Cerebrospinal Fluid Levels of Soluble Amyloid Precursor Protein and β-Amyloid 42 in Patients with Multiple Sclerosis, Neuromyelitis Optica and Clinically Isolated Syndrome

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Amyloid precursor protein (APP) accumulation in axonal ovoids is a sensitive marker for acute axonal injury in multiple sclerosis (MS) lesions. This study measured levels of α-cleaved soluble APP (αsAPP) and β-amyloid 42 (Aβ42) in the cerebrospinal fluid (CSF) of 42 MS, 10 neuromyelitis optica and 25 clinically isolated syndrome patients and 21 healthy controls, and analysed the correlation between αsAPP and Aβ42 levels and relevant clinical parameters. The CSF concentrations of αsAPP and Aβ42 in patients and controls were not significantly different. There was a significant inverse correlation in patients between CSF αsAPP concentration and the Expanded Disability Status Scale (EDSS), but no significant correlation between CSF Aβ42 concentration and EDSS. The concentration of αsAPP in the CSF of statin-treated patients was significantly higher than in those not treated with statins, suggesting that statins may have a neuroprotective effect. In conclusion, αsAPP was present at similar levels in the CSF of patients with neuromyelitis optica, MS and clinically isolated syndrome and healthy controls, and an inverse correlation existed between CSF αsAPP concentration and neurological disability.

KEY WORDS: MULTIPLE SCLEROSIS; NEUROMYELITIS OPTICA; CLINICALLY ISOLATED SYNDROME; AXONAL DAMAGE; AMYLOID PRECURSOR PROTEIN; β-AMYLOID 42

Introduction

Multiple sclerosis (MS) and neuromyelitis optica (NMO) are both inflammatory demyelinating diseases of the central nervous system, while clinically isolated syndrome (CIS) is defined as the first acute or subacute neurological event suggestive of MS (optic neuritis, spinal cord syndrome or brain stem syndrome) in the absence of any alternative diagnosis. The pathological hallmark of MS is sharply defined as a demyelinating plaque with the axons
relatively preserved while, in NMO, both axons and myelin are involved, resulting in necrotic cavitation.\(^1\) Evidence indicates that axonal damage is the major contributor to the permanent neurological deficits in both MS and NMO patients.\(^2\) – \(^4\)

Amyloid precursor protein (APP), a transmembrane protein, is known to be produced in neurons and transported by anterograde axonal transport and it can be cleaved through two pathways.\(^5\) – \(^7\) The subsequent cleavage by \(\beta\)- and \(\gamma\)-secretase generates \(\beta\)-cleaved soluble APP (\(\beta\)sAPP) and \(\beta\)-amyloid (A\(\beta\)) peptides simultaneously.\(^7\) – \(^9\) In contrast, cleavage by \(\alpha\)-secretase precludes A\(\beta\) formation and generates \(\alpha\)-cleaved soluble APP (\(\alpha\)sAPP).\(^7\) – \(^9\) Preliminary studies suggest that APP accumulation in axonal ovoids is a sensitive marker for acute axonal injury in MS.\(^10\) – \(^14\) In marmoset experimental allergic encephalitis (EAE), the animal model of MS, immunoreactivity for APP is detected mostly in early active lesions compared with late active lesions and normal-appearing white matter.\(^11\) One immunocytochemistry study on postmortem brain tissues from 18 patients with MS suggested that APP expression was most pronounced in acute lesions and at the border of active chronic lesions, but seldom in chronic lesions.\(^14\) Another study of 22 patients with MS and 18 EAE model rats showed that the highest incidence of acute axonal injury, defined by APP accumulation, was found in active demyelinated lesions but not in inactive demyelinated plaques and remyelinated shadow plaques.\(^10\) Although the underlying molecular mechanisms for axonal damage are not well defined, it has been suggested that APP expression in damaged axons is correlated with the extent of inflammation but is independent of demyelinating activity in MS.\(^12\),\(^13\) Another study indicated that nitric oxide and its metabolites may contribute to axonal pathology in EAE.\(^15\) One study revealed that, in the acute lesions of EAE, axonal injury occurred at sites of coexpression of the sodium channel Na\(_\text{v}1.6\) and the Na\(^+\)/Ca\(^{2+}\) exchanger\(^16\) while, in chronic lesions, axonal injury is independent of these two molecules.\(^17\)

