Use of cerebrospinal fluid biomarker analysis for improving Alzheimer's disease diagnosis in a non-specialized setting

Malnar, Martina; Kosiček, Marko; Bene, Raphael; Petek Tarnik, Iva; Pavelin, Sanda; Babić, Ivana; Brajenović-Milić, Bojana; Hećimovic, Hrvoje; Titlić, Marina; Trkanjec, Zlatko; ...

Source / Izvornik: **Acta Neurobiol Exp, 2012, 72, 264 - 271**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:184:933151

ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research Paper Acta Neurobiol Exp 2012, 72: 264–271

Use of cerebrospinal fluid biomarker analysis for improving Alzheimer's disease diagnosis in a non-specialized setting

Martina Malnar[1#], Marko Kosicek[1#], Raphael Bene[2#], Iva Petek Tarnik[3], Sanda Pavelin[4], Ivana Babic[5], Bojana Brajenovic-Milic[5], Hrvoje Hecimovic[2], Marina Titlic[4], Zlatko Trkanjec[2], Vida Demarin[2], and Silva Hecimovic[1]*

[1]Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia, *Email: silva.hecimovic@irb.hr; [2]Department of Neurology, University Hospital Sestre milosrdnice, Zagreb, Croatia; [3] Department of Nuclear Medicine and Oncology, University Hospital Sestre milosrdnice, Zagreb, Croatia; [4] Department of Neurology, University Hospital Split, Split, Croatia; [5]Department of Biology and Medical Genetics, School of Medicine, University of Rijeka, Rijeka, Croatia

[#]Authors equally contributed to the work

Low levels of amyloid-β42 (Aβ42) and high total-tau (t-tau) or phosphorylated-tau (p181-tau) levels in cerebrospinal fluid (CSF) were shown to be characteristic for Alzheimer's disease (AD) patients and for mildly cognitively impaired (MCI) or non-demented individuals who will progress to AD. The goal of this study was to evaluate the benefit of CSF biomarker testing in a setting with no specialized dementia centers, in order to improve the accuracy of AD diagnosis and to identify individuals with incipient AD. Using ELISA assay we analyzed CSF Aβ42, t-tau and p181-tau levels among clinically diagnosed non-demented individuals, AD patients and individuals with uncertain dementia *(n*=36). CSF cut-off values of low Aβ42 (≤530 pg/mL) and high t-tau (≥350 pg/mL) or p181-tau (≥52 pg/mL) were used to identify individuals with AD/MCI-CSF profile, regardless of clinical diagnosis. *APOE* genotyping was performed using PCR-RFLP method. In accord with previous studies we detected significantly decreased levels of CSF Aβ42 and increased tau and p181-tau levels in clinically diagnosed AD group vs. non-demented controls. CSF profiling identified individuals with a typical AD/MCI-CSF pattern in clinically referred non-demented group (9%) and among patients with uncertain dementia (41.7%). *APOE ε4*-allele was associated with the CSF biomarker changes typical for AD. This study shows that in a non-specialized setting CSF biomarker testing may be used as a screening tool for improving the accuracy of AD diagnosis and for predicting individuals with incipient Alzheimer's disease who need to receive further clinical follow-up.

Key words: Alzheimer's disease, amyloid-β, biomarkers, cerebrospinal fluid, diagnosis, tau

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia with no test available that would accurately diagnose the disease during lifetime and no adequate treatments that would cure, delay its onset or retard progression of the disease. Currently, there are 18 million people with Alzheimer's disease worldwide. With increasing life expectancy across the world, AD is a rapidly growing socioeconomic and medical problem. It is estimated that by the year 2040 there will be 81 million people affected with AD (Ferri et al. 2005). It is believed that novel therapies for AD will benefit the most mildly cognitively impaired individuals (MCIs) or preclinical AD cases that do not show gross pathological changes in the brain (Morris 2005). In this respect, neuroimaging techniques as well as biomarkers have been investigated in order to identify such cases, to improve the accuracy of clinical diagnosis of Alzheimer's disease and to monitor disease-modifying therapies. This is especially important in non-specialist settings where the clinical diagnostic accuracy is lower and where more expensive imaging techniques (PET) are not available. Until now, apolipoprotein E (*APOE*) *ε4* allele remains the strongest genetic risk factor for Alzheimer's disease (Corder et al. 1993, Saunders et al. 1993).

