Cerebrospinal fluid biomarkers in patients with epilepsy in Alzheimer's disease: a nation-wide study

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Abstract

Alzheimer's disease is the most common neurodegenerative dementia. A subset of Alzheimer's disease patients develop epilepsy. The risk is higher in young-onset Alzheimer's disease, but pathophysiological mechanisms remain elusive. The purpose of this study was to assess biomarkers reflecting neurodegeneration in Alzheimer's disease patients with and without epilepsy. By cross-referencing the largest national laboratory database with Swedish national patient registers, we could identify cerebrospinal fluid biomarker results from 17901 Alzheimer's disease patients, and compare levels of neurofilament light, glial fibrillary acidic protein, total tau, phosphorylated tau, and amyloid beta 42 in patients with (n=851) and without epilepsy. The concentrations of total tau and phosphorylated tau were higher in Alzheimer's disease patients with epilepsy than Alzheimer's disease patients without epilepsy and amyloid beta 42 levels were significantly lower in Alzheimer's disease patients with epilepsy. No differences in the levels of neurofilament light and glial fibrillary acidic protein were observed. Our study suggests that epilepsy is more common in Alzheimer's disease patients with more pronounced Alzheimer's pathology, as determined by the CSF biomarkers. Further studies are needed to investigate the biomarker potential of these CSF markers as predictors of epilepsy course or as indicators of epileptogenesis in Alzheimer's disease.

Running title: Epilepsy in Alzheimer's disease

Key words: Alzheimer's disease; Epilepsy; Cerebrospinal fluid; Biomarkers

Abbreviations: AD = Alzheimer's Disease; $A\beta_{42}$ = amyloid beta 42; CSF = Cerebrospinal fluid; GFAP = Glial fibrillary acidic protein; ICD = International Classification of Diseases; NfL = Neurofilament light; NPR = National Patient Register; P-tau = phosphorylated tau; T-tau = Total tau.

Introduction

Alzheimer's disease (AD) carries an increased risk of epilepsy.[1,2] The pathophysiology remains elusive. Theoretically, AD-associated epilepsy could result from an individual predisposition to seizures triggered by neurodegeneration in vulnerable individuals, or from specific features of the neurodegenerative process. Studies of clinical characteristics seem to favor the latter explanation; young-onset and clinically severe AD are risk factors for epilepsy, and persons with AD and epilepsy may have higher cognitive decline and faster progression of symptoms.[3]

Cerebrospinal fluid (CSF) reflect brain changes and quantification of CSF biomarkers is increasingly used in AD.[4,5] Total tau (T-tau), phosphorylated tau (P-tau), and amyloid beta 42 ($A\beta_{42}$) are key pathological biomarkers for the diagnosis and staging of AD.[6] Neurofilament light (NfL) reflects axonal degeneration and injury.[7] Glial fibrillary acidic protein (GFAP) reflects astroglial activation or blood-brain barrier dysfunction across a broad range of acute and chronic neurological diseases.[8] Whether biomarker profiles differ in persons with or without AD-related epilepsy remains unknown.

In an attempt to elucidate whether epilepsy develops in patients with AD because of degenerative changes or individual predisposition, we asked if biochemical marker levels differed between AD patients with and without epilepsy. We used 20 years of laboratory data at Sahlgrenska University Hospital, the largest national provider of CSF biomarker analyses, and comprehensive national patient registers to identify 17901 individuals diagnosed with AD and compared CSF profiles of AD patients with and without epilepsy.

Materials and methods

Registers and study cohort

The Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital was among the first in Sweden to analyze CSF biomarkers in AD and for many years the sole national provider of these analyses. The laboratory database was searched for all individuals with an entry for CSF tau (any

form). The search identified 73370 individuals. For these individuals, we obtained all CSF analyses for brain injury markers. The data was sent to the National Board of Health and Welfare to identify individuals with a diagnosis of Alzheimer's disease in the National Patient Register (NPR). NPR contains information on all inpatient hospital admissions since 1987 and hospital-based outpatient visits since 2001. Diagnoses of AD and epilepsy were ascertained by identification of relevant International Classification of Diseases, 10th version (ICD-10) criteria; code F00 or G30 for AD and code G40 for epilepsy. Comorbidities that could also cause epilepsy or affect biomarker levels were identified by the relevant ICD-10 codes: stroke (I60-I69), traumatic brain injury (S00-S09), and CNS-neoplastic disease (C71, C793, D430, D32, D330). Information on anti-seizure medication (ASM) was obtained from the Drug Register, which contains information on all dispensed drugs in Sweden since 2005. The final study cohort included 17901 AD patients, out of which 851 also had epilepsy (Fig. 1- Flow chart).

