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ORIGINAL ARTICLE

Ratio $A\beta_{1-42}$:p-Tau: a Possible Diagnostic Tool in Differentiating Dementias

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SUMMARY

Background: **Patients with Alzheimer's disease (AD) present a typical biochemical profile of biomarkers: low concentration of β amyloid 1-42 ($A\beta_{1-42}$), high concentration of total Tau (t-Tau) and phosphorylated Tau at threonine 181 (p-Tau). Several neurodegenerative diseases may overlap with AD, both in regards to clinical symptoms and neuropathology. Many data suggest that Alzheimer's disease (AD) pathophysiology can be identified using biomarkers. It has been hypothesized that subjects with dementia due to AD showed low levels of $A\beta_{1-42}$ combined with the highest levels of total Tau and phosphorylated Tau; moreover, it has been hypothesized that the ratio $A\beta_{1-42}$:p-Tau further help in discriminating Alzheimer's disease from other diagnoses. The aim of this work is to verify this hypothesis in our cohort of patients and to investigate if the same ratio could be a sensitive index able to discriminate MCI due to neurodegenerative factors (MCId) from MCI due to vascular factors (MCIv).**

Methods: **Two hundred sixty-two patients meeting the NIA-AA and NINDS-AIREN criteria were diagnosed as follow: AD in 120 patients [mean age 71.6 (42 - 87)], FTD in 23 patients [mean age 67.3 (46 - 78)], LBD in 17 patients [mean age 73.2 (58 - 83)], VAD in 9 patients [mean age 71.2 (60 - 81)]. According to the criteria proposed by Petersen RC, 24 patients had the diagnosis of MCId [mean age 71.8 (59 - 81)], 38 MCIv [mean age 69.3 (55-82). The comparison between the ratio of $A\beta_{1-42}$/p-Tau among the six groups was done using *t*-test for independent samples. A p-value < 0.05 was considered to represent statistical significance. The ROC (Receiver Operating Characteristic) curve analysis was made using R-studio software.**

Results: **The ratio $A\beta_{1-42}$:p-Tau was significantly lower in AD and MCId with respect to all the other groups and the difference was also statistically significant between MCId and MCIv.**

Conclusions: **$A\beta_{1-42}$:p-Tau ratio has potential for being implemented in the clinical routine for differential diagnosis between AD and other dementias and to distinguish underling pathology such as neurodegenerative or vascular disease.**

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KEY WORDS

dementias, biomarker, $A\beta_{1-42}$:p-Tau ratio

INTRODUCTION

Detecting AD (Alzheimer's disease) at the earliest possible stage is the big challenge that clinicians have to face in the ageing global population. AD is the most common cause of dementia: usually affecting people over the age of 65 years and resulting in progressive

cognitive and functional decline. Considerable efforts have been invested in the identification and replication of biomarkers for this purpose. The hallmark histological signs of AD have been known for more than a century and include atrophy of the brain due to neuronal and synaptic/axonal degeneration and loss, extracellular accumulation of amyloid plaques, and intra-neuronal neurofibrillary tangles consisting of phosphorylated tau [1]. AD is characterized by abnormal patterns in structural and functional imaging as well as by pathological cerebrospinal fluid (CSF) signature [2]. The pathological CSF signature is defined by decreased CSF concentrations of the peptide amyloid-beta 1-42 ($A\beta_{1-42}$) and increased levels of the proteins total Tau (t-Tau) and Tau phosphorylated at threonine 181 (p-Tau). These CSF biomarkers reflect AD neuropathological changes with high accuracy [3,4]. Biomarkers enable the identification of AD pathophysiology in pre-dementia stages of the disease, such as the stage of mild cognitive impairment (MCI) [5]. The term "mild cognitive impairment" refers to patients with objective cognitive impairment and normal capabilities for activities of daily living, who do not meet the criteria for dementia [6]. MCI is a risk factor for dementia and may represent the prodromal phase of AD or other neurodegenerative disorders i.e., vascular dementia (VAD), frontotemporal dementia (FTD), Parkinson disease (PD), dementia with Lewy bodies (LBD). Indeed, MCI can be caused by different diseases (e.g., AD, cerebrovascular disease, depression, frontotemporal lobar degeneration, etc.). As a consequence, only some patients with MCI progress to dementia due to AD or other dementia within limited time periods, while cognitive functioning remains stable or even reverses to normal in others [7,8]. It has been hypothesized that subjects with dementia due to AD showed low levels of $A\beta_{1-42}$ combined with the highest levels of total tau and phosphorylated tau; moreover, it has been hypothesized that the ratio $A\beta_{1-42}$:p-Tau further help in discriminating Alzheimer's disease from other diagnoses [9]. We further hypothesized that the same ratio could be a sensitive index able to discriminate MCI due to neurodegenerative factors (MCId) from MCI due to vascular factors (MCIv). We sought to evaluate the strength of this hypothesis given the importance of defining the pathophysiology of the disorder, when it presents in the earliest phases.

