Cerebrospinal fluid neuron-specific enolase: a further marker of Alzheimer’s disease?

Barbara Palumbo, MD a
Donatella Siepi, BSc a
Isabella Sabalich, MD a
Cristina Tranfaglia, MD a
Lucilla Parnetti, MD, PhD b

a Nuclear Medicine Section, Department of Radiological Sciences, University of Perugia, Italy
b Department of Neurosciences, University of Perugia, Italy

Corresponding author: Barbara Palumbo
Nuclear Medicine Section
Department of Surgical, Radiological and Odontostomatology Sciences, University of Perugia, Policlinico Monteluce
Via Brunamonti - 06122 Perugia - Italy
E-mail: mednuc@unipg.it

Received: January 2008
Accepted for publication: January 2008

Summary

To investigate whether neuron-specific enolase (NSE) plays a role in dementia, we measured cerebrospinal fluid (CSF) concentrations of NSE, Abeta42 and total protein tau (h-tau) in different dementia patients. We studied 159 patients: 76 with Alzheimer’s disease (AD), 35 with mild cognitive impairment (MCI), 28 with fronto-temporal dementia (FTD), and 20 with Lewy body disease (LBD). Thirty healthy age-matched subjects were studied as controls. NSE was measured by immunoradiometric assay, Abeta42 and h-tau were dosed by ELISA assay.

Mean CSF NSE was significantly higher in AD (15.1±9.9 ng/ml) than in controls (8.3±3.5 ng/ml, p<0.01), FTD (9.1±6.1 ng/ml, p<0.05) and MCI (9.7±7.8 ng/ml, p<0.05). Abeta42 was significantly lower in AD (413.8±163.7 pg/ml) than in MCI (708.4±422.1 pg/ml, p<0.001) and controls (914.4±277.1 pg/ml, p<0.05); it was also significantly reduced in FTD (497.1±221.9 pg/ml) versus MCI (p<0.05) and controls (p<0.001); and in LBD patients (477.1±225.7 pg/ml) compared with MCI (p<0.05) and controls (p<0.001).

H-tau concentration was significantly higher in AD (607.9±372.3 pg/ml, p<0.001) than in MCI (383.8±277.9 pg/ml, p<0.05), controls (176.6±43.9 pg/ml, p<0.001) and LBD (472.3±357.7 pg/ml, p<0.05); it was also increased in FTD (541.7±362.8 pg/ml) versus controls (176.6±43.9 pg/ml, p<0.001).

Furthermore, NSE was inversely correlated with Abeta42 (r=0.333, p=0.0001) and directly correlated with h-tau (r=0.370, p=0.0001). In conclusion, CSF NSE emerged as a specific indicator of AD and showed the same behaviour as the other accepted markers of AD, being correlated with both biomarkers.

KEY WORDS: Alzheimer’s disease, cerebrospinal fluid, fronto-temporal dementia, Lewy body disease, mild cognitive impairment, neuron-specific enolase.

Introduction

The identification of biochemical markers is an important issue in Alzheimer’s disease (AD). Cerebrospinal fluid (CSF) levels of β-amyloid (1-42) protein (Abeta42), the main component of neuritic plaques, are significantly reduced in AD patients, while CSF levels of protein tau, a constituent of neurofibrillary tangles, are significantly increased and the frequency of ε4 allele of apolipoprotein-E is raised (1-7). Furthermore, it has been shown that a monoclonal antibody (mAb 22.212), raised against a synthetic C-terminal peptide of β/A4-protein (residues 28-40) labelling senile plaques in AD after proteolysis of tissue section, is able to cross-react with a soluble protein found in brain homogenates, which was found to be NSE (10). Therefore, the cross-reaction of a conformational NSE epitope with a monoclonal antibody raised against β/A4-protein in the AD brain suggests a possible association between NSE and amyloid plaques (10).

With the aim of evaluating the possible role of NSE as biological marker of dementia disorders, we measured the CSF concentration of NSE in 159 patients with different dementia disorders (probable AD; mild cognitive impairment, MCI; fronto-temporal dementia, FTD; and Lewy body disease, LBD) and in 30 control subjects also undergoing Abeta42 and total tau (h-tau) measurement.

