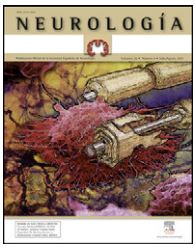
Neurología. 2012;27(1):28—33

NEUROLOGÍA

www.elsevier.es/neurologia



ORIGINAL ARTICLE

Patients with mild cognitive impairment and a reduced CSF $A\beta_{1-42}$ protein progress rapidly to Alzheimer's disease☆,☆☆

J.A. Monge-Argilés[a,*], J. Sánchez-Payá[b], C. Muñoz-Ruiz[c], A. Pampliega-Pérez[a], M.J. Gómez-López[d], E. Rodríguez Borja[c], J. Montoya-Gutiérrez[a], C. Leiva-Santana[a]

[a] *Servicio de Neurología, Hospital General Universitario de Alicante, Alicante, Spain*
[b] *Servicio de Medicina Preventiva, Hospital General Universitario de Alicante, Alicante, Spain*
[c] *Laboratorio de Inmunología, Hospital General Universitario de Alicante, Alicante, Spain*
[d] *Clínica Neuropsicóloga, Hospital General Universitario de Alicante, Alicante, Spain*

Received 18 December 2010; accepted 27 March 2011
Available online 9 February 2012

KEYWORDS
CSF biomarkers;
Mild cognitive impairment;
Alzheimer's disease;
$A\beta_{1-42}$ protein;
T-tau protein;
P-tau_{181p} protein

Abstract
Introduction: Some studies have shown that CSF amyloid-beta 1-42 ($A\beta_{1-42}$), total tau (T-tau) and tau phosphorylated at threonine 181 (P-tau_{181p}) proteins are useful diagnostic markers for distinguishing between clinically stable mild cognitive impairment (MCI) patients and those who will develop Alzheimer's disease (AD).

Our objective was to test the ability of this technique to discriminate in our cohort of MCI patients, according to the clinical outcome, one year after the lumbar puncture.

Material and methods: A total of 36 MCI patients were included from the local hospital memory clinic. Using INNO-BIA Alzbio-3 reagents from Innogenetics, we measured CSF $A\beta_{1-42}$, T-tau and P-tau_{181p} proteins, and calculated the T-tau/$A\beta_{1-42}$ and P-tau_{181p}/$A\beta_{1-42}$ ratios. This project was approved by the local ethics committee.

Results: One year after the lumbar puncture, 14 MCI patients (38%) developed AD. These patients had lower $A\beta_{1-42}$ protein levels (285.3 ng/ml vs 377 ng/ml, $P < .02$) and higher P-tau_{181p}/$A\beta_{1-42}$ ratio (0.25 vs 0.16, $P < .02$) than the clinically stable patients.

Conclusions: Our MCI patients with lower $A\beta_{1-42}$ protein levels and an increased P-tau_{181p}/$A\beta_{1-42}$ ratio progressed quickly to AD. These results may help to identify those MCI patients with a poorer prognosis.

☆ Please cite this article as: Monge-Argilés JA, et al. Pacientes con deterioro cognitivo leve y reducción de la proteína $A\beta_{1-42}$ en LCR evolucionan rápidamente a enfermedad de Alzheimer. Neurología. 2011;28—33.

☆☆ This work was presented at the Annual Meeting of the Spanish Neurology Society in 2010.

* Corresponding author.
E-mail address: monge_jos@gva.es (J.A. Monge-Argilés).

2173-5808/$ - see front matter

PALABRAS CLAVE
Biomarcadores en LCR;
Deterioro cognitivo leve;
Enfermedad de Alzheimer;
Proteína $A\beta_{1-42}$;
Proteína T-tau;
Proteína P-tau_{181p}

Pacientes con deterioro cognitivo leve y reducción de la proteína $A\beta_{1-42}$ en LCR evolucionan rápidamente a enfermedad de Alzheimer

Resumen

Introducción: En muchos artículos recientes, el análisis de las proteínas $A\beta_{1-42}$, tau total (T-tau) y tau fostorilada (P-tau) en LCR puede discriminar entre los pacientes con deterioro cognitivo leve (DCL) estables y aquellos otros que van a progresar a enfermedad de Alzheimer (EA). Nuestro objetivo fue comprobar la capacidad de estas proteínas del LCR para discriminar, entre nuestros pacientes DCL, según la evolución clínica en el año siguiente a la punción lumbar.