Correspondence should be addressed to S. Hećimović
Email: silva.hecimovic@irb.hr

Received 08 February 2012, accepted 19 May 2012

The key pathological features of Alzheimer's disease are amyloid plaques (extracellular aggregates of amyloid-β (Aβ) peptides) and neurofibrillary tangles (intracellular aggregates of hyperphosphorylated tau protein) in the brain. It appears that pathological processes that cause these aggregates begin 20−30 years before the onset of cognitive symptoms or significant neuronal loss (Morris 2005). Decreased concentrations of amyloid-β42 peptide (Aβ42) in the cerebrospinal fluid (CSF) have been consistently demonstrated in AD as well as in MCI cases together with increased concentrations of total-tau (t-tau) and/or phosphorylated tau protein at position threonine181 (p181-tau) (Hulstaert et al. 1999, Riemenschneider et al. 2002, Hansson et al. 2006, Fagan et al. 2007, Mattsson et al. 2009). Most importantly, these biomarker changes were observed in non-demented individuals who will progress to AD (Fagan et al. 2007). The recently revised research criteria for AD have incorporated CSF biomarkers as supportive features in the diagnosis of AD (Dubois et al. 2007, Gauthier et al. 2008). The latest survey across 23 European countries has shown that CSF biomarker testing is considered to be an important part of the dementia diagnostic work-up in most countries (Hort et al. 2010). Since several drug candidates for Alzheimer's disease are being evaluated in clinical trials, there will be a demand for accurate and early diagnosis of AD and for monitoring disease-modifying effects.

In Croatia it is estimated that there are 40 000–50 000 individuals with Alzheimer's disease (www.alzheimer.hr). The diagnosis of Alzheimer's disease is made through a routine clinical work-up usually within the Departments of Neurology or Departments of Psychiatry. In Croatia there are no memory clinics that would enable diagnosis of AD through a medical team specialized in dementia. In this study, we tested the benefit of analysis of the three proteins (Aβ42, t-tau and p181-tau) in the CSF of clinically diagnosed non-demented individuals, Alzheimer's disease patients and patients with uncertain type of dementia in a setting with no specialized dementia centers, such as in Croatia. We show that in non-specialized setting CSF biomarker testing may improve the diagnosis of AD and may detect early AD cases giving thus the chance for potential preventive therapy.

METHODS

Patients

Thirty-six patients of the Departments of Neurology at the University Hospitals in the two largest cities in Croatia, Zagreb and Split, were involved in this study.

