Total tau and S100b proteins in different types of multiple sclerosis and during immunosuppressive treatment with mitoxantrone


Introduction

Multiple sclerosis (MS) is considered a chronic inflammatory demyelinating disease of the central nervous system (CNS) of autoimmune etiology. In recent years, axonal pathology has been recognized as the major pathological substrate of permanent clinical disability. Simultaneously, research has been carried out searching for biochemical methods that may help to monitor the disease progression and therapy response. A candidate biochemical surrogate markers to monitor neuronal damage in vivo are total Tau protein (tTau) and S100b protein. Tau is microtubule-associated structural protein localized in axons and is considered to be important for maintaining the stability of axonal microtubules. The S100b protein is a subunit of the S100 protein, which is mainly localized to glia cells. Following neuronal damage, these proteins are released into the extracellular space and can be measured in the cerebrospinal fluid (CSF) and serum as a potent biochemical markers of axonal or glial degeneration and MS activity (1, 2).

In CSF of patients with MS, tTau levels are higher than that in control groups (3–7); moreover, it was shown that the levels of tTau protein correlate with an increased disability in patients with early MS and may predict the short-term progression of the disease, particularly when CSF markers are combined with MRI (8, 9). Concentration of S100b was found increased both in CSF...
and in serum of patients with MS and the increase in S100b levels correlated with the activity of the disease (10–12). However, conflicting data have previously been reported as well (13–16). Therefore, the question of whether axonal or glial proteins are useful clinical markers of neuronal damage in patients with MS remains controversial. Moreover, testing of CSF samples is not useful for monitoring of therapy. Therefore, in our study, we have investigated potential biochemical markers to monitor axonal damage in vivo both in CSF and in serum.

Subjects and methods

The study population included 54 patients with definite MS diagnosed according to the revised McDonald’s criteria (17). Depending on the clinical symptoms, patients with MS were divided into the following subgroups.

Group I – 30 patients with remitting–relapsing MS (RRMS). All patients were in the stable stage of disease – they had no relapse within the 3 months preceding the study. None of the patients with RRMS had ever received immunosuppressive or immunomodulatory therapy.

Group II – 24 patients with secondary progressive MS (SPMS). All patients received mitoxantrone therapy (iv, every 3 months, the cumulative dose $90 \text{mg/m}^2$).

Group III – 30 healthy controls without known neither neurological nor psychiatric conditions.

Paired CSF and blood samples were collected from all subjects. For patients with SPMS, CSF samples were obtained before the first dose of Mitoxantrone and moreover serum samples after 6, 12 and 24 months of therapy. The control samples of serum and CSF were collected during spinal anesthesia prior to surgical procedures owing to lower limb varices or urinary conditions. No medication was injected to the intrathecal space before CSF was drawn.

The study was approved by the local ethics committee, and all subjects gave informed consent.

The baseline characteristics of the patients are summarized in Table 1.

Serum and CSF analysis

Paired CSF and blood samples were collected into sterile polypropylene tubes. After collection, the routine analyses were performed (including the levels of albumin in serum and CSF), and the CSF/serum albumin ratio (QAlb) was calculated to evaluate the integrity of the blood–brain barrier. Samples for tTau and S100 estimation were immediately centrifuged to remove cells and stored at $-80^\circ\text{C}$ until analysis. Samples were thawed just prior to assay and protein concentrations were calculated at room temperature. The levels of the tTau protein were determined in duplicate using a commercial specific sandwich ELISA (Innotest hTAU-AG, Innogenetics, Ghent, Belgium), which is constructed to measure total human tau. In this assay, the wells of the polystyrene microtiter plates were coated with the solid-phase antihuman tau monoclonal antibody (AT120). The test samples were incubated in these wells along with two separate biotinylated tau monoclonal antibodies (HT7 and BT2) that recognize different tau epitopes. Samples were rinsed with assay buffer and then incubated with peroxidase-labeled streptavidin. Samples were then incubated with tetramethylbenzidine and 0.006% hydrogen peroxide. The reaction was stopped with diluted sulfuric acid, and optical density measurements were read at 450 nm using a microplate reader. The assay sensitivity was 60.0 pg/ml (standards range 75–1200 pg/ml). S100b concentrations were assayed in duplicate using the sandwich ELISA method. The assay was performed according to the manufacturers’ instructions with the commercial kit from Fujirebio Diagnostics AB, Sweden. Calibrators and unknown samples were incubated over 2 h together with biotinylated Anti-S100b monoclonal antibody and horseradish peroxidase-labeled Anti-S100b MAbS3 in Streptavidin-coated microtiter strips. After washing,