For the analysis of CSF biomarkers, we included all AD-epil patients with onset of epilepsy after AD diagnosis and age >55 at AD onset. Case ascertainment in this analysis was based solely on the diagnostic code. Age- and sex-matched controls were selected from AD patients without epilepsy. In one sensitivity analysis, individuals with epilepsy and controls with a diagnosis of trauma or stroke before the CSF test were excluded, since such CNS insults may alter biomarker levels. In an additional sensitivity analysis, patients with CNS neoplastic disease were excluded.

Ethical approval

This study was approved by Swedish Ethical Review Authority (approval number 2020-05717). The National Board of Health and Welfare anonymized all data after linkage and before we had access to them. All handling of personal data was done in agreement with Swedish data protection laws.

CSF Biomarkers

The CSF samples were obtained by lumbar puncture according to standard procedures as described previously.[9] Biomarkers were measured at the Clinical Neurochemistry Laboratory at Sahlgrenska

University Hospital. CSF was analyzed continuously as part of routine clinical practice. The samples were analyzed using commercially available enzyme-linked immunosorbent assays (ELISA) to determine the levels of T-tau, Aβ42, P-tau (INNOTEST, Fujirebio, Ghent, Belgium), and NfL (UmanDiagnostics, Umeå, Sweden). GFAP levels were quantified using an in-house ELISA based on polyclonal antibodies.[10] The biomarker measurements were performed by board-certified laboratory technicians who were blind to clinical data and used protocols accredited by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC).

Statistical analysis

Biomarkers were compared in matched analyses where each AD patient with epilepsy was matched with a control (AD without epilepsy) with the same sex and closest age. If more than one sample of a biomarker was available for a patient, the sample taken closest to the date of AD onset was used for analysis. Subgroups analyzed were onset before or after age 65. The matching was then performed to match each AD patient with epilepsy with a control. In sensitivity analyses, patients with stroke or traumatic brain injury before a CSF test were removed (if for a patient, an earlier test was available before stroke or trauma, that test was used) and a separate analysis removed patients with CNS neoplastic disease before the CSF test. CSF biomarker levels were assessed using Student's *t*-test. Levels were considered significantly regulated at $p < 0.05$. A standard indicator of statistical significance was used in the figures (ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$). Data were analyzed using IBM SPSS Statistics version 26.0 for Windows and statistical analysis was performed using R software (Version 4.0.2).

Data Availability

The dataset for this study is protected by Swedish privacy laws and agreements between Sahlgrenska University Hospital and the register holder (National Board of Health and Welfare) and cannot be shared by the authors.

Results

Study cohort

The study cohort consisted of 17901 patients where 851 (4.75%) AD patients had epilepsy and 17050 (95.25%) were without epilepsy (Fig. 2A). The demographic and clinical characteristics of the patients are shown in Table 1. The age and sex distributions were comparable between the groups (Fig. 2B, Table 1), but stroke (28%) and trauma (31%) were more common in AD patients with epilepsy compared with AD patients without epilepsy (Table 1). Epilepsy onset in AD was more common between 60-80 years of age (Fig. 2C) and the first epilepsy diagnosis was often close to AD diagnosis (Fig. 2D). The time of the CSF analysis in relation to the AD diagnosis was similar in patients with and without epilepsy (Table 1).

CSF measures

CSF biomarker levels were analyzed in AD-ep patients, and age- and sex-matched controls (NfL: n = 226, GFAP: n = 83, T-tau: n = 384, P-tau: n = 364, Aβ42: n = 364 per group). The concentrations of T-tau and P-tau were higher in AD patients with epilepsy (p = 0.0019 and p = 0.0002, respectively) compared with AD patients without epilepsy (median, min-max; T-tau: 620 (107-6940) ng/L vs 567.5 (79-2480) ng/L, P-tau: 81 (18-253) ng/L vs 72.5 (13-197) ng/L) (Fig. 3C and D). The $A\beta_{42}$ levels were significantly lower (p = 0.0002) in AD patients with epilepsy (median, min-max; $A\beta_{42}$: 380 (144-1550) ng/L vs 437 (140-1140) ng/L) (Fig. 3E). There were no significant differences in the levels of NfL and GFAP (Fig. 3A and B).