MATERIALS AND METHODS

The studied population was composed as reported in Table 1. All 262 patients underwent the following tests: neurological and neuropsychological examination (consisting of MMSE, verbal fluency, verbal digit span, story recall, Raven's Progressive Matrices, Clock test, Rey figure, FAB (frontal assessment battery), and trail making tests A and B); biochemical tests; cerebrospinal fluid (CSF) analysis; brain MRI, and PET-FDG. At the end of our clinical and instrumental workup, according to NIA-AA and NINDS-AIREN criteria [10-12], we diagnosed AD in 120 patients [mean age 71.6 (42 - 87)], FTD in 23 patients [mean age 67.3 (46 - 78)], LBD in 17 patients [mean age 73.2 (58 - 83)], VAD in 9 patients [mean age 71.2 (60 - 81)]. Acording to the criteria proposed by Petersen RC [13], 24 patients had the diagnosis of MCId [mean age 71.8 (59 - 81)], 38 MCIv [mean age 69.3 (55 - 82)] (Table 1). Lumbar puncture was performed in the morning after overnight fasting and after informed consent was given by patients and/or relatives. CSF was collected in polypropylene tubes. All samples were centrifuged within 30 minutes at +4°C at 3,000 rpm for 10 minutes to remove cells and debris and stored at -80°C until analysis. The CSF sample was thawed only once just before biochemical determination. CSF $A\beta_{1-42}$, t-Tau, and p-Tau were measured using enzyme-linked immunosorbent assays (ELISAs) [INNOTEST® β-amyloid 1-42, INNOTEST® hTau and PHOSPHO-TAU (181P); Fujirebio] according to the manufacturer's instructions (intra- and interassay variances ≤ 10%). Data are presented as mean of concentration ± SD (pg/mL).

Statistical analysis

Data are presented as mean of concentration ± SD (pg/mL) and mean ratio of concentration. For statistical analysis the software STAT-SOFT® was used. The comparison between the ratios of $_{A\beta1-42}$/p-Tau among the six groups was done using *t*-test for independent samples. A p-value < 0.05 was considered to represent statistical significance. The ROC (Receiver Operating Characteristic) curve analysis was made by using R-studio software.

RESULTS

Data on age and gender are presented in Table 1. The mean CSF concentrations of $A\beta_{1-42}$, t-Tau and p-Tau_{181} in the different groups are reported in Table 2. The difference in the CSF concentration of $A\beta_{1-42}$ was statistically significant between AD versus MCIv, FTD, LBD, and VAD while there was no statistical difference between AD and MCId. Moreover, the difference of CSF concentration of $A\beta_{1-42}$ was significant between MCId and MCIv, and between MCId and FTD. The comparison between all the other groups showed no statistically significant results.

Considering the comparison of t-Tau and p-Tau concentration in the studied groups, the difference was statistically significant between AD and MCIv, FTD, LBD, and VAD while there was no statistical difference between AD and MCId.

Moreover, we evaluated the ratio $A\beta_{1-42}$:p-Tau, and the results are represented in Figure 1. The value of the ratio was significantly lower in AD and MCId with respect to all the other groups and the difference was also statistically significant between MCId and MCIv (data shown in Figure 2). We then searched for the best cut-

Table 1. Baseline demographic data.

	AD	FTD	LBD	MCId	MCIv	VAD
No. of cases (gender distribution)	120 (67F, 53M)	23 (9F, 14M)	17 (6F, 11M)	24 (13F, 11M)	38 (18F, 20M)	9 (2F, 7M)
Mean age	71.6 (42 - 87)	67 (46 - 78)	73.2 (58 - 83)	71.8 (59 - 81)	69.3 (55 - 82)	71.2 (60 - 81)

Table 2. CSF concentration (mean ± SD) of $A\beta_{1-42}$, t-Tau, and p-Tau_{181}.