These findings suggest that APP accumulation in axonal ovoids is a feature of the early stages of MS. To date, only a few studies on APP or APP-derived soluble proteins in the cerebrospinal fluid (CSF) or serum of patients with MS have been reported, but the results are contradictory.\(^18\) – \(^20\) Thus, in order to investigate the importance of APP for evaluation of axonal damage and prediction of prognosis in the early stages of MS, NMO and CIS, the present study investigated levels of \(\alpha\)sAPP and A\(\beta\)\(_{42}\) in the CSF of patients with MS, NMO and CIS, and determined whether there was any correlation between CSF levels of \(\alpha\)sAPP and A\(\beta\)\(_{42}\) and relevant clinical parameters such as disability, disease duration and brain atrophy.

**Patients and methods**

**STUDY POPULATION**

Blood and CSF samples were collected from sequential patients with MS (classic MS [CMS] and the opticospinal form of MS [OSMS]), NMO and CIS who met the inclusion criteria, and from healthy controls. All patients with MS, NMO and CIS were in- or outpatients at the Department of Neurology, The Fifth Affiliated Hospital of Sun Yat-Sen University (Zhuhai, China) or at the Department of Neurology, The Third Affiliated Hospital of Sun Yat-Sen University (Guangzhou, China) between January 2006 and November 2007.

Diagnosis of MS was made according to the 2005 revised McDonald criteria\(^21\) and
NMO was diagnosed according to the revised diagnostic criteria for NMO published in 2006.22 CIS was diagnosed as described by Pelidou et al.23 Patients with a clinically definite diagnosis of MS, NMO and CIS, those who had not received glucocorticoid pulse therapy within 30 days before enrolment and those who had no history of drug or alcohol abuse were included in the study. Patients with a history of using antidepressant, antipsychotic or antiepileptic drugs, who were pregnant, or had any other neurological or psychological disease were excluded.

Controls were selected from healthy volunteers without inflammatory diseases of the central nervous system who were attending The Third Affiliated Hospital of Sun Yat-Sen University (Guangzhou, China) for routine examination.

The study was approved by the Ethical Committee of The Third Affiliated Hospital of Sun Yat-Sen University and all procedures were carried out with the verbal consent of all participants.

MRI AND OLIGOCLONAL BAND EXAMINATION

Magnetic resonance imaging (MRI) of the brain, spinal cord or optic nerve was performed in patients with MS, NMO and CIS in the relapsing phase, using an MRI GE Signa Excite II 1.5-T scanner (GE Medical Systems, Waukesha, WI, USA). Estimations of oligoclonal banding in serum and CSF were performed by isoelectric focusing, as described by Link et al.24

CLINICAL EVALUATIONS

Functional disability of the patients with MS, NMO and CIS was assessed using the Expanded Disability Status Scale (EDSS).25–27

The blood–brain barrier (BBB) index was assessed; a BBB index > 7 reflects destruction of BBB function. The Delpech–Lichtblau index and Tourtellotte synthesis rate, two important indices of immunoglobulin (IgG) synthesis in the central nervous system, were also assessed; a Delpech–Lichtblau index > 0.7 or Tourtellotte synthesis rate > 3.3 mg/dl reflects a raised level of IgG synthesis in the central nervous system. These three indices were calculated according to IgG and albumin concentrations in paired serum and CSF samples (detected by nephelometry assay at the Central Laboratory of The Third Affiliated Hospital of Sun Yat-Sen University) using the following formulae: BBB index = albumin_{CSF}/albumin_{serum}, Delpech–Lichtblau index = (IgG_{CSF}/IgG_{serum})/(albumin_{CSF}/albumin_{serum}), Tourtellotte synthesis rate = [(IgG_{CSF} – IgG_{serum}/369) – (albumin_{CSF} – albumin_{serum}/230) × (IgG_{serum}/albumin_{serum}) × 0.43] × 5.