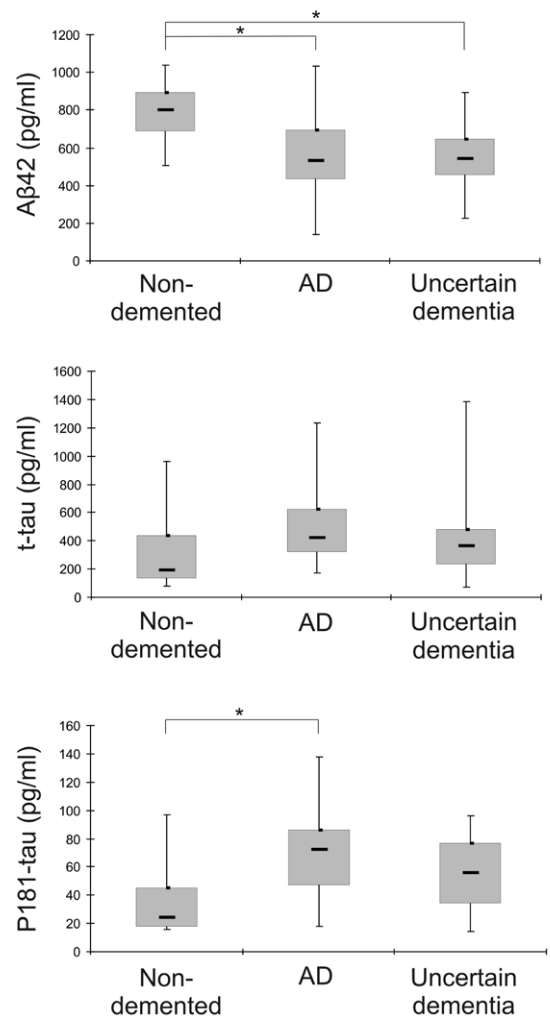

Fig. 1. CSF levels of Aβ42, t-tau and p181-tau as a function of clinical diagnosis. The levels of the three biomarkers were analyzed by ELISA assay (Innogenetics, Ghent, Belgium) among individuals clinically referred as non-demented (n=11), patients with AD (n=13) and individuals with uncertain type of dementia (uncertain dementia, n=12). The median levels together with 25–75th percentile range are shown. Comparisons between groups were performed using nonparametric Mann-Whitney U test (*P<0.05; ** P<0.01). (Aβ42) amyloid-β42; (t-tau) total tau; (p181-tau) phosphorylated-tau; (AD) Alzheimer's disease.

The patients clinically referred as non-demented (n=11) were patients who were examined because of other neurological problems that did not affect cognition such as headache, vertigo and back syndrome. Clinical diagnosis of probable Alzheimer's disease (n=13) was made according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition) and the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) diagnostic criteria (McKhann et al. 1984). Dementia severity was assessed using the Mini-Mental State Examination (MMSE) (Folstein et al. 1975) and/or the Mattis Dementia Rating Scale (MDRS) (Miller and Pliskin 2006). Patients that were clinically diagnosed as uncertain dementia (n=12) presented individuals with dementia without certain diagnosis of the dementia type. Patients with other causes of cognitive impairment, including brain tumor, vitamin B_{12} deficiency, folate deficiency, thyroid dysfunction, depression, head trauma, CNS infection and current alcohol abuse were excluded. All patients or their closest relative (if patients were judged unable to give informed consent) gave written informed consent to participate and the study was approved by the local medical ethical committees.

CSF analysis

CSF (2 mL, n=36) was collected in polypropylene tubes. CSF samples were free from any blood contaminations. Samples were gently inverted to avoid possible gradient effect and were aliquoted (0.5 mL) into polypropylene tubes before freezing at −80°C. Measurements of CSF concentrations of amyloid-β42 (Aβ42), total-tau (t-tau) and phosphorylated tau at position threonine 181 (p181-tau) were performed using commercially available ELISA assay (Innogenetics, Ghent, Belgium) according to the manufacturer's protocol and blinded to clinical diagnosis of the studied subjects.

APOE genotyping

DNA was isolated from peripheral blood by a standard salting out procedure (Miller et al. 1988). *APOE* genotyping was performed using a PCR-RFLP method as described previously (Hixson and Vernier 1990). Subjects were classified according to their *APOE* genotype as *APOE ε4*-negative (*ε2/ε3* and *ε3/ε3*) and *APOE ε4*-positive (*ε3/ε4* and *ε4/ε4*) subjects.

Statistical analysis

For statistical analysis software SPSS for Windows v 11.0 (Chicago, IL) was used. Comparisons between groups of subjects were performed using nonparametric Mann-Whitney U test. Results of the analyses were considered significant at $P<0.05$.

RESULTS

A summary of the demographic characteristics of the individuals tested is given in Table I. According to their clinical assessment individuals were grouped as non-demented, individuals with Alzheimer's disease

Table I

Demographic data of individuals tested

	Non-demented	Alzheimer's disease	Uncertain dementia
n	11	13	12
Age	63 ± 13.98*	76 ± 8.64	72.5 ± 11.29
Sex, F/M (%F)	8/3 (72.2)	6/7 (46.2)	7/5 (58.3)
MMSE score	28 ± 1.29	17 ± 4.72	19 ± 2.40
MDRS score	NA	106.5 ± 19.27	106.5 ± 12.98

The data are presented as median ± SD. The patients were grouped according to their clinical diagnosis: Non-demented, Alzheimer's disease, Uncertain dementia. (MMSE) Mini-Mental State Examination; (MDRS) Mattis Dementia Rating Scale; (NA) not available. * Note that non-demented group is younger that AD/uncertain dementia group.

