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Diagnosing prodromal Alzheimer's disease: Role of CSF biochemical markers

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Abstract

Mild cognitive impairment (MCI) is an aetiologically heterogeneous syndrome. A correct prediction of MCI conversion to Alzheimer's disease (AD) represents a primary goal in routine clinical practice. Since the presence of pathological levels in ≥2 cerebrospinal fluid (CSF) biomarkers; amyloid protein (Aβ42), total tau (h-tau) and phospho-tau (p-tau) seems to reliably identifying MCI subjects converting to AD, we report our experience in a routine clinical setting. In the period from January 2001 to June 2003, 273 consecutive patients referred to our Memory Clinic for diagnostic assessment of cognitive impairment. Of them, 180 underwent a complete diagnostic evaluation including CSF dosage of fragment 1–42 of amyloid protein, total tau and phospho-tau (ELISA Method, Innogenetics, Gent, Belgium), after vascular or other secondary causes of dementia could be excluded. At baseline, 38% of the MCI subjects (20/55) showed pathological levels in ≥2 CSF biomarkers. After 1 year, 11 MCI patients converted to dementia, 33 remained stable, 11 showed a further progression of cognitive impairment still not fulfilling the diagnostic criteria for dementia. Of the 11 converters, 10 showed ≥2 pathological values CSF biomarkers and in all of them, p-tau was high. On the contrary, 29 out of 33 stable MCI (88%) showed no or one pathological CSF value. We confirm that pathological levels in ≥2 CSF biomarkers reliably predict MCI conversion to AD and correctly identify the stable form of MCI.

Keywords: Mild cognitive impairment; Cerebrospinal fluid; β-Amyloid 1–42; Total tau; Phosphorylated tau; Alzheimer's disease

1. Introduction

Mild cognitive impairment (MCI) is an aetiologically heterogeneous syndrome characterised by cognitive impairment shown by objective measures adjusted for age and education in advance of dementia; approximately 12% of MCI convert to Alzheimer's disease (AD) or other dementia disorders every year. The amnestic subtype of MCI represents prodromal AD and should be adequately diagnosed (Dubois and Albert, 2004). The rapid improvement of therapeutical proposals aimed at interfering with AD pathogenetic aspects has highlighted the importance of early and accurate diagnosis of AD.

The degenerative process in AD brain starts 20–30 years before the clinical onset of the disease (Davies et al., 1988; Price and Morris, 1999). During this phase, plaques and tangles loads increase, and at a certain threshold the first symptom appears (Braak and Braak, 1995, 1998). Since the cerebrospinal fluid (CSF) is in direct contact with the extracellular space of central nervous system, biochemical changes taking place in the brain should be reliably reflected. CSF biomarkers of neurodegenerative processes taking place in AD brain are the 42 amino acid form of amyloid protein (Aβ42), total tau (h-tau) and phosphorylated tau (p-tau) protein. Diagnostic markers for AD can be divided into two groups: state markers, reflecting the disease process, and stage markers, reflecting the severity of disease (Blennow and Hampel, 2003); Aβ42, h-tau and p-tau belong to the former category.

Aβ42 is the major component of plaques (Masters et al., 1985). Several mechanisms have been proposed to explain the pathways by which Aβ induces neuronal cell death and specifically, Aβ42 fragment has a well documented neurotoxic effect (Parihar and Hemnani, 2004). A recent autopsy study

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(Strozyk et al., 2003) found a strong association between low CSF concentration of Aβ42 and high numbers of plaques in neocortex and hippocampus. Using Aβ42 protein alone yielded sensitivities varying from 78% to 100% and specificities from 47% to 81% when distinguishing AD from elderly controls (Hampel et al., 2004). Tau protein is located in the neuronal axons and increases in case of axonal damage. The concentration of tau protein in CSF reflects the intensity of neuronal degeneration and/or neuronal damage (Blennow and Hampel, 2003), as observed in different neurological acute and subacute conditions, namely acute stroke (Hesse et al., 2001) and Creutzfeld–Jacob disease (Otto et al., 1997). In differential diagnosis between AD and normal aging, CSF h-tau concentration shows specificity levels between 65% and 86% and sensitivity levels between 40% and 86% (Blennow et al., 2001). The CSF concentration of p-tau reflects the presence of hyperphosphorylated tau in the brain, i.e. the formation of tangles. As opposite to h-tau protein, there is no change in the concentration of p-tau protein after acute/subacute neuronal damage. When comparing AD patients to controls, p-tau$_{181}$ demonstrated 71% sensitivity and 94% specificity compared to h-tau with 63% sensitivity and 100% specificity (Hampel et al., 2004).

The aim of the present investigation was to confirm the usefulness of the CSF biomarkers (h-tau, p-tau and Aβ42) for distinguishing stable MCI from progressive MCI patients, with special interest on MCI converting to Alzheimer's disease, according to the routine clinical setting of our Memory Clinic.