The proportion of patients on statin or corticosteroid therapy was also measured.

DETECTION OF sAPP PROTEIN IN THE CSF

The level of sAPP in CSF was evaluated qualitatively by Western blot analysis. Protein concentration in the CSF samples was detected using a Model 550 microplate reader (Bio-Rad, Hercules, CA, USA) and a bicinchoninic acid (BCA)-100 Protein Quantitative Analysis Kit (Shanghai Biocolor Bioscience & Technology, Shanghai, China). Equal amounts of protein (35 µg) from the CSF samples were denatured by boiling (100°C) in 100 µl of loading buffer (7 ml Tris–HCl/sodium dodecyl sulphate [SDS] buffer [4×, pH 6.8], 3 ml glycerol, 1 g SDS, 0.93 g dithiothreitol and 1.2 mg bromophenol blue in deionized water adjusted to a final volume of 10 ml) for 5 min. They were then separated by Tris–Tricine 10% SDS–polyacrylamide gel electrophoresis and transferred...
electrophoretically to a nitrocellulose membrane at 110 mA for 2 – 3 h.

The membrane was then stained with Ponceau Red for 6 – 8 min to visualize the protein bands and blocked with 5% skimmed milk in Tris-buffered saline–TWEEN 20 (TBST; 20 mmol/l Tris–HCl, 150 mmol/l saline, 0.05% TWEEN-20 v/v; pH 7.5) for 60 min at room temperature. The blots were then incubated with the following primary antibodies overnight at 4°C: rabbit polyclonal anti-APP antibody, which recognizes amino acids 44 – 61 of human APP (1 : 500 dilution in 2% bovine serum albumin in TBST; Upstate USA Inc., Charlottesville, VA, USA); or mouse monoclonal anti-α-tubulin antibody as the internal control (1 : 1000 dilution; Sigma-Aldrich, St Louis, MO, USA). The membranes were washed three times for 10 min in TBST and incubated with horseradish peroxidase-conjugated goat antirabbit IgG and goat antimouse IgG (1 : 2000 dilution; Wuhan Boster Biological Technology, Wuhan, China) secondary antibodies for 60 min at room temperature. The membranes were washed a final three times for 10 min in TBST and immunoreactivity of the primary antibodies was detected using an enhanced chemiluminescence (ECL) detection kit (Lumi-Light® ECL; Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instruction, and exposure to film (Kodak, Rochester, NY, USA).

**DETECTION OF αsAPP BY SANDWICH ELISA**

The concentration of αsAPP in the CSF was assessed quantitatively by a sandwich enzyme-linked immunosorbent assay (ELISA) using a human APP immunoassay kit (BioSource, Camarillo, CA, USA). The capture antibody for this assay was bound to the N-terminal part of human APP and the detection antibody recognized the N-terminal part of Aβ peptide. Standard solutions, S0 – S7, of human APP were prepared according to the kit manufacturer's instructions (the concentration of S7 was 50 ng/ml, diluted 1 : 2 into S0 – S6 in standard diluent buffer supplied in the kit). Then, 100 µl of S0 – S7, control (standard diluent buffer) and CSF samples (diluted 1 : 100 in standard diluent buffer) were added to the wells of the kit microtitre strips, which were coated with a monoclonal antibody specific for human APP (capture antibody), and incubated for 2 h at room temperature. The strips were washed four times for 15 – 30 s each time in 1 x washing buffer (supplied with the kit) after each incubation step. After washing, 100 µl biotinylated monoclonal antibody specific for human APP (detection antibody) was added and incubated for 1 h at room temperature. After removal of excess detection antibody, 100 µl streptavidin–peroxidase was added and incubated for 30 min at room temperature. After the third incubation and washing to remove unbound enzyme, 100 µl of substrate solution (stabilized chromogen) was added. The colour reaction was quenched with 100 µl of stop solution (supplied in the APP immunoassay kit), and the absorbance was measured at 450 nm. The concentration of αsAPP in the CSF samples was calculated from the linear interval of each standard curve using a microplate reader (Model 550; Bio-Rad).