(AD) and individuals with uncertain type of dementia (uncertain dementia).

CSF biomarkers as a function of clinical diagnosis

The results of the levels of amyloid-β42 peptide (Aβ42), total-tau (t-tau) and phosphorylated tau at position 181 (p181-tau) in 36 CSF samples of clinically assessed individuals are shown in Figure 1. In clinically assessed AD group the median Aβ42 was 533.4 pg/mL and was significantly lower compared to the median level in non-demented controls (802.1 pg/mL, $P<0.05$). In addition, among clinically assessed individuals with uncertain type of dementia (uncertain dementia) median Aβ42 level (543.1 pg/mL) was also significantly lower ($P<0.05$) than the level in non-demented control group. In clinically assessed AD group median t-tau (426.6 pg/mL) and p181-tau levels (72.7 pg/mL, $P<0.05$) were higher than the median levels in the non-demented control group (191.2 pg/mL for t-tau and 24.4 pg/mL for p181-tau), although only levels of p181-tau showed significant increase. Median levels of t-tau (364.6 pg/mL) and p181-tau (56.3 pg/mL) in patients with uncertain type of dementia were also increased compared to the levels in non-demented controls, but this difference was not statistically significant (Fig. 1). These results show that CSF samples of both clinically assessed AD and uncertain dementia individuals display altered median levels of Aβ42, t-tau and p181-tau compared to the levels in the CSF of non-demented controls. In accord with previous studies (Hulstaert et al. 1999, Riemenschneider et al. 2002, Hansson et al. 2006, Fagan et al. 2007, Mattsson et al. 2009) our clinically assessed AD individuals showed decreased median Aβ42 levels and increased median t-tau or p181-tau levels in their CSF.

Utility of CSF biomarker cut-off values for improving accuracy of AD diagnosis and for identifying individuals with incipient AD

We applied externally established AD/MCI CSF cut-off values of low Aβ42 (≤530 pg/mL) and high t-tau (≥350 pg/mL) or high p181-tau (≥52 pg/mL) levels to our CSF samples (Hansson et al. 2006, Tapiola et al. 2009), regardless of their clinical status, to identify individuals with AD/MCI CSF profile who are at high risk for probable AD. We set three groups taking into account positivity for 0, 1 or 2/3 CSF AD/MCI biomarker cut-off values. Normal CSF group (n=10) had all three biomarkers within normal range (Aβ42>530 pg/mL, t-tau<350 pg/mL and p181-tau<52 pg/mL) (0/3 positivity). AD/MCI CSF group (n=12) had a pathological (or Alzheimer's disease indicative) CSF pattern of decreased Aβ42 (≤530 pg/mL) and increased t-tau (≥350 pg/mL) or p181-tau (≥52 pg/mL) levels (2/3 positivity). Other CSF group (n=14) showed a change in only one biomarker (1/3 positivity), i.e. had either