Material y métodos: Se incluyó a 36 pacientes DCL amnésico (criterios de Petersen 2006) procedentes de la consulta de deterioro cognitivo del Hospital General de Alicante. Usando los reactivos INNO-BIA Alzbio-3 (Innogenetics), cuantificamos las proteínas $A\beta_{1-42}$, T-tau, P-tau_{181p} en LCR, y calculamos los cocientes T-tau/$A\beta_{1-42}$ y P-tau_{181p}/$A\beta_{1-42}$. El estudio fue aprobado por el comité ético de investigación del Hospital General de Alicante.

Resultados: En los 12 meses posteriores a la punción lumbar, 14 pacientes DCL (38%) evolucionaron a EA. Estos pacientes, presentaron menores niveles de $A\beta_{1-42}$ (285,3 vs. 377,7 ng/ml, $p < 0,02$), y un aumento en el valor del cociente P-tau_{181p}/$A\beta_{1-42}$ (0,25 vs. 0,16, $p < 0,02$) que los pacientes que se mantuvieron estables. No hubo diferencias significativas en el resto de variables estudiadas.

Conclusiones: Nuestros pacientes DCL que presentaron niveles reducidos de la proteína $A\beta_{1-42}$ y elevación del cociente P-tau_{181p}/$A\beta_{1-42}$ en LCR, evolucionaron rápidamente a EA. Estos resultados pueden ayudar a conseguir el objetivo de identificar de forma precoz a los pacientes DCL con peor pronóstico.

Introduction

Many authors consider mild cognitive impairment (MCI) as the most important risk factor for the development of Alzheimer's disease (AD).[1] Thus, 40—60% of patients with MCI develop AD within the next 5 years,[2] while another significant percentage maintain a stable form of cognitive impairment.[3]

Different complementary techniques have been used for several years in an attempt to identify MCI patients who will develop dementia, in order to treat them early or at least to delay and mitigate the devastating effects of the disease. Among the aforementioned complementary techniques the main technique is the analysis of $A\beta_{1-42}$, T-tau and P-tau proteins in cerebrospinal fluid (CSF), as well as the ratios of $A\beta_{1-42}$ and both tau proteins, as they reflect the relationship between both AD pathogenic pathways. Their study has led to numerous works being published[4—7] and also to different meta-analysis of their predictive ability for the diagnosis of AD.[8—10]

Moreover, recent publications have used these biomarkers to recognise the MCI patients who will progress rapidly to dementia,[11,12] and even to identify the patients already suffering dementia who will evolve more rapidly.[13]

The aim of this work is to verify whether this technique is capable of differentiating our stable MCI patients from those who progressed to AD in the months following the lumbar puncture (LP).

Materials and methods

Study design

This was a cohort study.

Study subjects

The study included 36 patients with amnestic MCI diagnosed according to 2006 Petersen criteria,[3] all from the cognitive impairment consultation at Hospital General Universitario in Alicante, some of whom had been monitored for several years. Their study included physical and neurological examinations, a neuropsychological study, Yesavage's geriatric depression scale (15 items), blood tests, brain structural imaging and LP. The patients were monitored in the outpatient clinic with periodic reviews at least every 6 months. These reviews evaluated the development of AD according to the NINCDS-ADRDA criteria[14] and GDS scale. According to the progression at 12 months after LP, we differentiated patients into those who were stable (s-MCI) and those who progressed to AD (p-MCI).

Inclusion criteria

Amnestic MCI patients over 55 years. They signed informed consent prior to inclusion in the study and performance of LP.

Exclusion criteria

The presence of dementia or any other neurological, psychiatric or systemic disease which could cause cognitive impairment, anticoagulant therapy and lack of informed consent were grounds for exclusion. An evaluation with Yesavage's geriatric depression scale over 5 points was also considered as reason for exclusion.

