PERIODICUM BIOLOGORUM
VOL. 110, No 1, 27–30, 2008

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Mini review

Cerebrospinal fluid phosphorylated tau proteins as predictors of Alzheimer's disease in subjects with mild cognitive impairment

GORAN ŠIMIĆ[1]
MARINA BOBAN[2]
PATRICK R. HOF[3]

[1] Department of Neuroscience
Croatian Institute for Brain Research
Medical School Zagreb, Šalata 12
10000 Zagreb, Croatia

[2] University Hospital Center »Rebro« Zagreb
Kišpatićeva 12, 10000, Zagreb, Croatia

[3] Department of Neuroscience
Mount Sinai School of Medicine
One Gustave L. Levy Place, New York,
NY 10029, U.S.A.

Correspondence:
Goran Šimić
Department of Neuroscience
Croatian Institute for Brain Research
Medical School Zagreb, Šalata 12
10000 Zagreb, Croatia

Abbreviations:
AD – Alzheimer's disease
CSF – cerebrospinal fluid
MCI – mild cognitive impairment

Received September 4, 2007.

Abstract

Major efforts are under way to define reliable biomarkers of Alzheimer's disease. Highly significant increases of hyperphosphorylated tau proteins in cerebrospinal fluid have been recently reported in Alzheimer's disease patients compared to controls by several independent groups, including ours. These findings support the notion that cerebrospinal fluid phosphorylated tau proteins may be very useful biomarkers in the early identification of Alzheimer's disease in patients with mild cognitive impairment.

INTRODUCTION

Mild cognitive impairment (MCI) is etiologically heterogeneous syndrome defined by cognitive impairment that can be shown by objective neuropsychological measures adjusted for age and education. About 40–60% of MCI patients develop Alzheimer's disease (AD) during the first 5 years (approximately 12–15% yearly; in contrast, only 1–2% of healthy older population convert to AD per year) *(1)*, whereas the rest of the MCI patients have a less progressive form of memory impairment *(1, 2)*. Other types of dementia, such as dementia with Lewy bodies and vascular dementia, may also be preceded by MCI *(2)*. The amnestic subtype of MCI (i.e., memory complaint with objective memory impairment, but with preservation of general cognitive functioning without or with minimum impairment of activities of daily living) mostly represents prodromal AD *(3)*. Clinically, AD cannot be diagnosed until dementia is present. Although the NINCDS-ADRDA criteria *(4)* have a relatively high accuracy rate (around 80–90%) *(5, 6)*, these percentages come from specialized expert research academic centres and are based on patients in later stages of the disease who were followed longitudinally for several years before autopsy. In clinical settings, there is no clinical method to determine which patients with MCI will progress to dementia, except for a very long clinical follow-up. On the other side, the introduction of new treatments and drug-modifying therapies that are supposed to have best effect if introduced early, as well as increased general knowledge on AD in the population, have facilitated patients and their families to seek medical advice earlier during the course of the disease. Therefore, in recent years, the preclinical phase of AD with mild memory impairment, but without overt de-

mentia, has been the topic of increased attention. In this article, we tried to evaluate the value of some CSF markers in predicting AD in MCI population.

SEARCH FOR AD BIOMARKERS

The pathogenic process in AD starts about 20–30 years before the clinical presentation of the disease *(7, 8)*. During this preclinical period there is gradual accumulation of a variable amounts of amyloid plaques and neurofibrillary tangles, and at a certain threshold the first symptoms appear *(9, 10)*. Although clinicopathological correlations show best correlation of dementia with the quantity of neurofibrillary tangles *(11)*, the pattern of synapse and neuron loss in AD does not necessarily match the pattern of tangle formation *(12)*. Because the cerebrospinal fluid (CSF) is in direct contact with the extracellular space of the central nervous system, it is hoped that pathological processes characteristic of AD are somehow reflected in CSF composition. It is also logical to assume that candidate biomarkers for AD should be proteins, or molecules that are intimately related with the key pathogenic processes in the brain, i.e. the aggregation of amyloid-ß with subsequent formation of plaques, and the hyperphosphorylation of microtubule-associated tau protein with subsequent formation of neurofibrillary tangles. Hence, several studied CSF biomarkers (total tau, amyloid-ß isoforms, in particular the amyloid-ß42 protein and especially several tau proteins phosphorylated on different epitopes (e.g., on threonine 181 and 231, and on serine 199) have been found to have the highest diagnostic potential.

