with patients with oligoclonal band-positive multiple sclerosis and expected number of mononuclear cells in cerebrospinal fluid, respectively. There were 26 patients with oligoclonal band-negative finding and 26 with ≥15 mononuclear cells/mm³ in cerebrospinal fluid. The two groups of patients did not overlap, except for one patient, who had 19 mononuclear cells/mm³ and was oligoclonal band-negative.

Results. The diagnosis was delayed in oligoclonal band-negative multiple sclerosis patients, their cerebrospinal fluid contained less leukocytes, and lower concentration of IgG. The patients with ≥15 leukocytes/mm³ in cerebrospinal fluid were diagnosed earlier and had increased cerebrospinal fluid protein and IgG concentrations.

Conclusion. Multiple sclerosis with negative oligoclonal band or increased count of leukocytes in cerebrospinal fluid were found in approximately 10% of patients with the disease. Because of the absence of oligoclonal band and less active cerebrospinal fluid, the diagnosis in these patients may be delayed.

Key words: cerebrospinal fluid; immunoglobulins; leukocytes, mononuclear; multiple sclerosis
which is more demanding in patients with unexpected results of examinations.

We retrospectively analyzed the frequency and clinical and laboratory features of patients with definite diagnosis of multiple sclerosis with uncommon cerebrospinal fluid findings (absent oligoclonal band or increased leukocyte count). We compared clinical features and cerebrospinal fluid findings of patients with oligoclonal band-positive and oligoclonal band-negative multiple sclerosis, and patients with multiple sclerosis and ≥15 vs <15 leukocytes per mm³ of cerebrospinal fluid.

**Patients and Methods**

A neurologist (UR) with a special interest in multiple sclerosis retrospectively analyzed the hospital records of patients admitted to the Department of Neurology, Ljubljana Medical Center, between January 1, 1990, and December 31, 1999, and discharged with the diagnosis multiple sclerosis. Out of 233 patients with suspected multiple sclerosis (138 women and 75 men, women-to-men ratio 2.1:1) in whom a lumbar puncture was performed for cerebrospinal fluid analysis, 229 fulfilled the Poser’s criteria (16) for clinically definite or laboratory supported diagnosis of multiple sclerosis, and four had probable (oligoclonal band-negative) primary progressive multiple sclerosis according to the criteria proposed by Thompson et al (17). Patients with bilateral optic neuritis, complete transverse myelitis, extensive spinal cord MRI lesions, or polymorphonuclear cerebrospinal fluid pleocytosis were excluded from the study.

The median age of patients was 37 (range, 16-71) years. The median age at the beginning of multiple sclerosis was 32 (range, 16-59) years. There were 148 (63.5%) patients with relapsing-remitting multiple sclerosis, 45 (19.3%) with secondary progressive multiple sclerosis, and 40 (17.2%) with primary progressive multiple sclerosis.

The total cerebrospinal fluid protein and IgG concentrations were determined with automatic immunonephelometry (DOSACAT nephelometer, DOSATEC GmbH, Munich, Germany) (18). The total cerebrospinal fluid protein concentration between 0.15 and 0.45 g/L was considered to be within normal value range. IgG concentration was expressed as a percentage of total protein concentration (normal values, <1%) (6). Leukocyte count in cerebrospinal fluid (normal value, <3 leukocytes/mm³) was determined by using Fuchs-Rosenthal chambers. Isoelectric focusing of concentrated cerebrospinal fluid and undiluted serum for determination of oligoclonal band was performed on polyacrylamide gels (19). Four or more oligoclonal bands at alkaline region were considered a positive finding. MRI was performed on Siemens Magnetom 63 SP 1.5 T imager (Siemens, Munich, Germany) with conventional T1, T2, and proton density-weighted sequences. A neuroradiologist considered the MRI findings supportive for multiple sclerosis if they fulfilled the criteria proposed by Fazekas et al (24).

Electrolytes, urea, creatinine, full blood count, erythrocyte sedimentation rate, proteinogram, anticardiolipin antibodies, antinuclear antibodies, serum and cerebrospinal fluid Borelia and syphilis serology, urine analysis, and chest X-ray were done to exclude other diseases. Vitamin B₁₂ and folic acid were also determined in patients with progressive multiple sclerosis.

**Statistical Analysis**

Statistical analysis was performed by using SPSS statistical package for Windows (SPSS Inc, Release 10.0.0, Chicago, IL, USA). Chi-square test with Yates correction and Student t-test for equality of means (after testing variables for normality) were used where appropriate to compare oligoclonal band-positive and oligoclonal band-negative groups of patients with multiple sclerosis. The same tests were used for comparison of patients with ≥15 and <15 cells/mm³ of cerebrospinal fluid. The level of statistical significance was set at $p<0.05$.

### Results

Total cerebrospinal fluid protein concentration and leukocyte count were determined in 233 patients. IgG levels were determined in 213 patients, and oligoclonal bands in 203 patients.

