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Brief Communication

Increased CSF tau is associated with a higher risk of seizures in patients with Alzheimer's disease

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ABSTRACT

Introduction: Neurofibrillary tangles and tau protein, the neuropathological hallmarks of Alzheimer's disease (AD), have been identified in patients with epilepsy. Tau protein was also associated with the modulation of neuronal excitability in animal models of AD.
Materials and methods: We evaluated in 292 patients with AD the association between the risk of seizure development and AD cerebrospinal fluid (CSF) biomarkers, demographic characteristics, baseline Mini-Mental State Examination (MMSE) score, comorbidities, and apolipoprotein E status.
Results: The development of seizures was associated with younger age at dementia's onset, lower baseline MMSE, and higher CSF total tau protein levels, but only MMSE (hazard ratio [HR] = 0.935; 95% confidence interval [CI] = [0.903, 0.968]; $p < 0.001$) and CSF tau (HR = 1.001; 95%CI = [1.001, 1.002]; $p = 0.001$) were independent predictors on multivariate analysis.
Discussion: While CSF tau and lower baseline MMSE association with seizure development could in part be explained by a greater degree of cortical damage, the role of tau in the modulation of neuronal excitability may also play a role and should be further investigated.

1. Introduction

Tau, a microtubule-associated protein, is natively unfolded in human brains and has an important role in microtubule assembly and stabilization [1]. Hyperphosphorylated tau (P-tau) is a key component of neurofibrillary tangles, which are central to the diagnosis and staging of Alzheimer's disease (AD) [2].

Neurofibrillary tangles have been identified in surgical specimens of dyplastic regions from patients with focal cortical dysplasia and medically refractory epilepsy [3]. In one postmortem study, chronic epilepsy was associated with increased tau neurofibrillary tangles [4]. Additionally, in tissues from refractory temporal lobe epilepsy surgery, 94% of the patients had P-tau in the resected tissue [5].

In AD mouse models, tau has been implicated in the regulation of excitability and synchronization of neuronal networks [6], and its genetic removal decreases interictal spiking and spontaneous seizures [7]. These findings have been extended to mouse and *Drosophila* non-AD genetic models of epilepsy [8]. In one study, endogenous tau concentrations modulate seizure susceptibility [9], with mice with higher levels of tau were more susceptible to neuronal hyperexcitability.

Considering that increased neuronal activity may increase secretion of amyloid-β and tau [10,11], a vicious cycle augmenting the aberrant aggregation and spread of these disease proteins could be hypothesized [12]. In fact, patients with AD have an increased risk rate of epilepsy, especially in early-onset cases, compared with age-matched healthy controls [13].

We aimed to study the role of AD CSF biomarkers (amyloid-β 1-42 peptide (Aβ42), total tau (T-tau), and P-tau proteins) as predictors of the risk of seizures in patients with AD.

2. Materials and methods

2.1. Patient selection

Our study included patients that fulfilled the current diagnostic criteria for AD [14], recruited at the Dementia Outpatient Clinic of the Centro Hospitalar e Universitário de Coimbra (Portugal). Defined inclusion criteria mandated a full neuropsychological evaluation and CSF biomarkers analysis. Patients who developed a structural cause for a

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seizure after the CSF collection were excluded (namely stroke, either ischemic or hemorrhagic, or other tumors). History of traumatic brain injury was also an exclusion criterion. Patients with a previous history of seizures were excluded. Patients who had a previous stroke were included. For the survival analysis, we considered the interval between the time of the lumbar puncture (LP) – baseline – and the time of the seizure, of the last appointment or of death.

These patients are part of a prospectively evaluated cohort at our center. For the purpose of this study, we collected demographical data (age of onset, age at LP, gender, and years of formal education), Mini-Mental State Examination (MMSE) score at LP, other comorbidities (hypertension, diabetes, renal failure, alcohol abuse, and history of stroke), and apolipoprotein E status: patients with one or two ε4 alleles were considered as carriers and the remainder as noncarriers.

Occurrence of seizures was retrospectively determined by consultation of the patients' files, both from our outpatient clinic and from the emergency departments of our and other hospitals. A seizure was determined clinically, with the support of other studies, namely electroencephalography, when considered necessary by the patients' consultant.