AMYLOID BETA

Amyloid plaques are extracellular aggregates with 42 aa amyloid-ß peptide (amyloid-ß42) as a major component *(13)*. The usual finding of decreased concentration of amyloid-ß42 in CSF of patients with AD is explained by its accumulation in plaques. This is supported by a strong correlation between the number of plaques found in neocortex and hippocampus and low concentration of amyloid-ß42 in CSF taken from intraventricular space *(14)*. Amyloid-ß42 has shown high sensitivity levels (78–100%) with relatively low specificity (47–81%) in differentiation of patients with AD and healthy controls *(15, 16)*.

TAU PROTEIN

Contrary to amyloid-beta, concentrations of microtubule-associated protein tau in CSF are increasing in cases of axonal damage and therefore potentially reflect neuronal degeneration and/or neuronal damage *(16, 17)*. This can happen not only in AD, but in a variety of acute and subacute neurological disorders, such as acute stroke *(18)* and Creutzfeldt-Jakob disease *(19)*. When comparing AD patients and healthy controls CSF total tau protein reached a sensitivity of 40–86% and a specificity of 65–86% *(20)*. Total tau concentration in CSF roughly correlates with the transition towards marked cognitive impairment *(16)*.

PHOSPHORYLATED TAU PROTEIN

In patients with AD tau protein is hyperphosphorylated and oxidized. Due to hyperphosphorylation tau loses its ability to bind to the microtubules and promote their assembly *(21)*. More than 30 phosphorylation sites have been described on tau in the brain *(22)*. In contrast to concentration of total tau protein, concentrations of phosphorylated tau protein are not increased in CSF of patients with acute stroke *(18)* and Creutzfeldt-Jakob disease *(23)*. With specificity of 92% in differentiating AD patients and healthy controls, average sensitivity for phosphorylated tau was 80% *(17)*. In comparison with total tau, phosphorylated tau has also showed higher specificity in distinguishing AD from other types of dementia (tau protein phosphorylated on threonine 181 showed sensitivity of 71% and specificity of 94% when differentiating AD patients to healthy controls in comparison to total tau protein which reached sensitivity of 63% with specificity of 100% for same group differentiation) *(15)*.

CSF BIOMARKERS IN MCI

With the advent of new drugs such as gamma-secretase inhibitors, early detection of elderly subjects with mild cognitive impairment (MCI) who are destined to develop AD is becoming increasingly important. The three CSF biomarkers mentioned above (total tau, amyloid-ß42 protein and phosphorylated tau protein) have been evaluated in numerous studies *(16, 17, 20)*. Most of these investigations confirmed that CSF markers have high sensitivity to differentiate early and incipient AD from normal aging, major depressive disorder, alcoholic dementia and Parkinson's disease, but lower specificity against other primary causes of dementia syndrome, such as frontotemporal and Lewy body dementia. Up to this moment, only a few studies that evaluated cerebrospinal fluid biomarkers and followed MCI patients for mainly 1–2 years have been published. It has been shown that in group of MCI patients, high total tau levels discriminate MCI patients that progress to AD from those with benign form of MCI (that do not progress to AD) with sensitivity of 90% and specificity of 100% *(24)*. In another study, low amyloid-ß42 and high total tau protein in cerebrospinal fluid were found in 90% of the MCI cases that progressed to AD as compared with the 10% stable MCI cases *(25)*. Similarly, higher concentrations of tau protein phosphorylated on threonine 231 were found in MCI cohort that progressed to AD compared to those with stable MCI *(26)*. Additionally, Maruyama *et al. (27)* followed 57 MCI patients for a period of approximately 2 years and found higher levels of total tau protein in subjects with the progressive form of MCI. One investigation confirmed previous studies and showed that concentrations of amyloid-ß42 and total tau protein from cerebrospinal fluid could be valuable biomarkers for early detection of AD in MCI cohort with approximate follow-up period of 8.4 months *(28)*. Finally, Parnetti *et al. (29)* have evaluated significance of three CSF biomarkers (total tau protein, amyloid-ß42 protein, tau protein phosphorylated on threo-

nine 181) in prediction of conversion to AD in patients with MCI after one year follow-up period and reported that pathological levels in two or more CSF biomarkers reliably predict MCI conversion to AD and correctly identify the stable form of MCI.