The mean total protein concentration was 0.41 ± 0.17 g/L. One hundred and sixty-one (69.1%) patients had normal and 72 (30.9%) had increased total protein concentration. The mean number of cells was 7.16 ± 2.60 per mm³ of cerebrospinal fluid. Mild mononuclear cell pleocytosis was found in 144 (60.9%) patients, whereas 26 (11.2%) patients had ≥15 cells/mm³ of cerebrospinal fluid. The mean percentage of IgG in total protein concentration was 15.75 ± 9.75%, with 151 (70.9%) patients having increased cerebrospinal fluid IgG fraction. One hundred and seventy-seven (87.2%) patients were oligoclonal band-positive, and 26 patients (12.8%) were oligoclonal band-negative. The two groups of patients with uncommon cerebrospinal fluid findings did not overlap, except for one oligoclonal band-negative female patient with 19 mononuclear cells/mm³ in cerebrospinal fluid and positive brain MRI. Brain MRI was performed in 153 patients, of whom 146 (95.4%) had typical high signal lesions on T2-weighted images.

The oligoclonal band-negative patients had statistically significant delay to the diagnosis and their

### Table 1. Comparison of clinical and laboratory features of 203 patients with oligoclonal band-positive and oligoclonal band-negative multiple sclerosis

<table>
<thead>
<tr>
<th>Feature*</th>
<th>Oligoclonal band in patients with multiple sclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive (n=177)</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>118/59</td>
</tr>
<tr>
<td>Age (median, range, years)</td>
<td>37 (19-71)</td>
</tr>
<tr>
<td>Duration of disease (median, range, years)</td>
<td>4 (0-25)</td>
</tr>
<tr>
<td>Age at onset (median, range, years)</td>
<td>32 (16-59)</td>
</tr>
<tr>
<td>RR-to-secondary progressive</td>
<td>113:31</td>
</tr>
<tr>
<td>No. (%) of primary progressive cases</td>
<td>33 (18.6)</td>
</tr>
<tr>
<td>Early-to-late ratio</td>
<td>140:37</td>
</tr>
<tr>
<td>Total cerebrospinal fluid protein concentration (mean ± SD, g/L)</td>
<td>0.42 ± 0.17</td>
</tr>
<tr>
<td>Leukocyte count/mm³ (mean ± SD)</td>
<td>7.74 ± 7.57</td>
</tr>
<tr>
<td>IgG fraction (mean ± SD, %)</td>
<td>17.19 ± 9.93</td>
</tr>
</tbody>
</table>

*Abbreviations: RR-to-secondary progressive – ratio of patients with relapsing-remitting multiple sclerosis to patients with secondary progressive multiple sclerosis; early-to-late ratio – ratio of patients with early onset multiple sclerosis (aged <40 years) to patients with late onset multiple sclerosis (aged >40 years); MRI – magnetic resonance imaging.

Critical Note: The critical analysis of this text reveals that there is a significant misalignment and potential error in the data presented. The statistical analysis and conclusions presented in Table 1 are based on a sample size of 203 patients, yet the mean total protein concentration and leukocyte count are provided for 233 patients, which could indicate an error in the documentation or data collection process. Further, the reported p-values for some comparisons are not consistent with the expected outcomes based on the sample size and variability. This discrepancy suggests a need for thorough verification of the data and analysis methods to ensure accurate and reliable conclusions are drawn. Additionally, the text contains inconsistencies in the reporting of certain parameters, which may affect the validity of the presented results.
cerebrospinal fluid analysis showed lower white cell number and IgG concentration (Table 1).

The comparison of the clinical features and laboratory findings in patients with ≥15 cells/mm³ and those with <15 cells/mm³ showed that the patients with increased count of leukocytes in cerebrospinal fluid were diagnosed earlier (Table 2). They were more often women, younger, and suffered from relapsing-remitting multiple sclerosis, but this was not statistically significant. Their total protein concentration and IgG fraction were also higher.

**Discussion**

Our results showed that approximately 70% of our patients with definite diagnosis of multiple sclerosis had normal cerebrospinal fluid protein concentration. The study also revealed that in 60% of patients we could expect mild cerebrospinal fluid mononuclear pleocytosis. Approximately 70% patients had increased cerebrospinal fluid IgG fraction and 87% had positive oligoclonal band. More than 95% of our patients had positive brain MRI, which is in accordance with the general agreement that MRI has a greater sensitivity than cerebrospinal fluid investigations in the diagnosis of multiple sclerosis (6).

In a 10-year study period, there were 26 (12.8%) patients with oligoclonal band-negative multiple sclerosis. In comparison with oligoclonal band-positive patients, they had delayed diagnosis, lower cerebrospinal fluid white cell number, and low IgG concentration.

The reason for the delay to the diagnosis in oligoclonal band-negative patients with multiple sclerosis was either a mild symptomatology, which did not bring the patient to the neurologist, or a reluctance of neurologists to establish the diagnosis in oligoclonal band-negative cases. Rudick et al (4) identified the absence of oligoclonal band as one of the five “red flag” features that should cast doubt on a correct diagnosis. Recent studies have revealed that the oligoclonal band-negative multiple sclerosis can be even rarer than expected (2). Studies performed in the 1980s showed that oligoclonal bands were positive in 85-95% of multiple sclerosis patients (1,6). Zeman et al (2), however, demonstrated that only 3% of their patients with clinically definite multiple sclerosis were oligoclonal band-negative.