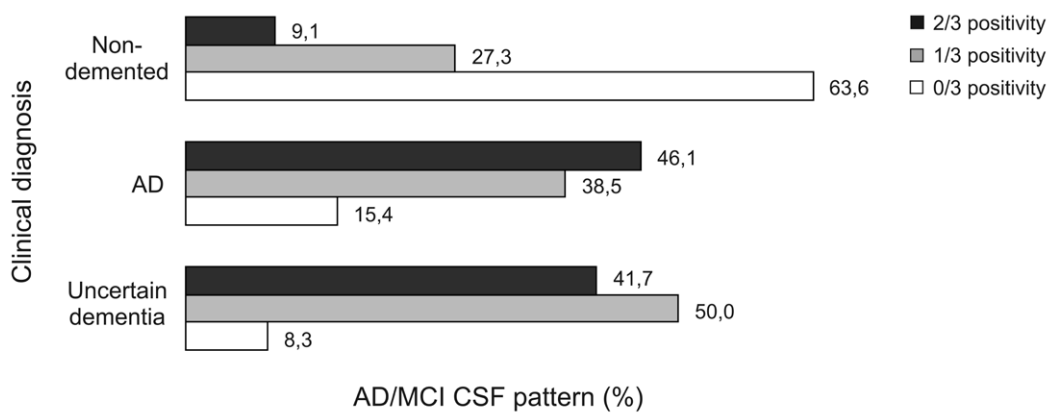

Fig. 2. Comparison between clinical diagnosis and CSF profiling. Among clinically diagnosed groups, three CSF profiles were identified taking into account positivity for 0, 1 or 2/3 CSF AD/MCI biomarker cut-off values (Aβ42≤530 pg/mL, t-tau≥350 pg/mL or p181-tau≥52 pg/mL): normal CSF profile (0/3 positivity), other CSF profile (1/3 positivity) and AD/MCI CSF profile (2/3 positivity). (AD) Alzheimer's disease; (CSF) cerebrospinal fluid; (MCI) mild cognitive impairment; (Aβ42) amyloid-β42; (t-tau) total tau; (p181-tau) phosphorylated-tau.

decreased Aβ42 (≤530 pg/mL) or increased t-tau (≥350 pg/mL) or p181-tau (≥52 pg/mL) levels.

Applying these CSF cut-off values revealed that in each clinically assessed group there may be false positive and/or false negative cases (Fig. 2). In more detail, among 11 clinically assessed non-demented individuals the set CSF cut-offs identified 1 individual (9.1%) who showed a typical AD/MCI CSF pattern (2/3 positivity) and 3 individuals (27.3%) who showed a change in only one biomarker (1/3 positivity) (Fig. 2). MMSE/MDRS scoring of these four individuals was repeated and indicated that they may have probable Alzheimer's disease (mean MMSE 16.67, mean MDRS 103). Follow-up clinical assessment of these individuals is underway. Among clinically diagnosed AD and uncertain dementia cases, the set cut-off values identified AD/MCI-CSF pattern (2/3 positivity) in approximately 45% of patients in both groups (Fig. 2). Surprisingly, CSF profiling revealed individuals with normal CSF pattern (0/3 positivity) in both clinically diagnosed AD and uncertain dementia cases.

Next, in clinically assessed groups (non-demented, AD and uncertain dementia) we analyzed all three biomarkers in a two-dimensional format by plotting the p181-tau concentration (x-axis) *versus* the IATI index [Innotest Amyloid Tau Index, IATI = Aβ42/(240 + 1.18 t-tau), y-axis] (Fig. 3). The IATI<1 was reported for individuals with a typical AD biomarker profile, while IATI>1 was found to be characteristic for healthy control subjects (Riemenschneider et al. 2002). Indeed, all our subjects who had normal CSF profile (0/3 positivity, black) had IATI>1 and those with AD/MCI CSF pattern (2/3 positivity, light gray) had IATI<1 (Fig. 3), suggesting that the applied CSF profiling and/or IATI index can be useful for discriminating probable AD patients from non-demented individuals. Furthermore, majority of individuals (9/14, 64.3%) showing only 1 biomarker positivity of the AD/MCI CSF cut-off values (1/3 positivity, dark gray) showed IATI<1, indicating that in this group majority of patient may indeed have Alzheimer's type dementia which needs to be clarified by further clinical follow-up. Furthermore, 2/13 (15.4%) clinically diagnosed AD patients showed normal CSF profile (0/3 positivity, black) and IATI>1 characteristic for healthy individuals. Additionally, 3/11 (27.3%) clinically referred non-demented control subjects showed IATI<1 a typical AD biomarker profile. Applying IATI index confirmed a possible false positive and false negative clinically diagnosed cases.

When p181-tau cut-off value (≥52 pg/mL) was applied to IATI index (Fig. 3), we identified that in AD/MCI CSF group 4/12 samples (33.3%) had p181-tau<52 pg/mL, implying that, in contrast to IATI index (Aβ42 and t-tau values), p181-tau biomarker may not be