	AD	FTD	LBD	MCId	MCIv	VAD
$A\beta_{1-42}$	428.2 ± 177.5	902.7 ± 307.4	785 ± 390.7	681.1 ± 281.6	917.7 ± 277.7	748.9 ± 119.7
t-Tau	635.8 ± 361.9	358.8 ± 290.7	304.7 ± 183.6	504.7 ± 285.2	295.6 ± 216.2	430.7 ± 260.5
p-Tau	81.5 ± 50.7	53.8 ± 46.9	52.6 ± 31.8	85.8 ± 75.9	45.7 ± 25.2	70.6 ± 52.5

Table 3. $A\beta_{1-42}$:p-Tau ratio in the different studied groups.

	AD	FTD	LBD	MCId	MCIv	VAD
Value of $A\beta_{1-42}$:p-Tau ratio.	7.05 ± 6.1	23.0 ± 13.4	19.5 ± 16.0	10.7 ± 8.7	30.1 ± 41.5	14.1 ± 7.7

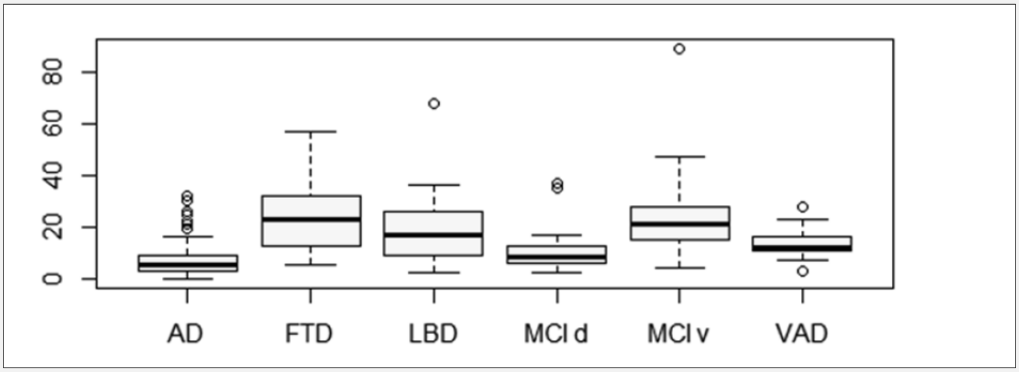

Figure 1. Value distribution of the $A\beta_{1-42}$:p-Tau ratio for all diagnoses.

off value of this parameter to be able to distinguish the different underlying pathology (degenerative or vascular) in this group of patients with MCI. ROC curve (Receiver Operating Characteristic curve) analysis was made. AUC (Area Under the Curve) was 0.8395 (Figure 3) with the best cutoff at 15. AUC would be considered to be "good" at separating MCId from MCIv.

By contrast the difference between MCIv and all the other groups did not reach statistical significance. The difference in the CSF concentration of $A\beta_{1-42}$ was statistically significant between AD and all the other groups. As for $A\beta_{1-42}$, the difference in the CSF concentration of total Tau and p-Tau between MCId and MCIv was statistically significant.

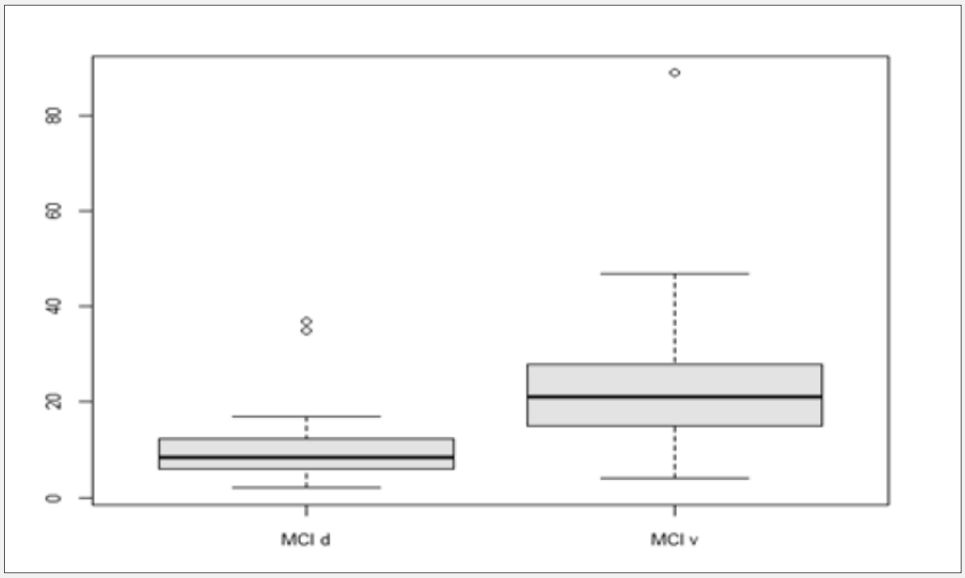

Figure 2. Distribution of the $A\beta_{1-42}$:p-Tau ratio in MCId and MCIv groups. The difference was statistically significant between the two groups ($p < 0.05$).