We also collected the data on drugs that the patient was taking, namely memantine. All the patients that did not have a seizure during the study interval but took the drug at some point during the interval of follow-up were considered as takers. In the case of the patients that had a seizure, they were considered takers if they were taking memantine before having a seizure, with the exception of the patients that had a seizure and had not been taking memantine in the previous week, who were considered as nontakers, even if they had taken it before.

All patients who did not have a seizure (but were treated for an infection during the study timeline) were considered as having that risk factor. In the case of the patients that had a seizure, infection was considered as a risk factor if diagnosed in the previous or the following week.

The study was conducted according to the revised Declaration of Helsinki and Good Clinical Practice guidelines. Informed consent was given by all study participants or their legal next of kin.

2.2. CSF biomarkers

CSF samples were collected as part of every patient routine clinical diagnosis investigation. Preanalytical and analytical procedures were done in accordance with the Alzheimer's Association guidelines for CSF biomarker determination [15]. CSF samples were collected in sterile polypropylene tubes, immediately centrifuged at 1800*g* for 10 min at 4 °C, aliquoted into polypropylene tubes, and stored at −80 °C until analysis. CSF $A\beta_{42}$, T-tau, and P-tau were measured separately by commercially available sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kits (Innotest, Fujirebio, Belgium), as previously described [16,17]. External quality control of the assays was performed under the scope of the Alzheimer's Association Quality Control Program for CSF Biomarkers [15].

2.3. Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) statistical software (version 20.0; SPSS Inc., Chicago, IL, USA). Categorical data are presented as frequency (percentage) and were compared using χ^2-test. Ordinal or discrete risk factors are represented using median values and compared using Mann–Whitney *U* test. Comparison of the measured variables between the different groups was performed using a Student's *t*-test or γ^2 test.

To assess for independent associations with seizure occurrence, a Cox regression was performed, adjusted for the variables for which there was an association with seizure in the univariate analysis (we considered all the variables with $p < 0.25$): CSF biomarkers levels, age at onset, age at LP, MMSE at the time of LP, history of taking memantine, and history of high blood pressure. History of renal failure was not included because of the reduced amount of cases (1 case in the group of patients who seized). Mortality was not included because it is a posterior event and not a cause. All the assumptions of these models were verified. A Benjamini–Hochberg correction for multiple comparisons was performed, calculating an adjusted α of 0.0023.

3. Results

We included 292 patients. Mean follow-up duration was of 5.35 (±3.43) years. Mean age of onset was of 63.8 (±8.9) years old. Female patients composed 63.4% of the cohort. Median education was of 4.0 (interquartile range [IQR] = 4.0) years. Of 237 patients with apolipoprotein E genotyping, 48.1% had at least one ε4 allele. From the whole sample, 52 (17.8%) patients had a seizure during the follow-up. Table 1 presents the comparison of patients that had a seizure and those who did not in terms of the studied variables.

In univariate analysis, seizure development was associated with the age at dementia's onset, baseline MMSE, and CSF T-tau. There was a trend towards significance of association between seizure development and CSF $A\beta_{42}$ and history of hypertension. Differences between patients that had a seizure and those who did not are depicted on Table 1.

In Cox regression (Table 2), the probability of having a seizure was associated with MMSE at baseline (hazard ratio [HR] = 0.935; 95% confidence interval [CI] = [0.903, 0.968]; $p < 0.001$) and CSF T-tau (HR = 1.001; 95%CI = [1.001, 1.002]; $p = 0.001$).

4. Discussion

We report a moderate sized sample of thoroughly studied patients with AD with CSF biomarkers. We found an association between CSF T-tau and the risk of having a seizure, replicating the findings in animal models [7–9].

In our cohort, patients who developed seizures had, on average, an earlier onset of the dementia. In fact, association of earlier age at onset with higher seizure risk had a trend towards significance (HR = 0.948; 95%CI = [0.912, 0.984]; $p = 0.006$) in multivariate analysis. This may be associated with an additional pathogenic mechanism in early-onset patients, maybe concurring to both an earlier onset of symptoms and a higher risk of seizures. Patients who had seizures

Table 1
Comparison of the two groups in terms of the studied variables. AD: Alzheimer's disease. LP: lumbar puncture. MMSE: Mini-Mental State Examination.

