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The added value of Aβ42/Aβ40 in the CSF signature for routine diagnostics of Alzheimer's disease

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ABSTRACT

The cerebrospinal fluid (CSF) signature of Alzheimer's disease (AD) includes abnormal levels of amyloid-β 1–42 (Aβ42), total tau (t-Tau) and phosphorylated tau (p-Tau). Several studies have reported that the CSF Aβ42/Aβ40 ratio could outperform CSF Aβ42 as a more accurate marker of brain amyloidosis, since it normalizes the CSF Aβ42 levels according to the total production of Aβ in the brain. In the present study, we wanted to assess the diagnostic utility of adding the Aβ42/Aβ40 ratio within the core AD CSF biomarkers for the classification of patients, according to NIA-AA criteria and Erlangen score. We consecutively recruited 168 patients (62 with AD and 106 with other neurological diseases) who referred to our Memory Clinic for diagnostic work- up from 2003 to 2016. The use of CSF Aβ42/Aβ40 ratio increased the percentage of correctly diagnosed AD patients from 72.0% to 82.8%. The high gain in sensitivity (from 75.8% to 85.5%) was obtained in face of loss of specificity (from 95.3% to 82.5%). Our study showed that the use of CSF Aβ42/Aβ40 could significantly improve the routine diagnostic work up of AD.

1. Introduction

According to current diagnostic criteria for Alzheimer's disease (AD) [1,2], the only validated cerebrospinal fluid (CSF) biomarkers are markers of brain amyloidosis (reduced amyloid-β 1–42, Aβ42) and markers of neurodegeneration (increased total tau, t-tau and phosphorylated tau 181,p-tau) [3]. Some authors have proposed different algorithms for the classification of CSF AD biomarkers profiles. According to the National Institute for Aging and Alzheimer's Association (NIA-AA) criteria for Mild Cognitive Impairment (MCI), the positivity of CSF AD biomarkers can help to classify patients as *MCI due to AD*, with varying levels of certainty (*high likelihood*, *intermediate likelihood* or *unlikely due to AD*). When amyloid and neurodegeneration markers are conflicting, the CSF profile is defined *uninformative* [4]. Also the Erlangen Score, a diagnostic algorithm to interpret CSF profile, takes into account the alterations of CSF Aβ42 and tau species (distinguishing between slightly alterations or clearly pathologic results), and assigns a numeric score ranging from 0 (low risk, non-AD) to 4 (high risk, probable AD) [5]. However, in some cases CSF profile is not discriminative for AD diagnosis, since it is possible to find a normal pattern in AD patients and vice versa. Many efforts have been made in order to improve the interpretation of CSF pattern in case of discrepancies among the three AD core biomarkers. In this context, several studies have reported that the CSF Aβ42/Aβ40 ratio is a very promising biomarker for AD [6–8]. It has been supposed that this ratio "normalizes" the CSF Aβ42 levels according to the total amyloid burden in the brain, reliably expressed by Aβ40 levels (the most abundant amyloid peptide in CSF). Recently, a prospective study documented a significant improvement of diagnostic accuracy in differential diagnosis between AD and non-AD disorders, using Aβ42/Aβ40 ratio when the CSF pattern was not discriminative for AD [9]. A recent paper by Baldeiras and colleagues supported the usefulness of Aβ42/Aβ40 ratio with respect to Aβ42 alone for the correct detection of an underlying AD pathophysiology in MCI patients [10].

On this background, we aimed to assess the performance of CSF Aβ42/Aβ40 ratio with respect to CSF Aβ42 alone in the reclassification of AD and non-AD subjects according to the Erlangen Score and NIA-AA criteria.

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2. Methods

2.1. Study population

We consecutively recruited 168 patients who referred to our Memory Clinic between 2003 and 2016 for diagnostic work-up. All patients underwent a thorough neuropsychological assessment, MRI or CT scan and lumbar puncture. In selected cases, also FDG-PET was performed. Patients with AD dementia were diagnosed according to the criteria for probable AD as defined by the National Institute on Aging-Alzheimer's Association [1]. On the basis of all available elements, patients were classified into two groups: AD and non-AD. Complex or unclear cases were discussed by a multidisciplinary team of neurologists, nuclear doctors, geriatricians and neuropsychologists, and revised taking into account clinical, neuroimaging and CSF data. Non-AD group included patients with neurological disorders other than AD, such as frontotemporal dementia, Lewy body dementia, Parkinson's disease, Creutzfeldt-Jakob disease normal pressure hydrocephalus, and psychiatric disturbances. All the diagnoses were made according to the most up-to date clinical criteria. This research study was approved by the local ethics committee. All patients signed an informed consent to agree with CSF collection, assessment and analysis.