Procedures

The neurologist in charge emitted a diagnosis of pure or multi-domain amnestic MCI, according to the Petersen criteria.[3] Subsequently, a neuropsychological study was conducted that included assessment of memory, language, executive functions, attention and visuo-constructive abilities through the Mini Mental State Examination (MMSE), Rey auditory—verbal learning test, trail making test (TMT) and informer test (INT). Alterations of functions were defined as a result *Z* of −1.5 or less, which represented at least 1.5 standard deviations below the mean of the control subjects, in at least 1 of the tests used to study each function. The criteria for considering the change from s-MCI to p-MCI was a decline in MMSE over 2 points and/or an in increase in INT over 7 points.

Neuroimaging was performed in all patients with MCI (cerebral MRI in most cases or only cerebral CT scan in 5 of them), in order to rule out other brain lesions which could be responsible for the clinical symptoms.

Obtaining and analysing cerebrospinal fluid

This was conducted between February 2008 and February 2009. The samples were obtained between 10:00 and 14:00 h. The LP was conducted by the neurologist responsible using a $20 \times 3(1/2)$ needle. The CSF was collected in standard tubes and was frozen at $-80\,^{\circ}C$ in less than 1 h. When the CSF contained blood, it was centrifuged prior to freezing. One tube was collected to perform biochemical analysis and cell count prior to obtaining the freezing tubes. In no case did this cell count show over 100 red blood cells.

After the LP, patients were advised to avoid Valsalva manoeuvres for at least 3 days.

Quantifying $A\beta_{1\text{-}42}$, T-tau and P-tau_{181p} cerebrospinal fluid protein levels

This was performed using Luminex xMAP technology with INNO-BIA AlzBio3 reagents from Innogenetics (Ghent, Belgium). The details of this combination of immunoassay reagents and analytical platform have been published previously.[15]

All samples were analysed simultaneously at the end of recruitment and in a blind manner for clinical data. The samples from 19 patients were analysed in duplicate.

Study variables

We studied the levels of CSF proteins $A\beta_{1\text{-}42}$, T-tau and P-tau_{181p}, as well as the ratios T-tau/$A\beta_{1\text{-}42}$ and P-tau_{181p}/$A\beta_{1\text{-}42}$. These ratios are being used by various authors widely, as they seem to reflect the relationship between the amyloid and tau pathways of the disease.[16]

Statistical analysis

The reliability of the technique was calculated using the intraclass correlation coefficient.

We used the Kolmogorov—Smirnov test to analyse the distribution type of each variable.

For comparison between 2 groups, we used the Student's *t*-test for parametric variables and the Mann—Whitney *U*-test for nonparametric variables.

In addition, we performed ROC curve analysis to determine the best cut-off values for the measurement of variables. The best cut-off value was defined in response to higher sensitivity above all. We then obtained the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for each cut-off point and each variable.

We used a $P < .05$ level of statistical significance for all hypothesis contrasts. The statistical analysis was conducted using the SPSS v.10.0 software.

Ethical criteria

The pharmaceutical companies that funded this project had no role in its design, data collection or interpretation or in the writing of this work.

This project was approved by the clinical research ethics committee of Hospital General Universitario in Alicante, after obtaining a civil liability insurance policy.

Results

Reliability of the technique

In the 19 cases analysed in duplicate, the intraclass correlation coefficient was 0.94 for protein $A\beta_{1\text{-}42}$, 0.96 for protein T-tau and 0.95 for protein P-tau.

Comparison of s-MCI group vs p-MCI group

By studying the evolution towards dementia of our patients in the first 12 months after the completion of a LP, we observed that 14 (38%) of them had progressed to dementia. When comparing these groups, we observed:

- Clinical-demographic characteristics (Table 1): there were no significant differences among the ages of both groups or among most of the variables studied. However, s-MCI patients had a higher percentage of history of depression (46% vs 18%) and a longer evolution time from the onset of symptoms until the completion of a LP.
- Comparison of protein levels and ratios (Table 2): distinctions between the 2 groups were notable for the protein $A\beta_{1\text{-}42}$ ($P < .02$) and the ratio P-tau/$A\beta_{1\text{-}42}$ ($P < .02$). The remaining variables studied did not reach statistical significance.