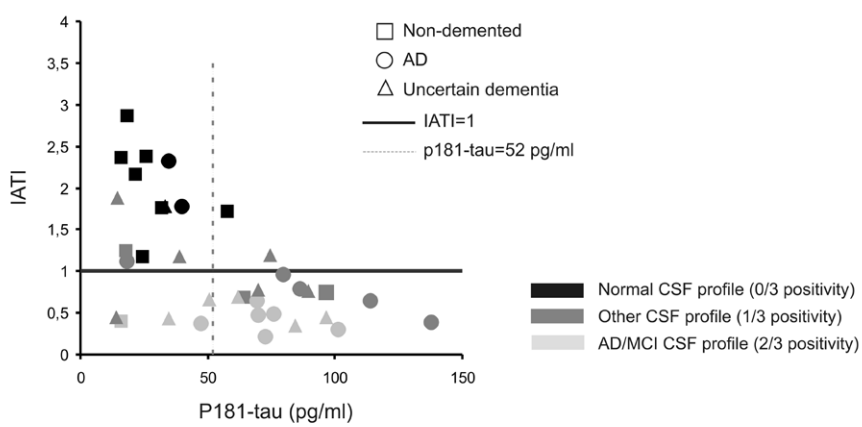

Fig. 3. Scatter plot of IATI index and p181-tau levels among clinically referred non-demented individuals, AD patients and individuals with uncertain dementia in relation to CSF profiling. All three biomarkers were analyzed in a two-dimensional format by plotting the p181-tau levels (x-axis) *versus* the IATI index [IATI = Aβ42/(240 + 1.18 t-tau), y-axis]. Clinical diagnosis of non-demented individuals (square), AD (circle) and uncertain dementia (triangle) patients is shown as a function of IATI index and the levels of CSF p181-tau. Filled shapes represent the results of CSF profiling: normal CSF profile (0/3 positivity, black), other CSF profile (1/3 positivity, dark gray) and AD/MCI CSF profile (2/3 positivity, light gray). (AD) Alzheimer's disease; (CSF) cerebrospinal fluid; (MCI) mild cognitive impairment; (p181-tau) phosphorylated-tau; (IATI) Innotest Amyloid Tau Index.

a perfect candidate to clearly discriminate AD/MCI from non-AD CSF pattern.

Apolipoprotein E genotype in relation to CSF biomarker profiling

Apolipoprotein E *ε4* allele is the strongest genetic risk factor for Alzheimer's disease detected so far (Corder et al. 1993, Saunders et al. 1993). Analysis of *APOE* genotype according to CSF biomarker profiling showed that all individuals having normal CSF profile (0/3 positivity, *n*=10) were *APOE ε4*-negative, while those with AD/MCI (2/3 positivity, *n*=12) and other (1/3 positivity, *n*=14) CSF profile were 71.4% and 30% *APOE ε4*-positive, respectively (Fig. 4). Although our sample size is too small to draw any strong conclusions our results are in agreement with previous genetic studies (Corder et al. 1993, Saunders et al. 1993) and support that *APOE ε4* allele is associated with the CSF biomarker changes typical for Alzheimer's disease (Riemenschneider et al. 2000).

DISCUSSION

If left underdiagnosed and untreated Alzheimer's disease will become a major health problem. In Western high income countries in the past several years an urged need for early diagnosis of AD has opened new avenues of AD research developing neuroimaging techniques and/or searching for biomarkers that will detect early pathological changes characteristic for AD. Unlike most of the CSF biomarker studies that were conducted in settings within specialized AD research centers or memory clinics (Hulstaert et al. 1999, Riemenschneider et al. 2002, Hansson et al. 2006, Fagan et al. 2007, Mattsson et al. 2009), we evaluated the benefit of the analysis of the three biomarkers (Aβ42, t-tau and p181-tau) in the CSF as a part of routine clinical work-up within Neurology clinics with no specialized dementia centers, such as in Croatia. In this study we showed that clinical diagnosis in Croatia is not very accurate (showing both false positive and false negative cases). Thus, we consider the usage of clinical diagnosis as a gold standard and the tool for measuring biomarker sensitivity and specificity inappropriate in this study. Sensitivity and specificity of CSF biomarkers and IATI index for distinguishing Alzheimer's disease from non-Alzheimer dementia and non-demented group were determined in more extensive studies by others (Hansson et al. 2006, Tapiola et al. 2009, Mattsson et al. 2009). The scope of our study was not to confirm reliability of CSF biomarkers, rather to show how they can aid in differential diagnosis of AD in a setting with no dementia centers/memory clinics. Indeed, we show that in such non-specialized settings clinical evaluation together with CSF biomarker testing when applied with a pathological (or AD/MCI-indicative) CSF cut-off values (Hansson et al. 2006, Tapiola et al. 2009) may improve the accuracy of AD diagnosis among individuals with diagnostic doubts and may identify non-demented individuals with early CSF pathological changes characteristic for AD (incipient AD). Thus, in such settings CSF biomarkers may, be useful as a screening tools selecting individuals at high risk of developing Alzheimer's disease who need to