2. Materials and methods

2.1. Subjects

During the period January 2001 and June 2003, 273 outpatients sent by GPs or other specialists for a suspected cognitive impairment, were consecutively examined at our Memory Clinic (Fig. 1). In order to exclude secondary causes, all patients underwent clinical examination, routine blood tests (including vitamin B12, folate, albumin, electrolytes, thyroid function), ECG and basic neuropsychological evaluation (Mini Mental State Examination (MMSE), Milan Overall Dementia Assessment (MODA), Global Deterioration Scale (GDS), Clinical Dementia Rating (CDR), assessment of activities of daily living, Neuropsychiatric Inventory (NPI), for behavioural disorders and Hamilton Depression Scale (HDS), CT scan). Patients with no secondary causes of cognitive impairment at the first screening underwent MRI, brain SPECT and lumbar puncture (LP) for CSF biomarkers determination. Patients fulfilling clinical and neuroradiological criteria for subcortical ischemic vascular dementia (SIVD) did not proceed to further evaluation (i.e., SPECT and/or LP).

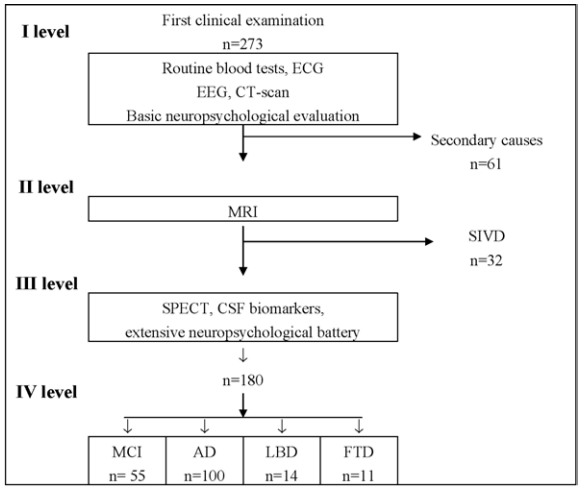

Fig. 1. Diagnostic screening of subjects consecutively referred to our Memory Clinic for suspected dementia. SIVD: subcortical ischemic vascular dementia. MCI: mild cognitive impairment; AD: Alzheimer's disease; LBD: lewy body dementia; FTD: frontotemporal dementia.

LPs were performed after informed consent had been obtained. CSF (10 ml) was collected in sterile polypropylene tubes. In the native CSF, determination of routine chemical parameters (leukocyte and erythrocyte cell count, glucose, lactate, total protein content, IgG index, TPHA) was performed.

The diagnostic criteria used were: for AD, the NINCDS-ADRDA criteria (McKhann et al., 1984); for frontotemporal dementia (FTD), the Lund-Manchester Consensus criteria (1996); for lewy body dementia (LBD), the criteria stated by McKeith et al., 1994; for MCI, the criteria by Petersen et al., 1999.

MCI patients underwent a complete clinical and neuropsychological evaluation after 1 year.

2.2. CSF measurements

The CSF was centrifuged for 10 min at 4000 × *g*, and aliquots of the remaining CSF supernatants were immediately frozen at −80 °C for later h-tau, p-tau and Aβ42 protein determination. CSF biomarkers are measured with the ELISA method. CSF h-tau levels were determined using an ELISA (Innotest hTAU-Ag, Innogenetics NV, Gent, Belgium). The levels of p-tau was determined using ELISA (Innotest pTAU$_{181}$-Ag, Innogenetics NV, Gent, Belgium). The levels of CSF Aβ42 were determined using ELISA (Innotest β-amyloid 1–42, Innogenetics) specific for Aβ42. According to Sjogren et al. (2001), the cutoff for CSF-Aβ42 protein, independent of age, was fixed at >500 pg/ml for healthy subjects; h-tau protein <300 pg/ml was considered normal for subjects 21–50 years old, <450 pg/ml for subjects between 51 and 70 years of age and <500 pg/ml for persons older than 71 years (Sjogren et al., 2001). For p-tau$_{181}$, we used the cutoff <80 pg/ml (Zetterberg et al., 2003).

2.3. Statistical analysis

Levels of CSF markers were compared between groups using nonparametric Kruskal–Wallis ANOVA as a test for the overall group differences, followed by the Mann–Whitney *U*-test for pairwise comparisons. Cutoff levels in our population were derived from receiver operating characteristic (ROC) curve analysis when the sum of specificity and sensitivity was maximized. Standard decision statistics such as specificity, sensitivity and positive predictive value (PPV) were generated by means of a logistic regression model. When comparing areas under the receiver operating characteristic curve, we used the algorithm suggested by DeLong et al., (1988). Statistical analyses were performed with the SAS and STATA software systems. Unless otherwise stated, all statistical tests are two-sided and have a 0.05 significance level.

3. Results

In Table 1, (mean ± S.D.) values, median values and range of CSF biomarkers in all the patients studied are reported. Lowest values of Aβ42 were observed in LBD and highest p-tau levels in AD patients. After 1 year, all MCI patients were re-evaluated. Eleven out of 55 converted to dementia; 33 remained stable; 11 showed a further progression of cognitive impairment, still not fulfilling the diagnostic criteria for dementia.

The biomarkers showed all a clear-cut alteration in converter MCI subjects as compared to stable MCI; furthermore, a significant difference between progressive MCI and converter