Table 1 Clinical and demographic characteristics of the s-MCI and p-MCI groups.

	s-MCI group	p-MCI group	*P*
Number of cases	22	14	
Gender (M/F)	9/13	3/11	
Age (years) Mean ± SD	73.43 ± 6.63	73.86 ± 6.95	NS
History			
DM	3	3	
AHT	9	7	
HPL	13	4	
Depression	11	3	
Toxic habits	No	No	
Family history of dementia	8	2	
Years of schooling	4.4	5.8	NS
Onset of symptoms (months), mean ± SD	38.22 ± 7.3	25.71 ± 5.6	.05
Initial Folstein MMSE	25	24	NS
Type of MCI	Amnestic: 6A/Multi-domain: 16	Amnestic: 4A/Multi-domain: 10	
Other neuropsychological tests			
INT	80 ± 6	81 ± 8	NS
TMT (s)	68 ± 26	70 ± 24	NS
Rey A—V test	24.7 ± 8.3	23.2 ± 7.6	NS

AHT: arterial hypertension; DM: diabetes mellitus; HPL: hyperlipidemia; INT: informer test; NS: not significant; Rey A—V test: Rey auditory—verbal learning test; TMT: trail making test.

Table 2 Comparison of the concentrations of the 3 biomarkers and quotients between biomarkers for s-MCI and p-MCI patients.

	s-MCI (n = 22)	p-MCI (n = 14)	Significance level ($P < .05$)
$A\beta_{1-42}$ (pg/ml)			
Mean ± SD	377.73 ± 130.77	285.37 ± 73.36	.02
T-tau (pg/ml)			
Mean ± SD	75.13 ± 34.85	88.30 ± 40.82	.3
P-tau_{181p} (pg/ml)			
Mean ± SD	52.57 ± 24.95	66.44 ± 23.87	.1
T-tau/$A\beta_{1-42}$			
Mean ± SD	0.23 ± 0.15	0.34 ± 0.19	.07
P-tau_{181p}/$A\beta_{1-42}$			
Mean ± SD	0.16 ± 0.11	0.25 ± 0.12	.02

— Table 3 shows the results of the ROC curves for each of the variables studied. The sensitivity and NPV values for the cut-off points selected were above 70% in 4 and 3 variables, respectively.

Discussion

Our MCI patients who progressed to AD in the first year after LP presented an average $A\beta_{1-42}$ protein level lower

Table 3 Results of the ROC curves for the different parameters used.

Parameters	$A\beta_{1-42}$	T-tau	P-tau	T-tau/$A\beta_{1-42}$	P-tau/$A\beta_{1-42}$
Limit value	320	77.5	54.5	0.18	0.17
Sensitivity (%)	71	57	79	91	71
Specificity	64	64	60	50	64
Positive predictive value (%)	56	50	55	52	71
Negative predictive value (%)	7	70	81	84	64

than that of the group of patients who were clinically stable. These results seem useful to identify the MCI patients with a worse prognosis and are in line with some studies published recently.[11,12] Landau et al.[11] described an increased P-tau$_{181p}$/Aβ_{1-42} ratio as a predictor of rapid cognitive decline and, in our experience, we observed this fact in MCI patients with poor prognosis. This ratio has been used by different authors as a reflection of the relationship between the amyloid and tau pathways,[11,16] both altered in AD.

Furthermore, we found a decrease in protein Aβ_{1-42} in this same group of patients. This finding is now considered as the first to appear in AD patients, even before they develop MCI, thus giving greater consistency to our results with respect to those obtained by Blom et al.[12] These authors reported an increase in the levels of T-tau and P-tau in patients who progressed to AD rapidly.