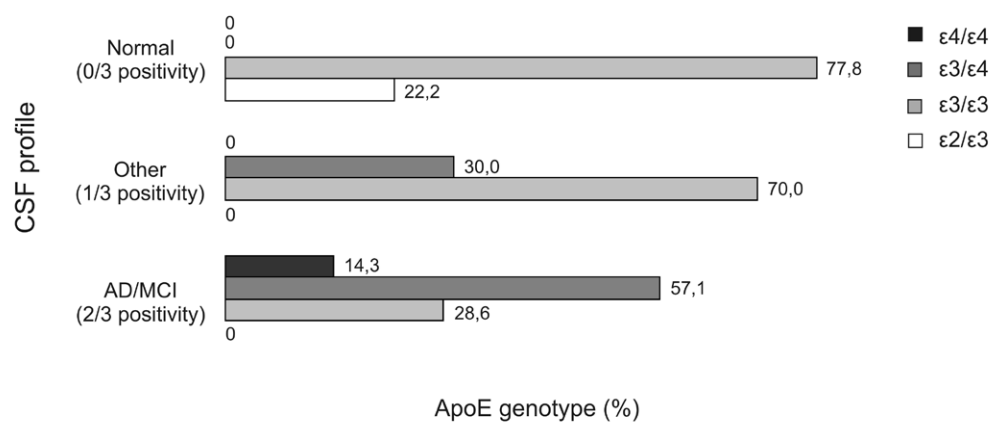

Fig. 4. *APOE* genotyping in relation to CSF profiling. Individuals were classified according to their *APOE* genotype as *APOE ε4*-negative (*ε2/ε3* and *ε3/ε3*) and *APOE ε4*-positive (*ε3/ε4* and *ε4/ε4*) subjects. (AD) Alzheimer's disease; (CSF) cerebrospinal fluid; (MCI) mild cognitive impairment; (ApoE) apolipoprotein E.

receive a detailed further clinical follow-up. Early changes in CSF biomarker profile before clinical symptoms appear have been reported in several studies that involved mildly cognitively impaired cases (MCIs) as well as non-demented individuals who will progress to AD (Riemenschneider et al. 2002, Hansson et al. 2006, Fagan et al. 2007, Mattsson et al. 2009). In accord with previous studies, we also showed that *APOE ε4* allele, the strongest genetic risk factor for AD, is associated with AD/MCI CSF profile. Although AD/MCI CSF pattern does not confirm the diagnosis of probable AD *per se*, it may help identifying and/or predicting individuals with incipient AD through their further clinical follow-up (Hansson et al. 2006). Due to a small sample size in this pilot study we acknowledge that our results need to be further validated on a larger sample size and confirmed with a follow-up clinical assessment of individuals who showed a characteristic AD/MCI CSF profile.

The newly revised research criteria for AD have incorporated CSF biomarker levels as supportive tests for the disease diagnosis, especially in cases of diagnostic doubts and in cases of incipient AD (Dubois et al. 2007, Gauthier et al. 2008, Sperling et al. 2011). Improving the inter-laboratory variability in CSF biomarkers for AD (Mattsson et al. 2010) and standardizing the cut-off values of the three CSF biomarkers is greatly appreciated, especially in routine CSF testing for identifying MCI individuals and even for predicting cognitive decline in non-demented adults. Since CSF tests are cheaper and more readily available than more expensive imaging techniques (PET, MRI), we conclude that they may become an important part of the dementia diagnostic work-up, especially in countries with non-specialist settings. In the light of upcoming therapies aimed at slowing the progression and/or treating AD, it is of particular interest to identify MCI and non-demented subjects with a highest risk for progression to AD.