SOME PROBLEMS

The major pitfall when studying CSF biomarkers to predict AD in MCI cohorts is the fact that conversion from MCI to AD is only about 12–15% per year. Therefore, only an extensive follow-up time (>5 years) of patients with stable MCI might further increase the specificity of CSF biomarkers. Two studies from a group from Finland that followed patients over period of 3 and 3.5 years, respectively, were recently published. In both of them CSF levels of amyloid-ß42, total tau and phosphorylated tau proteins were measured in patients with MCI and healthy controls *(30, 31)*. In the first study, the combination of amyloid-ß42 and phosphorylated tau reached the highest level of accuracy for prediction of MCI progression *(32)*. Whereas in the latter combination of four parameters (amyloid-ß42, total tau and phosphorylated tau protein as well as apolipoprotein e4 genotype) was highly predictive for the dementia in MCI patients with amnestic or executive symptoms *(33)*. The results of the probably most valuable study (because of the longest follow-up period of 4–6 years, rather large number of participants, as well as heterogeneity of possible dementia outcomes, such as progression of MCI to vascular, diffuse Lewy body dementia, frontotemporal dementia) suggested that by using the initial concentration of biomarkers (amyloid-ß42 protein, total tau and tau protein phosphorylated on threonine 181), it is possible to predict accurately the progression of MCI patients to AD *(34)*. Interestingly, initial concentrations of above mentioned biomarkers were useful not only to differentiate progressive MCI patients (those who progressed to AD) from stable MCI patients, but also progressive MCI group from those who progressed to other type of dementia. Yet in another study, the same authors showed the usefulness of the Aβ42/Aβ40 ratio as a predictive biomarker for AD and confirmed that amyloid precursor protein (APP) metabolism is disturbed early in the course of AD *(33)*. Our group has investigated usefulness of three CSF parameters (total tau protein, tau protein phosphorylated on threonine 181 and serine 199) in predicting MCI progression to AD. On a rather small sample size (13 MCI patients) that we followed for a period of at least two years (in that time 5 of them developed AD), our results were comparable to those from other, previously mentioned studies. CSF levels of total tau protein and tau protein phosphorylated on threonine 181 showed excellent sensitivity and specificity when comparing MCI patients that progressed to AD and stable MCI group (specificity when sensitivity was set at 85% or more was up to 100% for total tau protein and 80% for tau protein phosphorylated on threonine 181) (data not shown).

OBTAINING AND STORAGE OF CSF, PERFORMING ELISA

Lumbar puncture has to be performed to obtain CSF. This procedure can have quite unpleasant side effects causing postlumbar puncture headache, which is, however, in MCI patients older than 60 unusual with an incidence below 2% *(34, 35)* and hence a largely overestimated problem. CSF should be collected in polypropylene tubes and separated in aliquots in separate polypropylene tubes to avoid repeated freeze-thaw cycles which could lead to decrease in protein concentration. At the end, tubes should be stored at –80°C until analysis. For measurement of CSF biomarkers we are using commercially available ELISA kits (e.g. Innotest Phospho-Tau$_{(181p)}$; Innogenetics, Ghent, Belgium for measuring CSF levels of tau protein phosphorylated on threonine 181).

CONCLUSION

From the above mentioned studies, it is evident that CSF biomarkers are positive early in development of AD and might be ancillary tool in differentiation and early detection of patients prone to develop AD (which would, in turn, accelerate proper treatment). Evidently, more longitudinal studies are needed to clarify this issue. Additionally, increasing numbers of investigators are trying, by using proteomic analysis, to discover novel proteins and peptides able to differentiate progressive MCI patients from stable MCI group. A recent study discovered several potential biomarkers among which 5 were relevant to the pathogenesis of AD *(36)*. This kind of investigations could in the future help in better understanding of the intricate molecular pathways underlying AD pathogenesis.