In a sensitivity analysis, we excluded patients with other insults that could have affected CSF biomarker levels like stroke or traumatic brain injury before the CSF test (NfL: n = 185, GFAP: n = 73, T-tau: n = 320, P-tau: n = 302, $A\beta_{42}$: n = 302 per group). This did not alter the results; levels of T-tau (p = 0.0047) and P-tau (p = 0.0004) were still higher in AD patients with epilepsy (median, min-max; T-tau: 640 (108-6940) ng/L vs 577.5 (79-2480) ng/L, P-tau: 83 (21-253) ng/L vs 74.5 (16-197) ng/L), and the levels of $A\beta_{42}$ (p <0.0001) were lower (median, min-max; $A\beta_{42}$: 370 (144-1200) ng/L vs 446 (140-1450) ng/L) (Fig. 3H, I, and J). Similarly, we found no significant differences in concentrations of NfL and GFAP between the groups if patients with stroke or

traumatic brain injury before the CSF were excluded (Fig. 3F and G). In an additional sensitivity analysis, we excluded patients with codes for CNS neoplastic disease, which did not alter the results (supplementary table 1). We also performed analysis on patients under and over 65 years of age (under 65 years of age: NfL: n = 59, GFAP: n = 28, T-tau: n = 115, P-tau: n = 108, $A\beta_{42}$: n = 108 per group and over 65 years of age: NfL: n = 167, GFAP: n = 55, T-tau: n = 269, P-tau: n = 256, $A\beta_{42}$: n = 256 per group), where $A\beta_{42}$ was significantly lower in both groups (under 65 years of age p = 0.0151 and over 65 years of age p = 0.004) (Fig. 4E and J) and T-tau and P-tau levels were significantly higher under 65 years of age group ($p = 0.00245$ and $p < 0.0001$, respectively) (Fig. 4 C and D) (under 65 years of age: median, min-max; T-tau: 710 (150-6940) ng/L vs 562 (79-1870) ng/L, P-tau: 89.5 (25-253) ng/L vs 70 (16-189) ng/L, $A\beta_{42}$: 379.5 (144-952) ng/L vs 409 (200-1140) ng/L and over 65 years of age: median, min-max; T-tau: 581 (107-3400) ng/L vs 569 (134-2480) ng/L, P-tau: 76 (18-250) ng/L vs 73 (13-197) ng/L, $A\beta_{42}$: 380 (168-1550) ng/L vs 451 (140-1100) ng/L).

Discussion

In this study, we describe the profile of CSF biomarkers that reflect neurodegeneration and AD pathological processes in patients with and without epilepsy. This is the first and largest investigation of CSF biomarkers in this patient group, and the study offers insights into possible pathophysiological mechanisms of seizures in AD. We found epilepsy to be associated with increased levels of CSF T-tau and P-tau, and decreased levels of $A\beta_{42}$. These are key changes that are specifically associated with AD, and not associated with other neurodegenerative dementias,[4] which suggests that the development of epilepsy is linked to a more pronounced AD process. Put differently – patients with epilepsy in AD seem to have a more marked biochemical AD profile than patients who do not develop epilepsy.

The aggregation of hyperphosphorylated tau (also known as neurofibrillary tangles) in the cell body is a key pathological feature of AD. Increased phosphorylation and release of tau from neurons in CSF appears to reflect a neuronal response to Aβ deposition in AD.[11,12] Tau is attracting increasing interest in both epilepsy and dementia research; higher levels of tau aggregation have been described in brain tissue from AD patients with seizures, perhaps reflecting greater damage to

cortical neuronal networks or epileptogenesis in AD.[13-17] Interestingly, tau aggregation is also described in non-AD epilepsy and is linked to both seizure frequency and cognitive decline.[18-20] Our findings of higher levels of T-tau and P-tau in AD patients with epilepsy are in agreement with these observations, but whether the increased tau levels cause or reflect seizure activity remains to be determined.

Aβ is a secreted proteolytic cleavage product of the transmembrane amyloid precursor protein (APP), and accumulation of $A\beta_{42}$ into extracellular plaques in the brain is an early event in AD pathogenesis.[21] Aggregation of $A\beta_{42}$ in the brain parenchyma results in reduced concentration of the protein in CSF.[22] Low CSF $A\beta_{42}$ concentration is associated with late-onset epilepsy and subsequent development of AD,[23,24] but differences between AD patients with and without epilepsy have to our knowledge not been reported previously.

In summary, we found more pronounced biochemical evidence of AD pathology in AD patients with epilepsy. In contrast, we found no differences in NfL and GFAP levels between AD patients with and without epilepsy. These proteins are used as general markers of neurodegeneration and astrocytic activation and are not AD-specific.[25,26] The absence of an association of CSF NfL and GFAP with epilepsy in AD further underscores that epilepsy in AD is associated with AD pathology as such and not general neurodegenerative brain changes. A subgroup analysis of individuals younger vs. older than 65 years of age showed similar significant changes in the $A\beta_{42}$ levels, whereas T-tau and P-tau concentrations were significantly higher in the epilepsy compared with the non-epilepsy group only among younger individuals. This may be due to increased prevalence of subclinical AD pathology in older age groups, making AD biomarker results overall less informative in the elderly[27,28].