In our experience, although there are no statistically significant differences in P-tau$_{181p}$ protein levels between both groups, we observed that they were higher in the p-MCI group. The elevation of this biomarker in CSF is currently considered the most specific finding of AD, both in clinical studies[7,9] and in those confirmed by autopsy.[17]

Analysing the results of the ROC curves, we found high sensitivity and NPV for P-tau and the ratio T-tau/Aβ_{1-42}. The remaining results were more discreet, but we must not forget that the only difference between these patients may be their rate of progression to AD. In any case, these findings emphasise the importance of quantifying all 3 biomarkers, as well as the ratios used.

The new criteria for research in AD suggest that both MRI, PET and biomarkers in CSF may support the diagnosis of AD in amnestic MCI patients.[18] However, CSF analysis has some advantages over the other 2 techniques. On the one hand, it is cheaper and easier to obtain than PET. Furthermore, the latter currently studies only the amyloid pathway and its use is still very restricted. On the other hand, changes in CSF reflect AD pathology before volumetry.[19] Therefore, this technique seems especially preferable for a very early diagnosis of AD,[19] although both methods are complementary and most publications that combine the predictive ability of CSF analysis with other techniques such as MRI agree on this point.[20]

From a methodological point of view, the use of the Luminex xMAP technique seems to offer some advantages over CSF analysis by ELISA. The simultaneous quantification of the 3 biomarkers improves the technical management and quality control of tests, according to some authors.[21] In our case, conducting all quantifications simultaneously, obtaining CSF in a reduced time period and the small age range between the groups significantly decreased confusion factors. The results obtained in the intraclass correlation coefficients for the 3 biomarkers reflect the high reliability of the technique used.

Among the limitations of this study, we note:

- Short monitoring time. However, the differences obtained between patients who progressed in the first 12 months after the LP speak to a likely pattern of rapid evolution to AD, as described in recent literature.[11,12] This could support the performance of this test not only for diagnosis, but also for prognostic purposes.
- Not all patients included in the study had been diagnosed recently. However, we know that these biomarkers, especially protein Aβ_{1-42}, become altered very early in the evolution of AD and do not present significant changes when a control test is performed after a few years.[22]
- This is an invasive test. However, there have been no adverse effects on the population studied. This is consistent with the data published by other authors[23] when such tests are performed on patients under good conditions and specific efforts are subsequently avoided for a limited time.

In summary, patients with MCI who have reduced levels of protein Aβ_{1-42} progress rapidly to dementia, while those with higher levels remain stable for a longer time.

Conflict of interests

This work was funded by Novartis Laboratories Spain and Grunenthal Spain.

Acknowledgements

The authors wish to thank the Anaesthesia, Urology and Traumatology Services at Hospital General Universitario in Alicante for their help; as well as María de los Ángeles Miguelsanz, from the Immunology Laboratory, for the excellent management of the samples; and Margarita Ruiz Vegara for her generous collaboration (in memoriam).

References

1. Borroni B, Premi E, Di Luca M, Padovani A. Combined biomarkers for early Alzheimer disease diagnosis. Curr Med Chem. 2007;14:1171—8.
2. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol. 2006;5:228—34.
3. Artero S, Petersen RC, Touchon J, Ritchie K. Revised criteria for mild cognitive impairment: validation within a longitudinal population study. Dement Geriatr Cogn Disord. 2006;22:465—70.
4. Zetteberg H, Wahlund L, Blennow K. CSF markers for prediction of Alzheimer's disease. Neurosci Lett. 2003;352:67—9.
5. Hampel H, Teipel SJ, Fuchsberger T, Andreasen N, Wiltfang J, Otto M, et al. Value of CSF beta-amyloid$_{1-42}$ and tau as predictors of Alzheimer's disease in patients with mild cognitive impairment. Mol Psychiatry. 2004;9:705—10.
6. Herukka SK, Hallikainen M, Soininen H, Pirttila T. CSF Abeta42 and tau or phosphorylated tau and prediction of progressive mild cognitive impairment. Neurology. 2005;64:1294—7.
7. Ewers M, Buerger K, Teipel SJ, Scheltens P, Schröder J, Zinkowski RP, et al. Multicenter assessment of CSF-phosphorylated tau for the prediction of conversion of MCI. Neurology. 2007;69:2205—12.
8. Diniz BS, Pinto JA, Forlenza OV. Do CSF total tau, phosphorylated tau and beta amyloid 42 help to predict progression of mild cognitive impairment to Alzheimer's disease? A systematic review and meta-analysis of the literature. World J Biol Psychiatry. 2008;9:172—82.