2.2. CSF sampling and analysis

The lumbar puncture (LP) was performed from 8:00 a.m. to 10:00 a.m. after overnight fasting, following a standardized procedure and according to international guidelines. CSF (10–12 ml) was taken from the L3-L4 or L4-L5 interspace, immediately collected in sterile polypropylene tubes, and gently mixed to avoid possible gradient effects. The samples were centrifuged at 2000 $\times g$ for 10 min, aliquoted, and stored at −80 °C. Blood-contaminated samples were excluded from the analysis (cutoff of 50 red blood cells per microliter). Aβ42, Aβ40 were measured with commercially available enzyme-linked immunosorbent assays (ELISAs) purchased by Euroimmun (EUROIMMUN AG, Lübeck, Germany), while total tau (t-tau), and p-tau were detected using INNOTEST kits (Fujirebio Europe, Gent, Belgium) and according to previous reports. Operators blinded to the diagnosis performed the measurements.

2.3. Statistical analysis

R software version 3.4 was used for data analysis. CSF biomarkers cutoffs were determined based on the patients data included in the present study according to ROC analysis. Optimum cutoff values were determined using the highest Youden index. Erlangen Score and NIA-AA criteria were calculated to operationalize the AD diagnosis based on biochemical signature. The subjects characteristics were reported by diagnosis (AD vs. non-AD). The groups were compared using Student's *t*-test for continuous variables and chi-square test for categorical data. Non-parametric alternatives will be considered whenever appropriate. Erlangen score and NIA-AA criteria were calculated with and without the addition of Aβ42/Aβ40 ratio. Finally, we assessed the net reclassification index (NRI) for quantifying the improvement in diagnostic accuracy gained adding the Aβ42/Aβ40 ratio to the standard AD signature. A p-value $< .05$ was considered statistically significant.

3. Results

3.1. Demographical and clinical features

The demographic, clinical and biochemical data are reported in Table 1. There was a significant difference in the frequency of male sex among the groups ($p = .031$), with a lower percentage in the AD group. Also, there was a significant difference among groups in terms of age ($p < .001$), due to the higher mean age in the AD patients. As expected, the MMSE scores were significantly lower in AD patients ($p < .001$). CSF AD biomarkers levels were significantly altered in the AD group with a marked increase of t-tau and p-tau and lower levels of Aβ42 and Aβ42/Aβ 40 ratio to compared to non-AD group.

We have divided the cohort in three subgroups according to age: 55–65, 65–75, and 75–85. We have verified that the 95% CIs of the CSF biomarkers cut-offs calculated in each subgroup were largely overlapping (data not shown).

In Table 2 and Fig. 1, we reported the interpretation of CSF profile according to both NIA-AA research criteria and Erlangen Score. When Aβ42/40 ratio was included in the Erlangen algorithm, a clear improvement in the classification of AD subjects was revealed in face of slight worsening in the profiling of non-AD subjects. When considering

Table 1
Characteristics of the study population.

	AD (n = 62)	Non-AD (n = 106)	p-Value
Age (yr), mean ± SD	72.3 ± 7.2	66.6 ± 8.6	< 0.001
Male/Female	23/39	59/47	0.031
MMSE, mean ± SD	17.6 ± 4.5	24.8 ± 4.5	< 0.001
CSF Tau (pg/ml), mean ± SD	847.3 ± 344.5	379.7 ± 294.3	< 0.001
CSF pTau-181 (pg/ml), mean ± SD	104.9 ± 34.8	51.1 ± 22.1	< 0.001
CSF Aβ42 (pg/ml), mean ± SD	412.2 ± 161.0	676.4 ± 301.5	< 0.001
CSF Aβ40 (pg/ml), mean ± SD	7652.6 ± 2594.7	6408.1 ± 2314.7	0.002
Aβ 42/40 ratio (pg/ml), mean ± SD	0.05 ± 0.02	0.11 ± 0.04	< 0.001
NIA-AA = High	47 (75.8%)	5 (4.7%)	< 0.001
NIA-AA Aβ 42/40 = High	53 (85.5%)	8 (7.5%)	< 0.001
Erlangen Score > 2	50 (80.6%)	6 (5.7%)	< 0.001
Erlangen Score Aβ4240 > 2	60 (96.8%)	12 (11.3%)	< 0.001

Aβ: Amyloid-beta; AD: Alzheimer's disease; CSF: Cerebrospinal fluid; MMSE: Mini Mental State Examination; SD: Standard deviation.

Table 2
CSF interpretation.

Diagnostic algorithm	AD (n = 62)	Non-AD (n = 106)	p-value
NIA-AA			< 0.001
High	47 (75.8%)	5 (5.2%)	
Uninformative	14 (22.6%)	38 (39.6%)	
Unlikely	1 (1.6%)	53 (55.2%)	
NIA-AA with Aβ 42/40			< 0.001
High	53 (85.5%)	8 (8.3%)	
Uninformative	9 (14.5%)	28 (29.2%)	
Low	0 (0.0%)	60 (62.5%)	
Erlangen score			< 0.001
0	0 (0.0%)	48 (50.5%)	
1	0 (0.0%)	4 (4.2%)	
2	12 (19.4%)	37 (38.9%)	
3	12 (19.4%)	0 (0.0%)	
4	38 (61.2%)	6 (6.3%)	
Erlangen score with Aβ 42/40			< 0.001
0	0 (0.0%)	46 (48.4%)	
1	0 (0.0%)	6 (6.3%)	
2	2 (3.2%)	31 (32.6%)	
3	8 (12.9%)	0 (0.0%)	
4	52 (83.9%)	12 (12.6%)	