**DETECTION OF Aβ42 BY LIQUID ASSAY**

The concentration of Aβ42 in CSF was measured quantitatively by liquid assay using the Luminex™ system (MiraIBio, Heidelberg, Germany) with a Human Neuroscience Buffer Reagent Kit and an appropriate Human Neuroscience Antibody
Bead Kit (Biosource). Standard solutions, \( S_0 - S_7 \), of human \( \text{A}\beta 42 \) were prepared according to the manufacturer’s instructions (the concentration of \( S_7 \) was 5000 pg/ml, diluted 1 : 3 into \( S_0 - S_6 \) in the carbonate buffer supplied in the kit). After washing once for 15 – 30 s in 1 × washing buffer (supplied with the kit), a 25 µl antibody bead of human \( \text{A}\beta 42 \) was added into each well, except for G12 and H12, followed by 200 µl of wash solution (supplied in the kit). After soaking for about 15 – 30 s, vacuum filtration at low pressure was performed. The washing procedure was repeated twice. Then, 50 µl of detection antibody specific for human \( \text{A}\beta 42 \) was added to each well, followed by 50 µl of standard solutions \( S_0 - S_7 \) or CSF specimens. The wells were wrapped with aluminium foil and incubated for 3 h at room temperature. After repeating the washing procedure four times, 100 µl streptavidin–R-phycoerythrin was added. The wells were again wrapped with aluminium foil and incubated for a further 30 min at room temperature. The washing procedure was repeated four times, 100 µl of wash solution was added, and the plates were wrapped with aluminium foil and incubated for a further 3 min at room temperature. Finally, the samples were analysed on the Luminex™ system. The concentration of \( \text{A}\beta 42 \) in the CSF samples was calculated with MasterPlex QT 2.0 software (MiraiBio).

**STATISTICAL ANALYSES**

All statistical procedures were performed using SSPS® statistical package, version 16.0 (SPSS Inc., Chicago, IL, USA) for Windows®. The one-sample Kolmogorov–Smirnov test was performed to test whether distribution of the data (concentration of \( \text{A}\beta 42/\alpha s\text{APP} \) in the CSF, BBB index, Delpech–Lichtblau index, Tourtellotte synthesis rate and EDSS) was normal or non-normal. Between-group differences for the BBB index and Delpech–Lichtblau index were analysed by one-way analysis of variance; the data were log transformed prior to analysis due to a non-normal distribution. Between-group differences in the Tourtellotte synthesis rate were analysed by the Kruskal–Wallis \( H \) test. Between-group comparisons of protein concentration were determined by analysis of variance (data were log transformed prior to analysis due to a non-normal distribution). Pearson’s or Spearman’s correlation coefficients were used to analyse the correlation between CSF \( \alpha s\text{APP} \) and \( \text{A}\beta 42 \) levels and relevant clinical parameters. Positive rates of oligoclonal banding among groups were tested by the crosstabs \( \chi^2 \)-test. A value of \( P < 0.05 \) was considered to be statistically significant.

**Results**

Blood and CSF samples were collected from 42 patients with MS (26 CMS, 16 OSMS), 10 patients with NMO, 25 patients with CIS (four optic neuritis, 12 myelitis, nine brain stem syndromes) and 21 controls. Patient characteristics are shown in Table 1.