One important factor to consider when interpreting the results of this study is the timing of the CSF analysis. Lumbar puncture is a standard component of a dementia workup, with memory problems being the main indication. In general, the lumbar puncture in our material was performed before the AD diagnosis was made. Since our analysis only included individuals who were diagnosed with epilepsy after the AD diagnosis, the selective difference detected in our material for tau and $A\beta_{42}$ could indicate that epilepsy develops in patients with a more severe AD trajectory. It is well in line with clinical observations that seizures tend to develop in severe AD.[29] Unfortunately, we did not have access to dementia severity for our cohort, but an interesting future study for increased

patophysiological understanding could be to match cases with and without epilepsy of similar AD severity. Similarly, we did not have access to data on epilepsy severity and in future studies association between a a more pronounced AD CSF profile and seizure frequency would be very interesting. However, the correlation between CSF profile and positron emission topgrapy (PET) of tau brain pathology is not absolute, and tau-PET seems superior to CSF analysis when it comes to analysing disease progression,[30] so a multimodal approach including functional imaging would probably be of greatest value when trying to understand how AD pathology causes seizures. Regional distribution of AD pathology to particularly epileptogenic brain regions like the termporal lobe could also be interestig to explore in imaging studies.

To our knowledge, the combination of data from the largest national analysis provider with national register data has resulted in the largest study so far of biochemical markers in AD, covering CSF analyses between 2000 and 2021. Apart from the study size, another advantage of our approach is the unbiased detection of an administrative epilepsy diagnosis. Drawbacks include our reliance on administrative data. AD and epilepsy diagnoses are sometimes erroneous, but more importantly seizures are not seldom overlooked in patients with dementia. In general, the PPV of a dementia diagnostic code in the NPR is high (>80%), but the PPV for AD specifically is lower (56%),[31] so a subset of the patients with AD will have another reason for their dementia. However, given that our study population was examined with a lumbar puncture suggesting a dementia-interested center and that most patients received their AD diagnosis after the lumbar puncture, we suspect that the diagnostic accuracy is higher in our material. This is supported by the fact that the non-epilepsy group also had $A\beta_{42}$ levels below normal and indicative of true AD.[32] The PPV of a diagnosis of epilepsy is about 90%.[33] Importantly, diagnostic errors in the presence of absence of epilepsy are unlikely to be systematic with regard to the biomarker levels. Another way of interpreting our findings is that presence of epilepsy makes it more likely that a person with an AD diagnosis will have CSF biomarker results with a pronounced AD profile.

In summary, we found a more pronounced AD biomarker profile concerning the levels of T-tau, P-tau, and $A\beta_{42}$ in AD patients with epilepsy. More studies are needed. In addition to elucidating the pathophysiological processes underlying AD, an interesting question is whether the biochemical profile can contribute to increased clinical awareness of seizures in AD. With the

emergence of new drugs in dementia, a key question is also whether disease-modifying drugs can delay or prevent epileptogenesis.

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Competing interests

JZ has received consultancy fee from the Swedish Medical Products Agency, speaker honoraria from UCB and Eisai for non-branded education events, and as employee of Sahlgrenska University Hospital is or has been an investigator/ sub-investigator in clinical trials sponsored by GW Pharma, SK life science, UCB and Bial (no personal compensation). HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

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Figure legends

Figure 1 Study flow chart describing cohort's identification. After database search for CSF tau in Sahlgrenska University Hospital, the data was sent to National Board of Health and Welfare to identify individuals with a diagnosis of Alzheimer's disease in the National Patient Register (NPR). Diagnoses of AD and epilepsy were ascertained by identification of relevant ICD codes. The final study cohort included 17901 AD patients, out of which 851 had epilepsy and 17050 patients were without epilepsy.

Figure 2 The study cohort. (A) Population of AD with and without epilepsy and **(B)** sex distribution. **(C)** Histogram showing age of epilepsy onset (patients >55 years of age to the right of dashed line were included) and **(D)** days from AD to epilepsy diagnosis (patients with epilepsy onset after AD, to the right of the dashed line were included)**.**

Figure 3 Analysis of CSF biomarkers in AD patients with and without epilepsy (Fig. 3A-E). (NfL: $n = 226$, GFAP: $n = 83$, T-tau: $n = 384$, P-tau: $n = 364$, $A\beta_{42}$: $n = 364$ per group). Boxes show the median, first and third quartile, and minimum and maximum value (excluding outliers). Student's *t*-test T-Tau ($p = 0.0019$), P-Tau ($p = 0.0002$) and $A\beta_{42}$ ($p = 0.0002$). Excluded patients who had stroke and trauma before CSF test (Fig. 3F-J). (NfL: $n = 185$, GFAP: $n = 73$, T-tau: $n = 320$, P-tau: $n = 302$, $A\beta_{42}$: $n = 302$ per group). Boxes show the median, first and third quartile, and minimum and maximum value (excluding outliers). Student's *t*-test T-tau ($p = 0.0047$), P-tau ($p = 0.0004$) and $A\beta_{42}$ ($p < 0.0001$).