9. Mitchell AJ. CSF phosphorylated tau in the diagnosis and prognosis of mild cognitive impairment and Alzheimer's disease: a meta-analysis of 51 studies. J Neurol Neurosurg Psychiatry. 2009;80:966—75.
10. Monge-Argilés JA, Sánchez-Payá J, Muñoz-Ruiz C, Pampliega-Pérez A, Montoya-Gutiérrez FJ, Leiva-Santana C. Biomarcadores en LCR de pacientes con deterioro cognitivo leve: metaanálisis de su capacidad predictiva para el diagnóstico de la enfermedad de Alzheimer. Rev Neurol. 2010;50:193—200.
11. Landau SM, Harvey D, Madison CM, Reiman EM, Foster NL, Aisen PS, et al. Comparing predictors of conversion and decline in mild cognitive impairment. Neurology. 2010;75:230—8.
12. Blom ES, Giedraitis V, Zetterberg H, Fukumoto H, Blennow K, Hyman BT, et al. Rapid progression from mild impairment to Alzheimer's disease in subjects with elevated levels of tau in CSF and APOE $\varepsilon 4/\varepsilon 4$ genotype. Dement Geriatr Cogn Disord. 2009;27:458—64.
13. Kester MI, Van der Vlies AE, Blankenstein MA, Pijnenburg YA, Van Elk EJ, Scheltens P, et al. CSF biomarkers predict rate of cognitive decline in Alzheimer disease. Neurology. 2009;73:1353—8.
14. McKhann G, Drachman DA, Folstein M, Katzman R, Price DL, Stadlan EM. 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. 1984;34:939—44.
15. Olsson A, Vanderstichele H, Andreasen N. Simultaneous measurement of β-amyloid$_{1-42}$, total tau and phosphorylated tau in CSF by xMAP technology. Clin Chem. 2005;51:336—45.
16. Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, et al. Cerebrospinal fluid biomarkers signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol. 2009;65:403—13.
17. Koopman K, Le Bastard N, Martin JJ, Nagels G, De Deyn PP, Engelborghs S. Improved discrimination of autopsy-confirmed Alzheimer's disease from non-AD dementias using CSF p-tau 181p. Neurochem Int. 2009;55:214—8.
18. Dubois B, Feldman HH, Jacova C, Cummings J, DeKosky ST, Barberger-Gateau P, et al. Revising the definition of Alzheimer's disease: a new lexicon. Lancet Neurol. 2010;9:1118—27.
19. Hort J, Bartos A, Pirttila T, Scheltens P. Use of CSF biomarkers in diagnosis of dementia across Europe. Eur J Neurol. 2010;17:90—6.
20. Vemuri P, Wiste HJ, Weigand SD, Knopman DS, Trojanowski JQ, Shaw LM, et al. Serial MRI and CSF biomarkers in normal aging, MCI and AD. Neurology. 2010;75:143—51.
21. Lewczuck P, Kornhuber J, Vanderstichele H, Vanmechelen E, Esselmann H, Bibl M, et al. Multiplexed quantification of dementia biomarkers in the CSF of patients with early dementias and MCI: a multicenter study. Neurobiol Aging. 2008;29:812—8.
22. Bouwman FH, Van der Flier WM, Schoonenboom NS, Van Elk EJ, Kok A, Rijmen F, et al. Longitudinal changes of CSF biomarkers in memory clinic patients. Neurology. 2007;69:1006—11.
23. Andreasen N, Minthon L, Davidsson P, Vanmechelen E, Vanderstichele H, Winblad B, et al. Evaluation of CSF-tau and CSF-Abeta42 as diagnostic markers for Alzheimer disease in clinical practice. Arch Neurol. 2001;58:373—9.