A total of 59 patients (seven NMO, 28 MS and 24 CIS patients) underwent MRI scans of the brain, spinal cord or optic nerve in the relapsing phase; some did not undergo MRI, either for practical reasons (e.g. some were outpatients) or for costs reasons. Of the 59 patients evaluated, two NMO, 13 MS and four CIS patients were found to have brain or spinal cord atrophy.

Positive rates of oligoclonal banding among NMO, MS, CIS patients and controls were 28.57% (2/7 patients), 15.15% (5/33 patients), 19.05% (4/21 patients) and 11.76% (2/17 patients), respectively (no statistically significant between-group differences). Oligoclonal banding results were not obtained in all patients due to lack
of sample material for some patients. There were no statistically significant differences for BBB index, Delpech–Lichtblau index and Tourtellotte synthesis rate between the MS, NMO, CIS patients and control groups (Table 2).

A total of six NMO, 29 MS, 23 CIS patients and 21 controls had CSF specimens analysed by Western blot. This analysis was not carried out for all patients due to lack of sample material for some patients. The results revealed that one band corresponding to a molecular weight of 110 kDa (sAPP) was visualized in all NMO, MS, CIS patients and controls, with no significant differences in the grey scale intensity among the groups (Fig. 1).

There was no significant difference in the concentration of $\alpha$sAPP and A$\beta$42, measured by sandwich ELISA and liquid assay,

<table>
<thead>
<tr>
<th>Group</th>
<th>Male ($n$)</th>
<th>Female ($n$)</th>
<th>Age at onset (years)$^a$</th>
<th>Duration (years)$^b$</th>
<th>Relapse frequency$^c$</th>
<th>EDSS$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMO</td>
<td>2</td>
<td>8</td>
<td>33.40 ± 3.80</td>
<td>3.99</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2 days – 17 years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>17</td>
<td>25</td>
<td>37.95 ± 2.01</td>
<td>2.40</td>
<td>3.18</td>
<td>3.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(5 days – 13 years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIS</td>
<td>13</td>
<td>12</td>
<td>30.78 ± 3.40</td>
<td>2.01</td>
<td>1.00</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2 days – 4 years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>12</td>
<td>32.30 ± 2.25</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Data presented as: $^a$mean ± SD; $^b$median (range); $^c$median.

EDSS, Expanded Disability Status Scale.

### TABLE 2:
Blood–brain barrier (BBB) index, Delpech–Lichtblau index and Tourtellotte synthesis rate among the multiple sclerosis (MS), neuromyelitis optica (NMO) and clinically isolated syndrome (CIS) patients and healthy control subjects studied

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>BBB index$^a$</th>
<th>Delpech–Lichtblau index$^a$</th>
<th>Tourtellotte synthesis rate (mg/dl)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMO</td>
<td>7</td>
<td>4.5515</td>
<td>0.4704</td>
<td>–4.484</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.4598 – 8.9210)</td>
<td>(0.2849 – 0.5520)</td>
<td>(–29.4955 – 0.0656)</td>
</tr>
<tr>
<td>MS</td>
<td>33</td>
<td>4.6004</td>
<td>0.4900</td>
<td>–3.5469</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.8896 – 27.0286)</td>
<td>(0.1274 – 1.2095)</td>
<td>(–40.4746 – 22.1958)</td>
</tr>
<tr>
<td>CIS</td>
<td>21</td>
<td>6.0438</td>
<td>0.5029</td>
<td>–2.6200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.0192 – 39.3238)</td>
<td>(0.1694 – 0.9169)</td>
<td>(–58.3295 – 30.7386)</td>
</tr>
<tr>
<td>Control</td>
<td>17</td>
<td>5.9985</td>
<td>0.4844</td>
<td>–3.0486</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.5012 – 10.4392)</td>
<td>(0.4161 – 0.8584)</td>
<td>(–8.9616 – 6.7479)</td>
</tr>
</tbody>
</table>

Statistical significance$^b$

|               | NS | NS | NS |

$^a$Data presented as median (range).