Figure 4 Analysis of CSF biomarkers in AD patients with and without epilepsy under and over 65 years of age. (under 65 years of age: NfL: $n = 59$, GFAP: $n = 28$, T-tau: $n = 115$, P-tau: $n = 108$, $A\beta_{42}$: $n = 108$ per group and over 65 years of age: NfL: $n = 167$, GFAP: $n = 55$, T-tau: $n = 269$, P-tau: $n = 256$, $A\beta_{42}$: $n = 256$ per group). Boxes show the median, first and third quartile, and minimum and maximum value. Student's *t*-test under 65 years of age: T-Tau ($p = 0.0025$), P-Tau ($p < 0.0001$) and $A\beta_{42}$ ($p = 0.0151$) and over 65 years of age $A\beta_{42}$ ($p = 0.004$).

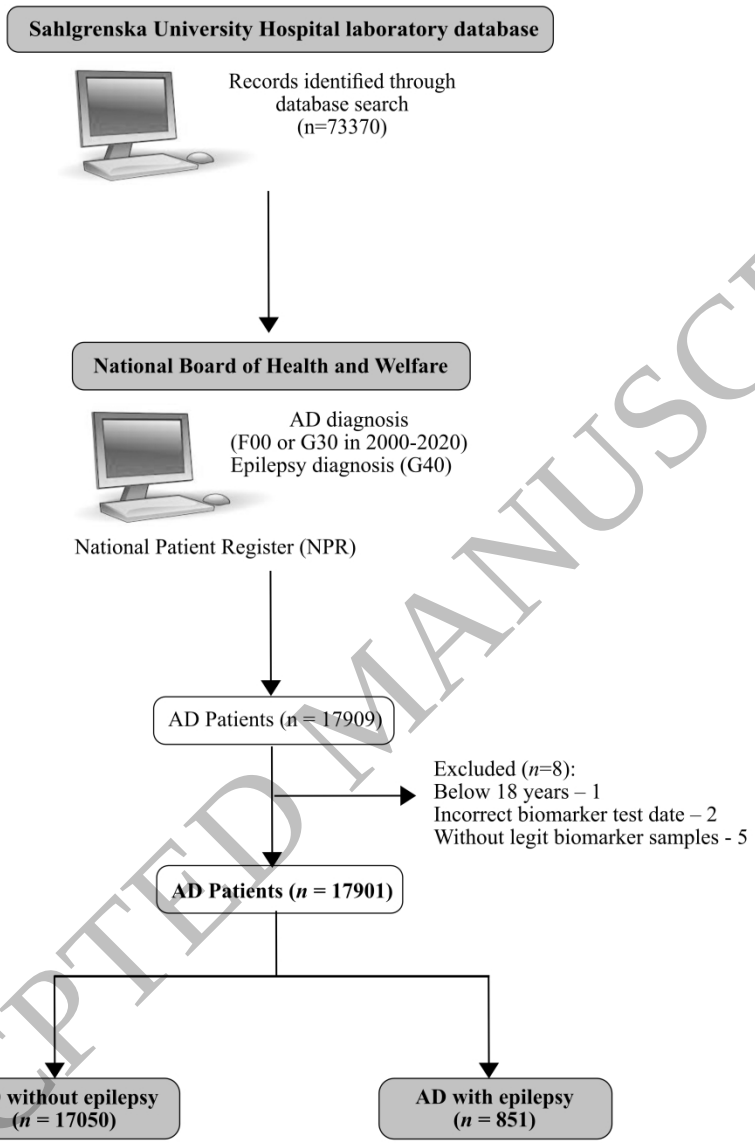

Figure 1 Study flow chart describing cohort's identification. After database search for CSF tau in Sahlgrenska University Hospital, the data was sent to National Board of Health and Welfare to identify individuals with a diagnosis of Alzheimer's disease in the National Patient Register (NPR). Diagnoses of AD and epilepsy were ascertained by identification of relevant ICD codes. The final study cohort included 17901 AD patients, out of which 851 had epilepsy and 17050 patients were without epilepsy.