Aβ: Amyloid-beta; AD: Alzheimer's disease; CSF: Cerebrospinal fluid; NIA-AA: National Institute of Aging Alzheimer Association criteria (high: both Aβ and tau species positive for AD; uninformative: one between Aβ and tau positive and the other one negative for AD; unlikely: both Aβ and tau species negative for AD); Erlangen score: 4: high alteration of both Aβ and tau; 3: a high alteration of one between Aβ and tau and a slight alteration of the other one; 2: a slight alteration of both Aβ and tau or a high alteration of one of these biomarkers and no alteration of the other one; 1 a slight alteration of one between Aβ and tau and no alteration of the other one; 0: no alteration of Aβ and tau. We considered slight alteration a deviation from cut-off of 10%.

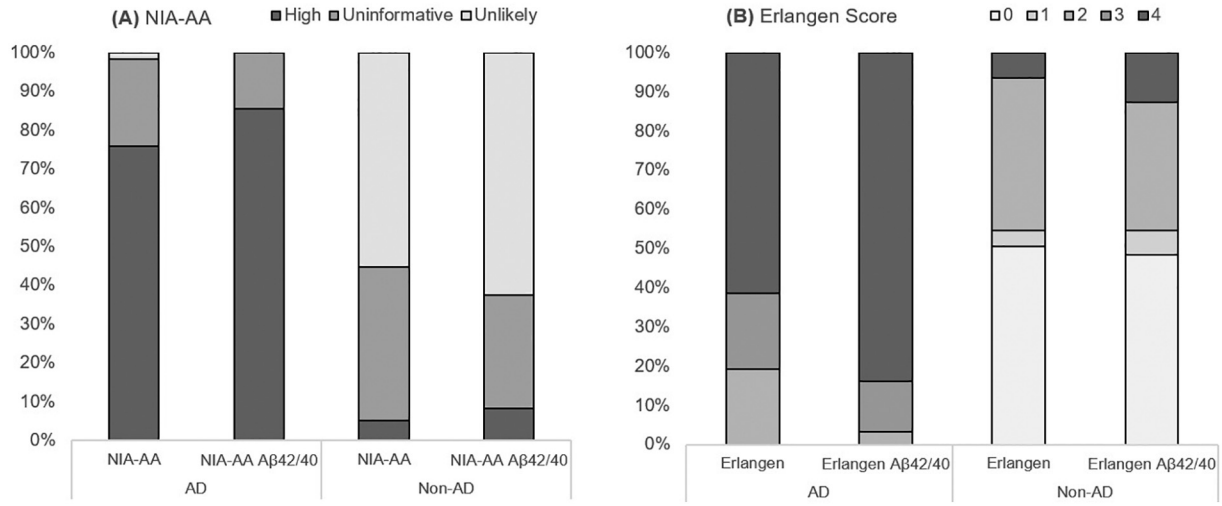

Fig. 1. Classification of AD and non-AD patients according to the CSF profile. CSF biomarkers profile used NIA-AA research criteria (A) and Erlangen Score (B). The barplots show the percentages of patients belonging to each categories: from Unlikely to High for NIA-AA research criteria; from 0 to 4 for Erlangen Score.

a cut-off of 2, the results in terms of NRI are equal to 9.8%. The same results hold for NIA-AA criteria, with a NRI of 6.5% considering "High" as pathological outcome.

4. Discussion and conclusions

In agreement with the available literature, our study supported the usefulness of CSF Aβ42/Aβ40 in the diagnostic work- up of patients with suspect Alzheimer's disease. In particular, our data showed a significant improvement in terms of sensitivity in spite of a little and not significant reduction of specificity.

The whole body of evidences was incorporated in the recent research framework of NIA-AA, where the Aβ42/Aβ40 ratio has been included in the diagnostic panel among pathophysiological AD biomarkers [11]. Also, the revised Erlangen score included the value of Aβ42/Aβ40 ratio for diagnostic classification [12].

A recent study by Lehman and colleagues [13] showed that an optimized PLMR scale (PLM ratio scale) including the Aβ42/Aβ40 ratio was able to increase the percentage of well- classified AD patients.

Consensus among studies that investigated the Aβ42/Aβ40 diagnostic accuracy was good, but it is still not clear whether the Aβ42/Aβ40 ratio is useful as diagnostic resolver when the CSF profile is not discriminative for AD [14], neither if it should be routinely used replacing Aβ42 [10]. Our results could be easily applied in the A/T/(N) research framework. In fact, there is an overlap between some categories in the NIA-AA criteria and A/T/(N) classification system as discussed in the paper of Kern and colleagues [15].

Our results support other approaches for taking into account the Aβ42/Aβ40 ratio in routine diagnostics for AD and in the enrichment strategy for clinical trials of disease-modifying drugs.

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