$^b$One-way analysis of variance was used to analyse between-group differences for BBB index and Delpech–Lichtblau index (data were log transformed prior to analysis due to a non-normal distribution); the Kruskal–Wallis test was used to analyse between-group differences for Tourtellotte synthesis rate. NS, not statistically significant ($P > 0.05$).
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CSF levels of sAPP and Aβ42 in MS, NMO and CIS patients

respectively, in the CSF of NMO (n = 7 and n = 8, respectively), MS (n = 31 and n = 25, respectively), CIS (n = 22 and n = 19, respectively) and controls (n = 9 and n = 11, respectively) (Table 3).

Pooling the data from the three groups of patients (NMO, MS and CIS patients) showed an inverse correlation between αsAPP concentration in the CSF and EDSS (r = -0.356, P = 0.007; bivariate correlation analysis), but no correlation between Aβ42 concentration in the CSF and EDSS (r = -0.093). There was no correlation between αsAPP or Aβ42 concentrations in the CSF and disease duration (r = -0.091 and r = -0.020, respectively; bivariate correlation analysis).

The αsAPP concentration in the CSF of patients (pooled data from NMO, MS and

![FIGURE 1: Western blot analysis of soluble amyloid precursor protein (sAPP) in the cerebrospinal fluid of the patients with multiple sclerosis (MS; classic MS [CMS] or the opticospinal form of MS [OSMS]), neuromyelitis optica (NMO) or clinically isolated syndrome (CIS) patients and healthy controls. One band (sAPP), corresponding to a molecular weight of 110 kDa was visualized in all MS, NMO, CIS patients and healthy controls, without any significant differences in the intensity (grey scale) of expression between the groups (tubulin used as the internal protein standard)](image)

### TABLE 3:

Concentrations of α-cleaved soluble amyloid precursor protein (αsAPP) and β-amyloid 42 (Aβ42) in cerebrospinal fluid (CSF) of the multiple sclerosis (MS), neuromyelitis optica (NMO) and clinically isolated syndrome (CIS) patients and healthy control subjects studied

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases (n)</th>
<th>αsAPP in CSFa (ng/ml)</th>
<th>Cases (n)</th>
<th>Aβ42 in CSFb (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMO</td>
<td>7</td>
<td>5.151 (3.462 – 30.713)</td>
<td>8</td>
<td>106.80 ± 25.70</td>
</tr>
<tr>
<td>MS</td>
<td>31</td>
<td>8.724 (1.221 – 52.54)</td>
<td>25</td>
<td>104.78 ± 13.73</td>
</tr>
<tr>
<td>CIS</td>
<td>22</td>
<td>8.659 (3.69 – 86.156)</td>
<td>19</td>
<td>134.13 ± 25.06</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>15.577 (6.093 – 29.251)</td>
<td>11</td>
<td>137.02 ± 23.35</td>
</tr>
</tbody>
</table>

Statistical significancec
F-valuec

Data presented as: a median (range); b mean ± SD.

^
One-way analysis of variance was used to analyse between-group differences (data were log transformed prior to analysis due to a non-normal distribution).

NS, not statistically significant (P > 0.05).
CIS patients) treated with statins was significantly higher than in patients not treated with statins ($P = 0.023$). There were no significant differences in the $\alpha$-APP and $\beta\text{-amyloid}$ 42 (A$\beta$42) concentrations in the CSF of patients with or without brain atrophy or with or without corticosteroid treatment (Table 4).