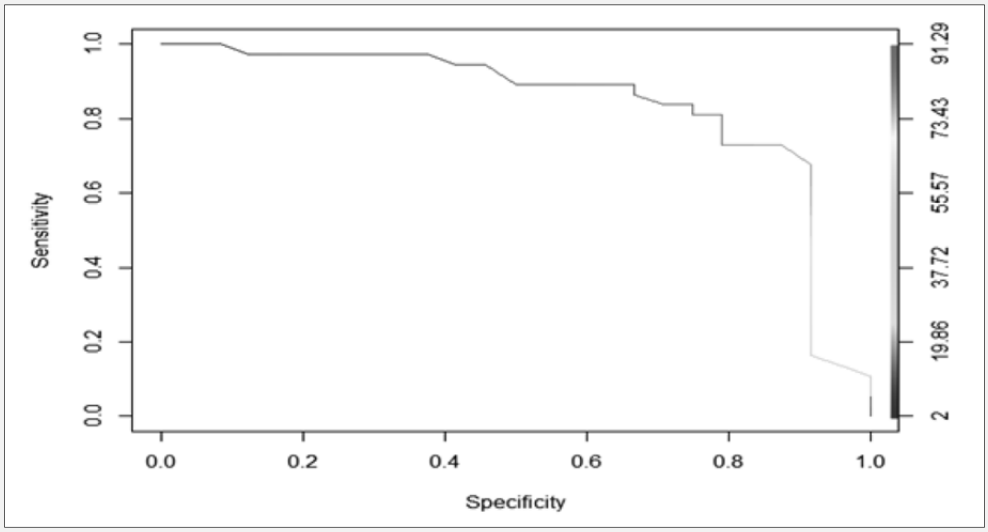

Figure 3. ROC Curve analysis for $A\beta_{1-42}$:p-Tau ratio in MCId and MCIv groups.

DISCUSSION

Sampling CSF is the least invasive direct method for assessing pathologic alteration occurring in the central nervous system (CNS). Bathing not only the superficial portions of the brain, spinal cord, and portions of the cranial and spinal nerves, CSF communicates directly with the cerebral ventricles and extracellular fluid of these structures. For these reasons, CSF provides an optimal source of various biomarkers for pathobiologi-

cal events occurring within the CNS. Proteolytic fragments of the amyloid precursor protein $A\beta_{1-42}$, in addition to t-Tau and p-Tau levels, are the most consistent CSF biomarkers correlated with AD.

As expected, and as reported in a previous study, low CSF concentration of $A\beta_{1-42}$ and high t-Tau and p-Tau are biomarkers for AD and have a discriminatory power in separating Alzheimer's disease from other disorders [14,15].

Alzheimer's disease starts silently and develops several years before clinical symptoms appear. Diagnostic markers for AD should be quantifiable, reliable, and must reflect the principle underlying process associated with the disease. CSF biomarkers are of great potential because they are considered to reflect the pathological process taking place in the brain. CSF biomarkers have proven to be of great diagnostic value in the diagnosis of AD, whereby levels of CSF t-Tau and p-Tau are increased and CSF $A\beta_{1-42}$ are decreased reflecting amyloid plaque load and severity of neurodegeneration, respectively [15]. This study investigates CSF biomarkers for dementia in a relatively large number of different disorders (AD, FTD, LBD, VAD, MCId and MCIv). In particular, we focused our attention on the ability of the ratio $A\beta_{1-42}$:p-Tau to enable the detection of the underlying pathology (degenerative or vascular) in patients with MCI. When individuals are diagnosed with MCI, the two most pressing questions relate to etiology and prognosis. Although the number of patients in some group are small, results obtained are encouraging for the purpose of this work, which was to understand if the ratio $A\beta_{1-42}$:p-Tau could be a sensitive index in discriminating MCI due to neurodegenerative factors (MCId) from MCI due to vascular factors (MCIv).

CONCLUSION

In this study, we emphasize the utility of CSF biomarkers for the differential diagnosis between AD and other forms of dementia. In particular, the combination of two of these biomarkers ($A\beta_{1-42}$ and p-Tau) of neurodegeneration can aid in identifying patients with MCI who are most likely to progress to AD dementia and patients with MCI who are most likely to show cerebrovascular pathology.

Declaration of Interest:
None.

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