Materials and methods

The study sample comprised 76 patients (37 males and 39 females; age range: 69-82 years; disease duration: 2-4 years) affected by probable AD, diagnosed according to NINCDS-ADRDA criteria (11). Their Mini Mental State Examination (MMSE) scores ranged from 10 to 24, while they had Clinical Dementia Rating (CDR) scores of 1-2, Hachinski Ischemic Scale scores <4, and Hamilton Depression Rating Scale scores <18. All these subjects had CT or MRI examinations that were negative for focal lesions.

Thirty-five patients (16 males and 19 females; age range: 62-84 years) had MCI diagnosed according to
Petersen’s criteria (12); their MMSE scores ranged from 22 to 26 and they all had a CDR score of 0.5 (questionable dementia).

Twenty-eight patients (12 males and 16 females; age range: 61-79 years) were affected by FTD, diagnosed according to the Lund and Manchester criteria (13); these patients’ MMSE scores ranged from 9 to 24 and they had CDR scores of 1-2.

Twenty patients (10 males and 10 females; age range: 63-80 years) suffered from LBD diagnosed according to the McKeith criteria (14); these patients had MMSE scores ranging from 13 to 24 and CDR scores of 1-2.

The control group consisted of 30 sex- and age-matched non demented (MMSE ≥ 27) subjects referred either to the urology or to the gynaecology department for minor surgical problems requiring spinal anaesthesia; their clinical history was negative for neuropsychiatric disorders and major medical diseases.

CSF samples were collected in the morning after a ten-hour overnight fast and were kept frozen at -80°C until assayed. NSE was measured by a solid phase two-site immunoradiometric assay (ELSA-NSE, CIS bio International SpA, Milan) as previously described (8). Aβ42 and h-tau were measured by ELISA assay (Innogenetics NV, Ghent, Belgium) as previously described (1,15,16). One-way ANOVA was carried out, taking p<0.05 as the level of significance.

### Results

The mean values of NSE, Aβ42 and h-tau in the different groups of subjects studied are reported in Table I, while figure 1 shows the distribution of the NSE concentrations in these groups.

The mean CSF NSE level was significantly higher in AD patients (mean±SD: 15.1±9.9 ng/ml) than in controls (mean±SD: 8.3±3.5 ng/ml, p<0.01), FTD (mean±SD: 9.1±6.1 ng/ml, p<0.05) and MCI subjects (mean±SD: 9.7±7.8 ng/ml, p<0.05). No statistically significant difference was observed between the mean NSE of the AD patients and that of the LBD subjects (mean±SD: 11.3±8.8 ng/ml). The NSE levels of MCI, FTD and LBD patients were not significantly increased with respect to controls.

As expected, Aβ42 was significantly lower in AD (413.8±163.7 pg/ml) than in MCI patients (708.4±422.1 pg/ml, p<0.001) and controls (914.4±277.1 pg/ml, p<0.05); it was also significantly reduced in FTD (497.1±221.9 pg/ml) versus MCI (708.4±422.1 pg/ml, p<0.05) and controls (914.4±277.1 pg/ml, p<0.001); furthermore, Aβ42 was significantly decreased in LBD patients (477.1±225.7 pg/ml) compared with MCI patients (p<0.05) and controls (p<0.001).

Total-tau (h-tau) was significantly increased in AD (607.9±372.3 pg/ml, p<0.001) compared with MCI (383.8±277.9 pg/ml, p<0.05), controls (176.6±43.9 pg/ml, p<0.001) and LBD (472.3±357.7 pg/ml, p<0.05); h-tau was also increased in FTD (541.7±362.8 pg/ml) with respect to controls (176.6±43.9 pg/ml, p<0.001); NSE showed a negative correlation with Aβ42 (r=-0.333, p<0.0001; Fig. 2) and a positive correlation with h-tau (r=0.370, p<0.0001, Fig. 3).

### Discussion

In the present study, CSF NSE was found to be a specific marker of neuronal damage in AD, being significantly increased in this group of patients compared with all the other categories except for LBD.

Blennow and co-workers (17) found significantly higher levels of CSF NSE in both AD and vascular dementia (VaD) subjects compared with all the other categories except for LBD.