**Discussion**

The accumulation of APP in axonal ovoids is a sensitive marker for acute axonal injury in MS lesions$^{2,14}$ but, to date, only a few studies have been reported on APP or APP-derived soluble proteins in the CSF or serum of patients with MS. Reports on the

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**TABLE 4:**

<table>
<thead>
<tr>
<th>Associations between $\alpha$-cleaved soluble amyloid precursor protein ($\alpha$sAPP) and $\beta$-amyloid 42 (A$\beta$42) concentrations in cerebrospinal fluid and disease disability (EDSS), disease duration, atrophy and therapy use in the patients studied (pooled data for patients with multiple sclerosis, neuromyelitis optica and clinically isolated syndrome)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>$\alpha$sAPP$^a$, ng/ml</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>A$\beta$42$^b$, pg/ml</td>
</tr>
</tbody>
</table>

| | Disease duration | Statistical significance$^c$ | F-value |
| | $\geq 1$ year | $< 1$ year | |
| $\alpha$sAPP$^a$, ng/ml | 8.724 | 8.465 | NS | 0.369 |
| | (2.780 – 52.540) | (1.221 – 86.156) | |
| A$\beta$42$^b$, pg/ml | 112.20 ± 15.70 | 136.62 ± 23.15 | NS | 0.747 |

<table>
<thead>
<tr>
<th>Atrophy on MRI</th>
<th>Statistical significance$^c$</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
<td></td>
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<tr>
<td>$\alpha$sAPP$^a$, ng/ml</td>
<td>7.685</td>
<td>8.724</td>
</tr>
<tr>
<td></td>
<td>(2.780 – 30.713)</td>
<td>(1.221 – 86.156)</td>
</tr>
<tr>
<td>A$\beta$42$^b$, pg/ml</td>
<td>110.24 ± 25.09</td>
<td>129.71 ± 16.99</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Corticosteroid therapy</th>
<th>Statistical significance$^c$</th>
<th>F-value</th>
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</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>$\alpha$sAPP$^a$, ng/ml</td>
<td>8.756</td>
<td>5.947</td>
</tr>
<tr>
<td></td>
<td>(2.780 – 52.540)</td>
<td>(1.221 – 86.156)</td>
</tr>
<tr>
<td>A$\beta$42$^b$, pg/ml</td>
<td>130.08 ± 17.00</td>
<td>109.17 ± 24.92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Statin therapy</th>
<th>Statistical significance$^c$</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>$\alpha$sAPP$^a$, ng/ml</td>
<td>11.907</td>
<td>7.766</td>
</tr>
<tr>
<td></td>
<td>(2.780 – 86.156)</td>
<td>(1.221 – 27.790)</td>
</tr>
<tr>
<td>A$\beta$42$^b$, pg/ml</td>
<td>135.45 ± 17.10</td>
<td>114.53 ± 22.34</td>
</tr>
</tbody>
</table>

Data presented as: $^a$median (range); $^b$mean ± SD.

$^c$One-way analysis of variance was used to analyse between-group differences (data were log transformed prior to analysis due to a non-normal distribution).

EDSS, Expanded Disability Status Scale; MRI, magnetic resonance imaging; NS, not statistically significant ($P > 0.05$).
concentrations of Aβ42, αsAPP and βsAPP in the CSF or serum of patients with MS have been contradictory, ranging from no significant change to reduced or increased. In the present study, no significant differences were observed in αsAPP and Aβ42 CSF levels among MS, NMO, CIS patients or the controls. These data do not support the original assumption that levels of sAPP and Aβ42 in the CSF of NMO, MS and CIS patients may be increased, but they do agree with the study by Hein Née Maier et al.

Previous research suggested a correlation between the numbers of APP-positive axons in MS lesions and disease duration. For example, most APP-positive axons were detected within the first year after onset, while only a few were detected in the lesions of patients with a disease duration ≥ 10 years. It was also observed that, in MS patients, β-site APP-cleaving enzyme 1 activity, which correlated weakly with the levels of Aβ42, α-sAPP and β-sAPP, was decreased over 10 years. In contrast, Valis et al. found no correlation between Aβ42 level and disease duration in patients with MS, CIS and controls. There were no differences in the αsAPP and Aβ42 concentrations in the CSF of patients in the present study based on disease duration (< 1 year versus ≥ 1 year). The reason for this finding is not clear, but might be because αsAPP and Aβ42 levels were compared for a group of individuals at different disease stages rather than in the same individual at different disease stages. Many factors, such as individual and environmental differences, might have influenced these results.