	Patients who had a seizure	Patients who did not have a seizure	p
Age at first seizure/end of follow-up	72.4 (8.3)	74.2 (9.7)	0.251
Age of AD onset (years)	**59.1 (9.4)**	**64.9 (8.5)**	**<0.001**
Female gender (%)	67.3	62.5	0.514
Age at LP (years)	68.4 (8.4)	68.1 (9.9)	0.762
Duration of follow-up (days)	1601.3 (1000.6)	2029.4 (1289.1)	0.025
Education (years)	6.3 (4.6)	5.9 (4.1)	0.442
MMSE at LP	**16.2 (6.4)**	**20.8 (7.4)**	**<0.001**
CSF amyloid-β (pg/ml)	358.06 (150.54)	441.38 (238.18)	0.038
CSF tau (pg/ml)	**619.31 (383.55)**	**444.95 (302.37)**	**0.001**
CSF hyperphosphorylated tau (pg/ml)	79.96 (43.50)	60.09 (82.96)	0.081
Apolipoprotein E carriers (%)	53.7	46.9	0.434
Memantine (%)	40.0	32.0	0.249
History of infection (%)	27.5	31.3	0.779
Hypertension (%)	48.0	63.9	0.036
Diabetes (%)	9.8	15.5	0.299
Renal failure (%)	2.0	7.7	0.136
Alcohol abuse (%)	0.0	0.0	–
History of stroke (%)	6.0	9.8	0.394
Mortality (%)	48.1	35.0	0.077

Bold data indicates $p < 0.05$ was considered statistically significant.

Table 2
Cox regression results of the variables associated with the probability of having a seizure. ($\gamma^2 = 29.740$; degrees of freedom [df] = 9; $p < 0.001$; −2 log likelihood = 167.652). LP: lumbar puncture. MMSE: Mini-Mental State Examination.

Variable	HR	95%CI	p
Age at onset (years)	0.948	0.912, 0.984	0.006
MMSE at LP	**0.935**	**0.903**, **0.968**	<**0.001**
High blood pressure	0.926	0.462, 1.856	0.829
Taking memantine	0.812	0.425, 1.551	0.812
CSF amyloid-β (pg/ml)	0.998	0.996, 1.000	0.083
CSF tau (pg/ml)	**1.001**	**1.001**, **1.002**	**0.001**
CSF hyperphosphorylated tau (pg/ml)	1.002	0.997, 1.006	0.447

Bold data indicates $p < 0.05$ was considered statistically significant.

during the study's interval had lower MMSE at baseline, meaning that they had a more advanced disease. These findings are in accordance with a large study with more than 3000 patients with AD, where younger age and greater cognitive impairment at baseline were independent risk factors for new-onset seizures in AD [18].

The fact that higher tau is associated with an increased probability of having a seizure is in line with the literature and may be in part explained by a greater damage of cortical structures. However, MMSE and CSF T-tau were independent predictors, meaning that the role of tau extends further than the simple stage of the disease. The $tau^{-/-}$ genotype and tau removal have been shown in numerous studies to be protective against excitotoxic insults [7,8,19]. This adds further evidence to the role of tau in the physiological regulation of aberrant neuronal excitability.

The fact that P-tau is not independently associated with seizures in our model may be due to the high colinearity with T-tau. In fact, despite the fact that P-tau is not an independent predictor of seizure risk in the multivariate model, there is a suggestion of association.

History of stroke was not associated with the risk of having a seizure in our study, possibly because of the low number of patients with history of stroke in this cohort. On the other hand, history of infection was also not associated with the risk of seizures, probably because of the high prevalence of infections in patients with AD. While the way we considered infections as a risk factor may also bias the results, there was no significant difference between groups in the prevalence of infection.

The main limitation of our study is its retrospective nature, with a mainly clinical diagnosis of seizure, often relying in the reports of a third person. This may bias the results, as nonmotor seizures may remain unnoticed and even motor seizures in patients with AD could be overlooked or not witnessed. However, the fundamental association of T-tau levels with seizure risk remains valid. Additionally, syncope may be mistaken with seizures, and patients taking anticholinesterase inhibitors may be at an increased risk of these.

5. Conclusions

In conclusion, higher levels of CSF tau are associated with a higher risk of developing seizures in patients with AD. While this could be partly explained by a greater degree of cortical damage, further supported by the association between baseline MMSE and seizure onset, CSF tau was an independent risk factor. This could suggest a role of tau in the modulation of neuronal excitability, as proposed in animal models of AD, and further studies should help clarify this association.

Declaration of Competing Interest

The authors have nothing to report.

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