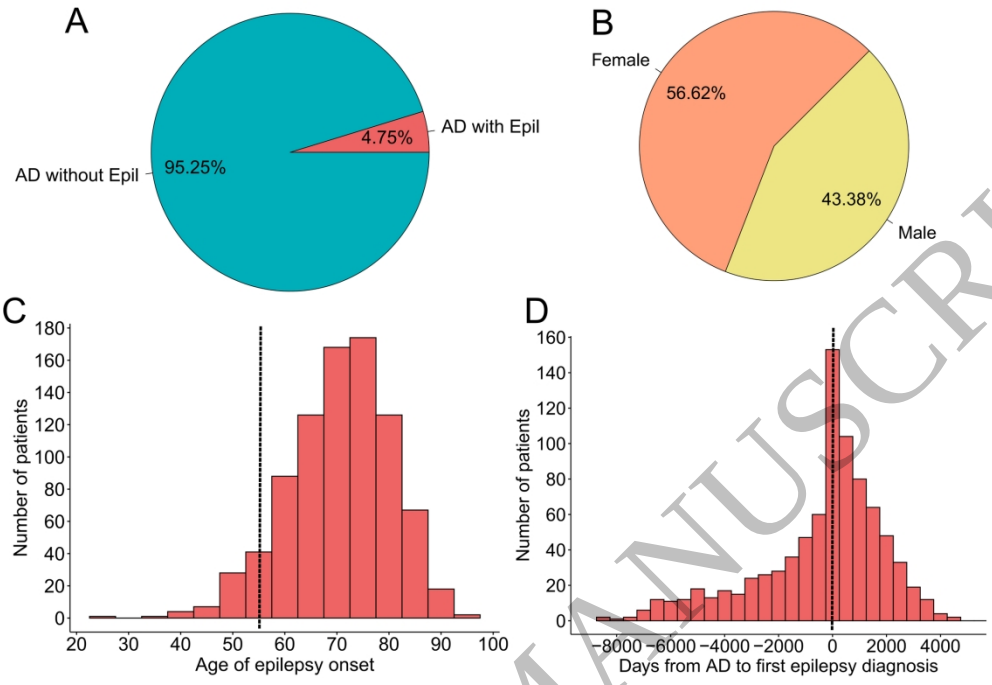

Figure 2 The study cohort. (A) Population of AD with and without epilepsy and (B) sex distribution. (C) Histogram showing age of epilepsy onset (patients >55 years of age to the right of dashed line were included) and (D) days from AD to epilepsy diagnosis (patients with epilepsy onset after AD, to the right of the dashed line were included).

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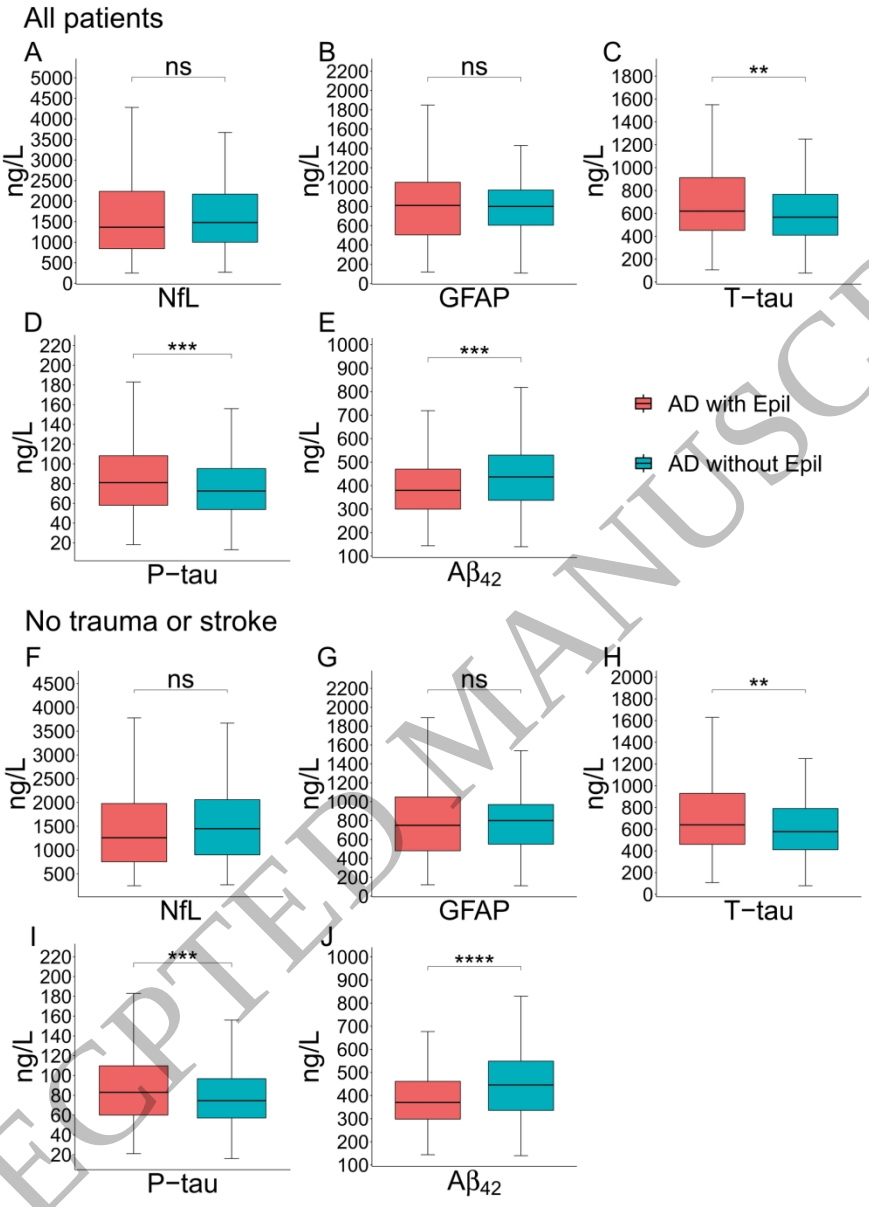