CONCLUSION

Early and accurate diagnosis of Alzheimer's disease is crucial for applying novel therapies to treat, prevent or slower progression of this devastating disorder. In this study we show that in settings with no specialized AD centers or memory clinics CSF biomarker profiling and/or IATI index (IATI = Aβ42/(240 + 1.18 t-tau) may improve the accuracy of clinical diagnosis of AD among individuals with diagnostic doubt and also may help identifying individuals with incipient Alzheimer's disease.

ACKNOWLEDGEMENTS

We would like to thank patients and their families for participation in this study. This work was supported by the grants of the Ministry of Science, Education and Sports, Republic of Croatia 098-0982522-2525 to S.H. and 134-134036-0035 to Z.T.

REFERENCES

Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261: 921–923.

Dubois B, Feldman HH, Jacova C, DeKosky ST, Barberger-Gateau P, Cummings J, Delacourte A, Galasko D, Gauthier S, Jicha G, Meguro K, O'brien J, Pasquier F, Robert P, Rossor M, Salloway S, Stern Y, Visser PJ, Scheltens P (2007) Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol 6: 734–746.

Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM (2007) Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. Arch Neurol 64: 343–349.

Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, Jorm A, Mathers C, Menezes PR, Rimmer E, Scazufca M (2005) Global prevalence of dementia: a Delphi consensus study. Lancet 366: 2112–2117.

Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12: 189–198.

Gauthier S, Dubois B, Feldman H, Scheltens P (2008) Revised research diagnostic criteria for Alzheimer's disease. Lancet Neurol 7: 668–670.

Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L (2006) Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol 5: 228–234.

Hixson JE, Vernier DT (1990) Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res 31: 545–548.

Hort J, Bartos A, Pirttila T, Scheltens P (2010) Use of cerebrospinal fluid biomarkers in diagnosis of dementia across Europe. Eur J Neurol 17: 90–96.

Hulstaert F, Blennow K, Ivanoiu A, Schoonderwaldt HC, Riemenschneider M, De Deyn PP, Bancher C, Cras P, Wiltfang J, Mehta PD, Iqbal K, Pottel H, Vanmechelen E, Vanderstichele H (1999) Improved discrimination of AD patients using beta-amyloid(1-42) and tau levels in CSF. Neurology 52: 1555–1562.

Mattsson N, Blennow K, Zetterberg H (2010) Inter-laboratory variation in cerebrospinal fluid biomarkers for Alzheimer's disease: united we stand, divided we fall. Clin Chem Lab Med 48: 603–607.

Mattsson N, Zetterberg H, Hansson O, Andreasen N, Parnetti L, Jonsson M, Herukka SK, van der Flier WM, Blankenstein MA, Ewers M, Rich K, Kaiser E, Verbeek M, Tsolaki M, Mulugeta E, Rosen E, Aarsland D, Visser PJ, Schroder J, Marcusson J, de LM, Hampel H, Scheltens P, Pirttila T, Wallin A, Jonhagen ME, Minthon L, Winblad B, Blennow K (2009) CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. JAMA 302: 385–393.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34: 939–944.

Miller JM, Pliskin NH (2006) The clinical utility of the Mattis Dementia Rating Scale in assessing cognitive decline in Alzheimer's disease. Int J Neurosci 116: 613–627.

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 1215.

Morris JC (2005) Mild cognitive impairment and preclinical Alzheimer's disease. Geriatrics Suppl: 9–14.

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.

Riemenschneider M, Schmolke M, Lautenschlager N, Guder WG, Vanderstichele H, Vanmechelen E, Kurz A (2000) Cerebrospinal beta-amyloid ((1-42)) in early Alzheimer's disease: association with apolipoprotein E genotype and cognitive decline. Neurosci Lett 284: 85–88.

Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ (1993) Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology 43: 1467–1472.

Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR, Jr., Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, Phelps CH (2011) Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7: 280–292.

Tapiola T, Alafuzoff I, Herukka SK, Parkkinen L, Hartikainen P, Soininen H, Pirttila T (2009) Cerebrospinal fluid {beta}-amyloid 42 and tau proteins as biomarkers of Alzheimer-type pathologic changes in the brain. Arch Neurol 66: 382–389.