Table 1 Demographic and clinical data (mean ± SD)

<table>
<thead>
<tr>
<th>Control</th>
<th>RRMS</th>
<th>SPMS</th>
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<tr>
<td>N = 30</td>
<td>N = 30</td>
<td>N = 24</td>
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<tr>
<td>Age – 39.3 ± 7.1 years</td>
<td>Age – 34.4 ± 6.1 years</td>
<td>Age – 42.9 ± 8.8 years</td>
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<tr>
<td>QAlb 5.1 ± 4.7</td>
<td>QAlb 5.7 ± 5.0</td>
<td>QAlb 6.7 ± 5.2</td>
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<tr>
<td>Disease duration – 6.8 ± 4.1 years</td>
<td>Disease duration – 11.8 ± 7.5 years</td>
<td>EDSS: 6.1 ± 2.5 – before MTX therapy</td>
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<tr>
<td>EDSS: 3.1 ± 1.2</td>
<td>5.8 ± 3.4 – after MTX therapy</td>
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RRMS, remitting–relapsing MS; SPMS, secondary progressive MS.
hydrogen peroxide and tetramethylbenzidine were added to each well, and the enzyme reaction was stopped with 0.12 M hydrochloric acid. Optical density measurements were read at 405 nm using a microplate reader. The limit of detection for S100b was 20 pg/ml (standards range 20–2500 pg/ml).

For each patient, tTau and S100b concentrations were assayed simultaneously by an independent technician. The inter-assay coefficient of variation for both proteins was <10%.

Statistical analysis

The distribution of parameters in the groups was tested by the Kolmogorov–Smirnov and Shapiro–Wilk tests. The data of patients with MS and control groups were compared using the non-parametric Mann–Whitney U test. ANOVA with Duncan’s post hoc test was used for comparisons during MTX therapy whereas the Spearman correlation coefficient test for correlations between the variables. The $P$ value was calculated by Bonferroni correction and was considered statistically significant when $P < 0.05$.

Results

All measurements of S100b concentration were in the range of detection both in serum and in CSF. The tTau level in serum was below the detection threshold in all samples of controls, whereas in RRMS and SPMS the percentages of results above the detection limit were 56% and 71%, respectively. In CSF, the percentages of results above detection limit were 62% in controls, 100% in RRMS and 85% in SPMS. If the level of tTau was below the detection limit of the kit, a concentration of 30 pg/ml was accepted for statistical calculations.

In serum of patients with MS, the S100b level was significantly higher than that in the control group. No differences were found between RRMS and SPMS patients. Similar results were observed for level of tTau in serum. In CSF, the S100b level in the RRMS group was significantly higher than that in control and SPMS groups. The tTau concentration in CSF of both MS groups was higher when compared to healthy subjects; however, no differences were observed between patients with SPMS and RRMS. The S100b and tTau levels in CSF and serum of patients with MS are presented in Table 2. In MS groups, no significant correlations between age, expanded disability status scale (EDSS), disease duration, tTau or S100b in serum and CSF were demonstrated.

During mitoxantrone therapy, decreases in both tTau and S100b concentrations were observed. For tTau, the difference was small, whereas for S100b, the difference was found statistically significant after 24 months (Fig. 1).

### Table 2 The levels of tTau and S100b in CSF and serum of patients with MS

<table>
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<th>Control Serum</th>
<th>RRMS Serum</th>
<th>SPMS Serum</th>
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<tbody>
<tr>
<td>S100b (pg/ml)</td>
<td>26.2 ± 10.8 [22] (20–56)</td>
<td>40.1 ± 22.9 [30.5]† (20–90)</td>
<td>47.9 ± 27.7 [38]† (20–83)</td>
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<td>396.7 ± 312.2 [395] (20–1200)</td>
<td>631.6 ± 392.1 [600]†† (20–1414)</td>
<td>401.1 ± 345.8 [285] (20–1075)</td>
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<td>tTau (pg/ml)</td>
<td>Serum bd</td>
<td>81.2 ± 90.4 [73]† (bd – 388.1)</td>
<td>74.2 ± 63.3 [61]† (bd – 216.3)</td>
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<td>CSF 104.0 ± 75.4 [99.7] (bd – 346)</td>
<td>246.4 ± 122.8 [213.6]† (92.9–566.4)</td>
<td>218.5 ± 96.1 [201]† (bd – 489)</td>
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MS, multiple sclerosis; RRMS, remitting–relapsing MS; CSF, cerebrospinal fluid; bd, below detection limit; SPMS, secondary progressive MS; tTau, total Tau protein.