Figure 3 Analysis of CSF biomarkers in AD patients with and without epilepsy (Fig. 3A-E). (NfL: n = 226, GFAP: n = 83, T-tau: n = 384, P-tau: n = 364, Aβ42: n = 364 per group). Boxes show the median, first and third quartile, and minimum and maximum value (excluding outliers). Student's t-test T-Tau (p = 0.0019), P-Tau (p = 0.0002) and Aβ42 (p = 0.0002). Excluded patients who had stroke and trauma before CSF test (Fig. 3F-J). (NfL: n = 185, GFAP: n = 73, T-tau: n = 320, P-tau: n = 302, Aβ42: n = 302 per group). Boxes show the median, first and third quartile, and minimum and maximum value (excluding outliers). Student's t-test T-tau (p = 0.0047), P-tau (p = 0.0004) and Aβ42 (p <0.0001).

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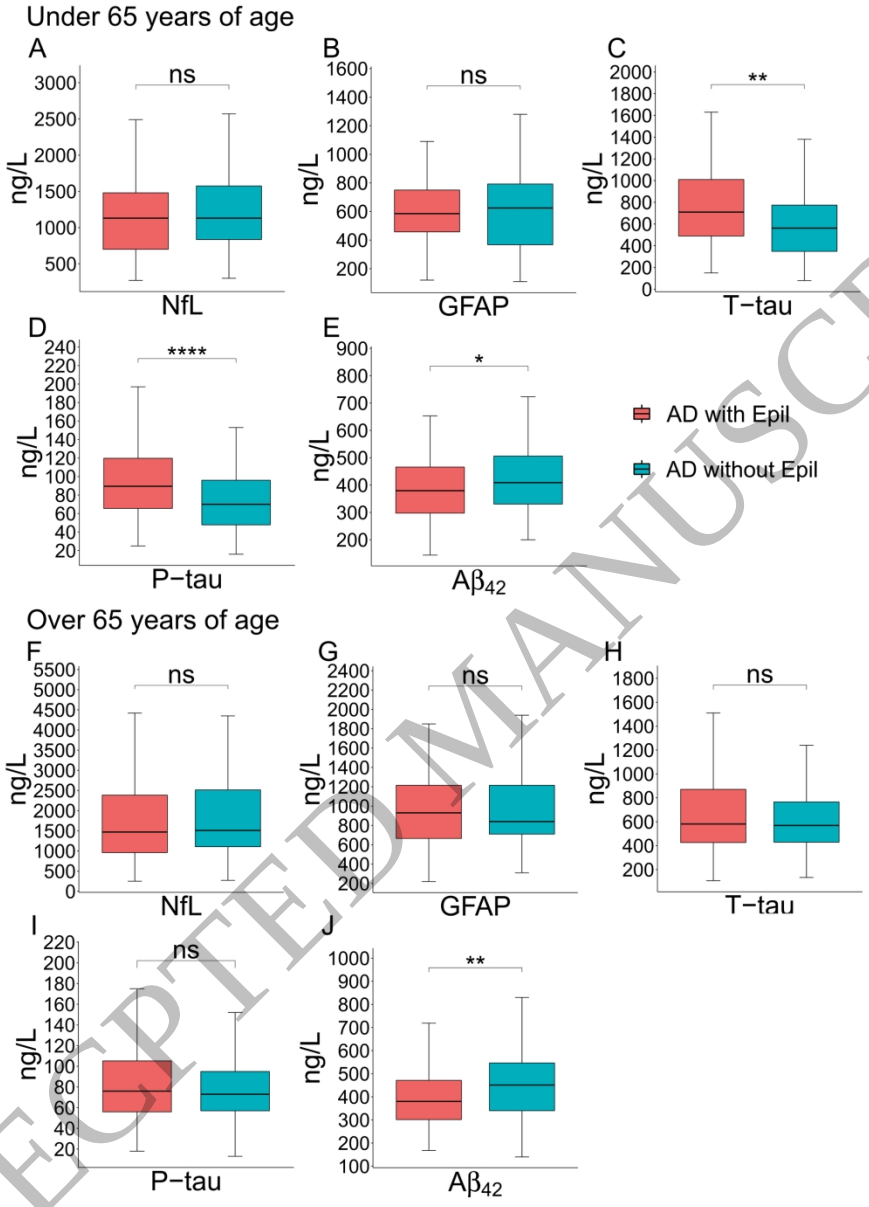