### Table I - Concentrations of NSE, Aβ42 and h-tau in the different groups of subjects studied.

<table>
<thead>
<tr>
<th></th>
<th>NSE (ng/ml) (mean±SD)</th>
<th>Aβ42 (pg/ml) (mean±SD)</th>
<th>h-tau (pg/ml) (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD (n=76)</td>
<td>15.1±9.9</td>
<td>413.8±163.7</td>
<td>607.9±372.3</td>
</tr>
<tr>
<td>MCI (n=36)</td>
<td>9.7±7.8</td>
<td>708.4±422.1</td>
<td>383.8±277.9</td>
</tr>
<tr>
<td>FTD (n=28)</td>
<td>9.1±6.1</td>
<td>497.1±221.9</td>
<td>541.7±362.8</td>
</tr>
<tr>
<td>LBD (n=20)</td>
<td>11.3±8.8</td>
<td>477.1±225.7</td>
<td>472.3±357.7</td>
</tr>
<tr>
<td>Controls (n=30)</td>
<td>8.3±3.5</td>
<td>914.4±277.1</td>
<td>176.6±43.9</td>
</tr>
</tbody>
</table>

Abbreviations and symbols: AD=Alzheimer’s disease; MCI=mild cognitive impairment; FTD=fronto-temporal dementia; LBD=Lewy body disease; * p<0.01 vs C, p<0.05 vs FTD and MCI; † p<0.05 vs MCI, p<0.001 vs C; • p<0.001 vs MCI, p<0.05 vs C; ‡ p<0.05 vs MCI, p<0.001 vs C.
that CSF NSE is not a disease-specific marker of the neuronal degeneration in dementia disorders. We carried out a similar investigation in a smaller number of patients investigating AD, VaD and control subjects (8). Although NSE was found to be increased in AD versus controls, statistical significance was not reached; however, a clear association between CSF NSE levels and degree of cognitive impairment was documented, again indicating that NSE might be a marker of the progressive neuronal loss taking place in AD.

In a more recent paper by Infante and co-workers (9), S-100 protein and NSE were measured in the CSF of different groups of patients (meningitis, dementia, polyneuropathy-motorneuron disease and acute cerebral infarction) and of control subjects. A significant increase in S-100 and NSE concentration was observed in dementia patients with respect to controls, while S-100 was also increased in the meningitis and acute cerebral infarction categories compared with the control group. This led the authors to conclude that these biological parameters could constitute sensitive markers of brain damage in different neurological disorders. In our opinion, the paper by Infante et al. seems to support a role of NSE in overt dementia.

In the present study, NSE was increased only in dementia of the Alzheimer’s type, while it was not significantly altered in MCI or in other kinds of dementia. This is of interest because it confirms the significance of NSE in AD. Furthermore, it is worth noting the presence of a correlation between NSE and the well-known markers of AD, Abeta42 and tau protein. In recent papers, a relationship between NSE and amyloid protein was described. The cross-reaction of a conformational NSE epitope with a monoclonal antibody raised against ß/A4-protein in the AD brain was clearly documented (10). Furthermore, Hwang et al. (18) showed that the regulation of amyloid precursor protein (APPsw) genes was expressed under the control of the NSE promoter in a brain-specific manner; in addition, Hwang et al. cited a previous paper which reported that tau phosphorylation was observed in NSE/APPsw transgenic mice, but was not present in the brains of Tg2576 mice at 12 months (19). They suggested that the discrepancy between the two reports was likely to be caused by the potential NSE promoter activity; on this basis, Hwang et al concluded that the NSE promoter is more important in the direct targeting of Alzheimer-causing genes than other promoters. Finally, Jee (20) provided experimental evidence that the over-expression of the APPsw genes in the hippocampal regions of transgenic mice leads to differential expression of a total of 119 genes. The magnitude of the change in expression of some of these genes (Xlr3b, Mup3, SerpinA9 and Ccr6) in the oligonucleotide-based analysis corresponded to those in the transgenic mice expressing the NSE-controlled APPsw gene.

In conclusion, in our study we demonstrated a selective increase in NSE in AD patients, a finding strengthened by a correlation of NSE with the classical markers of AD: amyloid beta and tau protein. If this result is confirmed in a larger series of patients, NSE might be considered as a further biological marker for early diagnosis of AD.

References

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