The data are expressed in pg/ml as mean ± SD (range) and median in square brackets.

†Statistically significant vs control group ($P < 0.05$, Mann–Whitney test).

‡Statistically significant vs SPMS stable group ($P < 0.05$, Mann–Whitney test).

Figure 1. The box plot shows S100b level in serum of patients with MS during immunosuppressive treatment. The box represents the 25th to 75th quartile, the whiskers represent the range, the square represents the median. The S100b level after 24 months of mitoxantrone therapy was significantly lower than that in the month 0 (ANOVA test $F = 2.804$, $P = 0.04$).
No significant correlations were found between tTau or S100b levels and demographic or clinical parameters. The correlations between the S100b level and EDSS during mitoxantrone therapy were found insignificant as well (Spearman test, \( P > 0.05 \)).

**Discussion**

The results of our study suggest that CSF levels of tTau and S100b are elevated in patients with MS and can reflect an axonal and glial pathology. Moreover, compared with clinical exams, the S100b level in serum may be useful for monitoring immunosuppressive therapy. The tTau level in serum is not suitable as a biomarker of MS activity.

Our findings demonstrated that the levels of S100b and tTau proteins in CSF were higher in patients with MS when compared to healthy individuals. The highest values were found in patients with RRMS, which is in accordance with the results published by other authors (3–7, 10, 11). Worth stressing that in our study, all patients with RRMS were in the stable stage; thus high S100b levels indicate the relapse-independent activity of astrocytes, whereas increases in tTau are suggestive of coexisting axonal pathology.

Measurements of S100b or tTau levels in CSF indicate the glial and axonal pathology in patients with MS. However, such determinations in CSF are of low usefulness in everyday clinical practice, particularly for disease monitoring and considering that measurements have to be repeated. Blood serum is found to be a better material; therefore, we determined S100b and tTau levels in serum. Increased serum S100b levels have already been described in patients with MS, yet the levels of Tau proteins have not been determined. Serum tTau levels were estimated in patients with stroke and head injury with intracranial lesions. The study findings demonstrated that serum tTau levels were significantly higher in patients with poor outcomes and could be used as an independent prognostic factor for severe head injury (18–20). Our results demonstrated that the serum levels of S100b and tTau were higher in patients with MS compared to healthy individuals. However, we did not find significant differences between the subtypes of disease. The S100b findings were in accordance with those reported by other authors and confirmed the usefulness of S100b as a marker of the activity of astrocytes (10, 11).

Furthermore, we evaluated the levels of S100b and tTau during the 24-month therapy with mitoxantrone. No significant differences in tTau levels were demonstrated, whereas S100b levels were found markedly lower after 24 months of therapy. The tTau concentrations reflect axonal loss most evident in rapidly progressive disease, e.g. traumatic brain injury and stroke (19, 20). In our study, patients had slowly progressive disease, which might have affected the lack of significant differences. Moreover, the tTau level above the detection limit was demonstrated during therapy only in a proportion of patients (from 43% to 61%, data not shown). Thus, the results indicate that serum tTau determinations are not sufficiently sensitive to assess the extent of axonal damage and to monitor the therapy efficacy. Extracellularly, S100b stimulates neuronal survival, differentiation, astrocytic proliferation, neuronal death via apoptosis, and in MS is related to inflammatory activity (11). Moreover, nanomolar levels of S100b are neurotrophic, while micromolar levels of S100b are cytotoxic (2). The earlier statement suggests that decreased S100b levels may be beneficial and suggestive of inhibition of inflammatory activity, hence of neuronal damage. However, no correlations between S100b levels and EDSS scores were observed. The group of affected patients had relatively high EDSS scores, which only slightly changed after therapy. The outcomes were mainly affected by the scores of two patients who substantially improved after therapy. In the remaining patients, stabilization of neurological status was mainly observed. S100b measurements were earlier used to assess the therapy outcomes by other authors, yet their usefulness was not demonstrated. Their study results, however, cannot be directly compared with our findings as different clinical parameters were assessed, and different drugs used (IFN beta); moreover, the clinical course of MS of their patients was different (12).

Determinations of biochemical markers of CNS damage in serum or CSF may be useful in everyday clinical practice. However, the available data do not explicitly confirm possible uses of single parameters and suggest that the panel of potential markers should be widened, and sensitivity as well as specificity of determination methods should be improved.

**References**

5. Bartosik-Psujek H, Archelos JJ. Tau protein and 14-3-3 are elevated in the cerebrospinal fluid of patients with multiple sclerosis and correlate with intrathecal synthesis of IgG. J Neurol 2004;251:414.