REFERENCES

1. DECARLI C 2003 Mild cognitive impairment: prevalence, prognosis, aetiology, and treatment. *Lancet Neurol 2:* 15–21
2. PETERSEN R C 2004 Mild cognitive impairment as a diagnostic entitiy. *J Intern Med 256:* 183–94
3. DUBOIS B, ALBERT M L 2004 Amnestic MCI or prodromal AD? *Lancet Neurol 3:* 246–248
4. MCKHANN G, DRACHMAN D, FOLSTEIN M, KATZMAN R, PRICE D, STADLAN E M 1984 Clinical diagnosis of AD: report of the NINCDS-ADRDA Work Group under the auspices of department of health and human services task force on AD. *Neurology 34:* 939–944
5. GALASKO D, HANSEN L A, KATZMAN R, WIEDERHOLT W, MASLIAH E, TERRY R, HILL L R, LESSIN P, THAL L J 1994 Clinicalneuropathological correlations in AD and related dementias. *Arch Neurol 51:* 888–895
6. JELLINGER K A 1996 Diagnostic accuracy of AD: a clinicopathological study. *Acta Neuropathol 91:* 219–20
7. PRICE J L, MORRIS J C 1999 Tangles and plaques in nondemented aging and »preclinical» AD. *Ann Neurol 45:* 358–368
8. DAVIES L, WOLSKA B, HILBICH C, MULTHAUP G, MARTINS R, SIMMS G, BEYREUTHER K, MASTERS C L 1988 A4 amyloid protein deposition and the diagnosis of AD: prevalence in aged brains determined by immunocytochemistry compared with conventional neuropathologic techniques. *Neurology 38*: 1688–1693
9. BRAAK H, BRAAK E 1995 Staging of AD-related neurofibrillary changes. *Neurobiol Aging 16*: 271–278
10. BRAAK H, BRAAK E 1998 Evolution of neuronal changes in the course of AD. *J Neural Transm (Suppl) 53*: 127–140