Axonal damage has been demonstrated to be the major contributor of permanent neurological deficits in MS patients, which has been shown to relate to atrophy of the spinal cord, cerebellum and cerebral cortex. Thus, in the present study, associations between αsAPP and Aβ42 levels and functional disability and brain atrophy were assessed. A weak inverse correlation between β-site APP-cleaving enzyme 1 activity and EDSS score has been reported in patients with MS. In contrast, no correlation between EDSS score and the concentration of Aβ was detected in MS and CIS patients. There was an inverse correlation between the CSF αsAPP concentration and EDSS in patients in the present study, which was in accord with the results of Mattsson et al. APP accumulation seems to occur predominantly in the early disease stage and persists in transected axons for a period of < 30 days. MS patients with serious disability are usually those who have experienced a long relapsing-remitting disease course or disease progression rather than those in the early stage of the disease and may be why an inverse rather than a positive correlation was observed between αsAPP level and disability. The present study demonstrated no significant differences in concentrations of αsAPP and Aβ42 in the CSF of patients with or without brain atrophy. Due to the use of two-dimensional MRI images, quantitative estimation of atrophy could not be made; only a qualitative classification of atrophy as either present or absent could be made. Further research using three-dimensional MRI images is needed to confirm whether there is an association between APP levels and the extent of brain atrophy.

Treatment with 3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitors (statins) has been associated with decreased Aβ production in animal models and a reduced prevalence of Alzheimer's disease in epidemiological studies. Putative
neuroprotective effects of statins in Alzheimer's disease have been suggested, but the detailed mechanisms remain unclear. Some clinical studies suggest that statin treatment may have an impact on APP processing\textsuperscript{34} and Aβ production\textsuperscript{35,36} through the lowering of cholesterol, while others suggest otherwise.\textsuperscript{7,37,38} Research has shown that αsAPP is probably involved in the regulation of key neural functions including cell excitability, synaptic transmission, long-term potentiation, learning and memory.\textsuperscript{39} The present study showed that the αsAPP concentration in the CSF of patients treated with statins was significantly higher than in those patients not treated with statins, suggesting that statins might have a neuroprotective effect. Those patients treated with statins received the routine dosage for hypercholesterolaemic therapy (20 mg/day). Further studies on the effects of statin treatment at different doses on CSF APP biomarker processing are needed.

There is evidence that, after corticosteroid treatment, Aβ\textsubscript{42} and APP levels in the CSF of patients with systemic lupus erythematosus are significantly decreased,\textsuperscript{40} indicating that corticosteroids might affect either the production of APP or the activity of secretases. It has also been demonstrated that anti-inflammatory drugs, such as corticosteroids, might directly modulate γ-secretase activity and thereby decrease Aβ\textsubscript{42} production.\textsuperscript{41,42} In contrast, another study in non-human primates demonstrated that glucocorticoid treatment might increase Aβ\textsubscript{42} levels in the brain by inhibiting the activity of insulin-degrading enzyme and its binding to insulin.\textsuperscript{43} The present study showed that CSF concentrations of Aβ\textsubscript{42} and αsAPP were not significantly different with or without corticosteroid treatment. The reason for this is not clear, but it is possible that corticosteroid treatment has different effects on the production and degradation of APP. The mechanism of action of corticosteroid treatment on APP production and degradation and the clinical relevance of these effects need to be investigated further.

In summary, the present study demonstrated that αsAPP is present at similar levels in the CSF of NMO, MS and CIS patients and healthy controls. There was an inverse correlation between the CSF αsAPP concentration and EDSS, indicating that APP, a sensitive marker of axonal damage, correlates with a lower level of neurological disability. Additionally, the CSF concentration of αsAPP in patients treated with statins was significantly higher than in patients not treated with statins, indicating that statins may have a neuroprotective effect.

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Conflicts of interest
The authors had no conflicts of interest to declare in relation to this article.

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