Less active cerebrospinal fluid found in patients in our study corresponds with the results of a neuropathological study performed by Farrell et al (20), who showed that the meninges of patients with oligoclonal band-negative multiple sclerosis contain fewer plasma cells.

We observed that the oligoclonal band-positive patients had relapsing-remitting multiple sclerosis more commonly than the oligoclonal band-negative patients. This is in accordance with the fact that the oligoclonal band-negative patients had a delay to diagnosis. Since the diagnosis in the oligoclonal band-negative patients was established late, more of them developed secondary progressive multiple sclerosis.

There were no differences between the two groups regarding sex, age at the onset of the disease, percentage of late onset cases, percentage of cases with primary progressive type of the disease, and the results of qualitative brain MRI.

A few studies of oligoclonal band-negative multiple sclerosis have been reported so far. Pirttila and Nurminko (21) included 13 oligoclonal band-negative patients in their analysis. The comparison with oligoclonal band-positive controls showed that their oligoclonal band-negative patients were more often men, older, and had secondary progressive multiple sclerosis. The authors, however, included patients with probable and definite multiple sclerosis and the percentage of oligoclonal band-negative cases was unreasonably high (25%). Two studies showed that oligoclonal band-negative multiple sclerosis was relatively benign (2,5). Zeman et al (2) established that oligoclonal band-positive patients had Expanded Disability Status Scale (EDSS) scores in the range of 4-6, whereas the EDSS score of oligoclonal band-negative patients was 3-4 (2). Using a rough functional scale, Stendhal-Brodin et al (5) demonstrated that oligoclonal band-negative patients were less disabled than oligoclonal band-positive ones.

There were a few drawbacks of our study. We were not able to determine our patients’ EDSS scores from their medical records, but the main purpose of our study was to specify the clinical and cerebrospinal fluid features of patients at the beginning of
multiple sclerosis and not to evaluate a prognostic significance of presence or absence of oligoclonal bands. The other drawback was methodological. The recommendations of an expert panel for oligoclonal band analysis include IgG immunofixation after isoelectric focusing (7), which was not performed in our laboratory for cerebrospinal fluid analysis. Therefore, there is a possibility that we lost some of the oligoclonal band-positive patients, but on the other hand, the percentage of our oligoclonal band-positive patients with definite multiple sclerosis was high (87%), which is in accordance with the results of previous studies (1,3).

We also compared clinical and laboratory features of patients with increased cerebrospinal fluid leukocyte count and patients with the expected number of cerebrospinal fluid leukocytes. Approximately 10% of our patients with definite diagnosis of multiple sclerosis had ≥15 leukocytes/mm³ of cerebrospinal fluid. Only 7 patients had > 30 leukocytes/mm³, and only two had > 35, ie, both had 36 leukocytes/mm³, which confirms that multiple sclerosis with a meningitic cerebrospinal fluid profile is indeed very rare.

We found that the diagnosis was established earlier in patients with increased leukocyte count in cerebrospinal fluid, who were younger, more often women, and more often suffered from relapsing-remitting multiple sclerosis than patients with normal leukocyte count in cerebrospinal fluid. They also had increased IgG concentration, which is logical since more cerebrospinal fluid leukocytes mean more immunoglobulin-producing cells. Our results also showed that the total cerebrospinal fluid protein concentration was higher in patients with increased number of leukocytes. Higher protein concentration was the consequence of an increased permeability of blood-brain barrier, which is usually seen during a relapse of multiple sclerosis. Some previous studies also showed that the patients with multiple sclerosis had increased cerebrospinal fluid leukocyte count if their cerebrospinal fluid was examined during the relapse (22,23).

In conclusion, we can say that the diagnosis of multiple sclerosis was delayed because of the less active cerebrospinal fluid and the absence of oligoclonal band, which was found in approximately 10% of our patients with multiple sclerosis. Since the efficacy of immunomodulatory treatment is greatest in early phases of the disease, the absence of oligoclonal band should be taken into account if timely diagnosis is to be reached. Medical history and clinical findings should be reviewed in suspicious cases. MRI, which showed over 95% sensitivity in our patients, should be performed or repeated in all, especially oligoclonal band-negative, patients. Cerebrospinal fluid should also be either re-examined because a repeated analysis often discloses oligoclonal band, or additionally tested for oligoclonal fractions of free light chains, according to the recommendations of some authors (2,21). Multiple sclerosis with ≥15 leukocytes/mm³ of cerebrospinal fluid is found in approximately 10% of patients with definite multiple sclerosis. We have demonstrated that more active cerebrospinal fluid increases the possibility of an early diagnosis. The patients are more often found in the relapsing-remitting phase of the disease, which is more responsive to immunomodulation.

References


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