11. BIERER L M, HOF P R, PUROHIT D P, CARLIN L, SCHMEIDLER J, DAVIS K L, PERL D P 1995 Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. *Arch Neurol 52:* 81–88
12. HOF P R, BUSSIERE T, GOLD G, KOVARI E, GIANNAKOPOULOS P, BOURAS C, PERL D P, MORRISON J H 2003 Stereologic evidence for persistence of viable neurons in layer II of the entorhinal cortex and the CA1 field in Alzheimer's disease. *J Neuropathol Exp Neurol 62:* 55–67
13. PARIHAR M S, HEMNANI T 2004 AD pathogenesis and therapeutic interventions. *J Clin Neurosci 11:* 456–67
14. STROZYK D, BLENNOW K, WHITE L R, LAUNER L J 2003 CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology 60:* 652–656
15. HAMPEL H, MITCHELL A, BLENNOW K, FRANK R A, BRETTSCHNEIDER S, WELLER W, MÖLLER M J 2004 Core biological marker candidates of AD – perspectives for diagnosis, prediction of outcome and reflection of biological activity. *J Neural Transm 111:* 247–72
16. STEFANI A, MARTORANA A, BERNARDINI S, PANELLA M, MERCATI F, ORLACCHIO A, PIERANTOZZI M 2006 CSF markers in Alzheimer's disease patients are not related to the different degree of cognitive impairment. *J Neurol Sci 251:* 124–128
17. BLENNOW K, HAMPEL H 2003 CSF markers for incipient AD. *Lancet Neurol 2:* 605–613
18. HESSE C, ROSENGREN L, ANDREASEN N, DAVIDSSON P, VANDERSTICHELE H, VANMECHELEN E, BLENNOW K 2001 Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. *Neurosci Lett 297:* 187–90
19. OTTO M, WILTFANG J, TUMANI H, ZERR I, LANTSCH M, KORNHUBER J, WEBER T, KRETZSCHMAR H A, POSER S 1997 Elevated levels of tau-protein in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. *Neurosci Lett 225:* 210–212
20. BLENNOW K, VANMECHELEN E, HAMPEL H 2001 CSF total tau, Abeta42 and phosphorylated tau protein as biomarkers for AD. *Mol Neurobiol 24*: 87–97
21. IQBAL K, ALONSO A D, GONDAL J A, GONG C X, HAQUE N, KHATOON S, SENGUPTA A, WANG J Z, GRUNDKE-IQBAL I 2000 Mechanism of neurofibrillary degeneration and pharmacologic therapeutic approach. *J Neural Transm (Suppl) 59:* 213–222
22. BUEE L, BUSSIERE T, BUEE-SCHERRER V, DELACOURTE A, HOF P R 2000 Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res Rev 33:* 95–130
23. RIEMENSCHNEIDER M, WAGENPFEIL S, VANDERSTICHELE H, OTTO M, WILTFANG J, KRETZSCHMAR H, VANMECHELEN E, FORSTL H, KURZ A 2003 Phospho-tau/total tau ratio in cerebrospinal fluid discriminates Creutzfeldt-Jakob disease from other dementias. *Mol Psychiatry 8:* 343–347
24. ARAI H, NAKAGAWA T, KOSAKA Y 1997 Elevated cerebrospinal fluid tau protein level as a predictor of dementia in memory-impaired patients. *Alzheimer Res 3:* 211–213
25. RIEMENSCHNEIDER M, LAUTENSCHLAGER N, WAGENPFEIL S, DIEHL J, DRZEZGA A, KURZ A 2002 Cerebrospinal fluid tau and beta-amyloid 42 proteins identify Alzheimer disease in subjects with mild cognitive impairment. *Arch Neurol 59:* 1729–1734
26. BUERGER K, TEIPEL S J, ZINKOWSKI R, BLENNOW K, ARAI H, ENGEL R, HOFMANN-KIEFER K, MCCULLOCH C, PTOK U, HEUN R, ANDREASEN N, DEBERNARDIS J, KERKMAN D, MOELLER H, DAVIES P, HAMPEL H 2002 CSF tau protein phosphorylated at threonine 231 correlates with cognitive decline in MCI subjects. *Neurology 59*: 627–9
27. MARUYAMA M, MATSUI T, TANJI H, NEMOTO M, TOMITA N, OOTSUKI M, ARAI H, SASAKI H 2004 Cerebrospinal fluid tau protein and periventricular white matter lesions in patients with mild cognitive impairment: implications for 2 major pathways. *Arch Neurol 61*: 716–20
28. HAMPEL H, TEIPEL S J, FUCHSBERGER T, ANDREASEN N, WILTFANG J, OTTO M, SHEN Y, DODEL R, DU Y, FARLOW M, MOLLER H J, BLENNOW K, BUERGER K. 2004 Value of CSF beta-amyloid1-42 and tau as predictors of AD in patients with mild cognitive impairment. *Mol Psychiatry 9:* 705–710
29. PARNETTI L, LANARI A, SILVESTRELLI G, SAGGESE E, REBOLDI P 2006 Diagnosing prodromal AD: role of CSF biochemical markers. *Mech Ageing Dev 127:* 129–32
30. HERUKKA S K, HALLIKAINEN M, SOININEN H, PIRTTILA T 2005 CSF Abeta42 and tau or phosphorylated tau and prediction of progressive mild cognitive impairment. *Neurology 64:* 1294–1297
31. HERUKKA S K, HELISALMI S, HALLIKAINEN M, TERVO S, SOININEN H, PIRTTILA T 2007 CSF Abeta42, Tau and phosphorylated Tau, APOE varepsilon4 allele and MCI type in progressive MCI. *Neurobiol Aging 28:* 507–14
32. HANSSON O, ZETTERBERG H, BUCHHAVE P, LONDOS E, BLENNOW K, MINTHON L 2006 Association between CSF biomarkers and incipient AD in patients with mild cognitive impairment: a follow-up study. *Lancet Neurol 5*: 228–34
33. HANSSON O, ZETTERBERG H, BUCHHAVE P, ANDREASSON U, LONDOS E, MIHTNOH L, BLENNOW K 2007 Prediction of AD using the CSF Abeta42/Abeta40 ratio in patients with mild cognitive impairment. *Dement Geriatr Cogn Disord 23:* 316–320
34. ANDREASEN N, MINTHON L, DAVIDSSON P, VANMECHELEN E, VANDERSTICHELE H, WINBLAD B 2001 Evaluation of CSF-tau and CSF-Aß42 as diagnostic markers for AD in clinical practice. *Arch Neurol 58:* 373–9
35. BLENNOW K, WALLIN A, HÄGER O 1993 Low frequency of post-lumbar puncture headache in demented patients. *Acta Neurol Scand 88:* 221–223
36. SIMONSEN A H, MCGUIRE J, HANSSON O, ZETTERBERG H, PODUST V N, DAVIES H A, WALDEMAR G, MINTHON L, BLENNOW K 2007 Novel panel of cerebrospinal fluid biomarkers for the prediction of progression to Alzheimer dementia in patients with mild cognitive impairment. *Arch Neurol 64:* 366–70