Figure 4 Analysis of CSF biomarkers in AD patients with and without epilepsy under and over 65 years of age. (under 65 years of age: NfL: n = 59, GFAP: n = 28, T-tau: n = 115, P-tau: n = 108, Aβ42: n = 108 per group and over 65 years of age: NfL: n = 167, GFAP: n = 55, T-tau: n = 269, P-tau: n = 256, Aβ42: n = 256 per group). Boxes show the median, first and third quartile, and minimum and maximum value. Student's t-test under 65 years of age: T-Tau ($p = 0.0025$), P-Tau ($p < 0.0001$) and Aβ42 ($p = 0.0151$) and over 65 years of age Aβ42 ($p = 0.004$).

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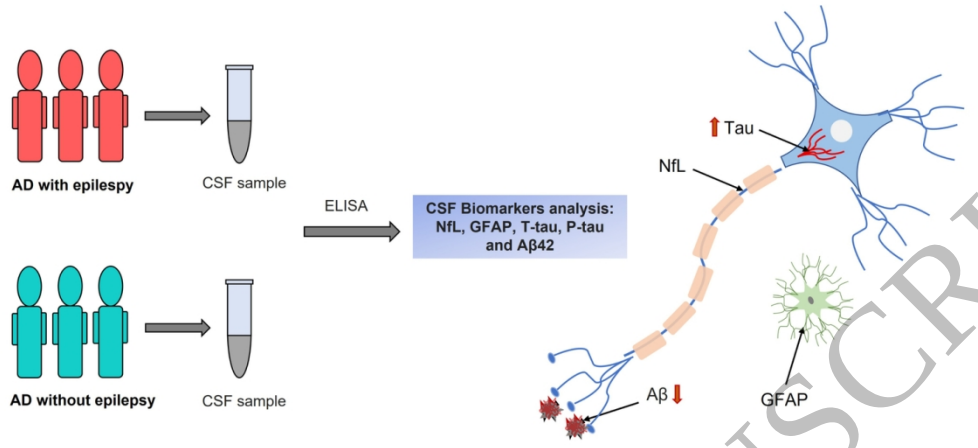
Table 1 Study cohort: description of demographics and clinical characteristics of the patients.

	AD without Epil	**AD with Epil**
N (%)	17050 (95.25%)	851 (4.75%)
Sex		
Male	7358 (43.2%)	408 (47.9%)
Female	9692 (56.8%)	443 (52.1%)
Age at export, mean ± SD	74.10 ±7.93	71.61 ±9.25
Epilepsy age (Age at Epi diagnosis)		70.51± 9.87
Age at CSF test, mean ± SD	73.34 ± 7.77	71.18 ± 9.16
Time from AD diagnosis to CSF test, mean ± SD	- 215.99 ± 600.53	- 239.18 ± 647.98
Deceased	9018 (52.9%)	505 (59.3%)
Comorbidities		
Stroke	2309 (13.5%)	238 (28.0%)
Trauma	3449 (20.2%)	265 (31.1%)
ASM	2467 (14.5%)	750 (88.1%)



Abbreviations: AD = Alzheimer's Disease; $A\beta_{42}$ = amyloid beta 42; CSF = Cerebrospinal fluid; ELISA = enzyme-linked immunosorbent assays; GFAP = Glial fibrillary acidic protein; NfL = Neurofilament light; P-tau = phosphorylated tau; T-tau = Total tau.

Graphical Abstract

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