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Diagnosis associated with Tau higher than 1200 pg/ mL: insights from the clinical and laboratory practice

Lehmann S* 1, Paquet C* 2, Malaplate-Armand C 3, Magnin E 4, Schraen S 5, Quillard-Muraine M 6, Bousiges O 7, Delaby C 1, Dumurgier J 2, Hugon J 2, Sablonnière B 4, Blanc F 8, Wallon D 9, Gabelle A 10, Laplanche JL 11, Bouaziz-Amar E 11, Peoc'h K 11,12 and ePLM group

**These authors collaborated equally to the manuscript*

¹ CHU de Montpellier and Université de Montpellier, IRMB, CRB, Laboratoire de Biochimie et Protéomique Clinique, 80 Avenue Augustin Fliche, 34295 Montpellier, France

²Centre de Neurologie Cognitive, Groupe Hospitalier Saint-Louis Lariboisière Fernand-Widal APHP, INSERM U942, Université Paris Diderot, France

³ Laboratoire de Biochimie et Biologie Moléculaire, UF Oncologie - Endocrinologie - Neurobiologie, Hôpital Central, Centre Hospitalier Universitaire, Nancy, France

⁴ Centre Mémoire Ressources Recherche Besançon Franche-Comté, Département of Neurology, CHU Besançon, Besançon, France

⁵ Univ.Lille, Inserm, CHU-Lille, UMR-S1172 and Neurobiology Unit, Centre de Biologie-Pathologie, Lille, France

⁶ Laboratoire de Biochimie, Rouen University Hospital, Rouen, France

⁷ Laboratoire de Biochimie et de Biologie Moléculaire, Hôpital de Hautepierre, Hôpitaux Universitaires de Strasbourg, Strasbourg, France ; Laboratoire de Neurosciences cognitives et Adaptatives (LNCA) UMR7364 Unistra/CNRS, Strasbourg, France

⁸ 2ICube laboratory and FMTS (Fédération de Médecine Translationnelle de Strasbourg), team IMIS-Neurocrypto, University of Strasbourg and CNRS, Strasbourg, France

⁹ Inserm U1079, University of Rouen, Department of Neurology

¹⁰ Centre Mémoire Ressources Recherche, CHU de Montpellier, Hôpital Gui de Chauliac, Montpellier, and Université Montpellier, Montpellier, France

¹¹ Service de Biochimie et Biologie moléculaire, GH Saint-Louis-Lariboisière-Fernand Widal, APHP, Paris, France

¹² APHP, HUPNVS, Hôpital Beaujon, Biochimie clinique, Clichy, France

Corresponding author

Katell Peoc'h

Biochimie Clinique, Hôpital Beaujon

100 bd du Général Leclerc

92118 Clichy Cedex

Tel: 33140875368

Katell.peoch@aphp.fr

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Conflict of interest statement

The authors have no conflict of interest to declare.

ABSTRACT

Context

Cerebrospinal fluid (CSF) biomarkers are valuable tools for the diagnosis of neurological diseases. We aimed to investigate within a retrospective multicentric study the final diagnosis associated with very high CSF Tau levels and to identify patterns of biomarkers that would differentiate them in clinical practice, to help clinical biologists into physicians' counseling.

Patients and methods

Within the national multicentric network ePLM, we included 1,743 patients from January 1, 2008, to December 31, 2013, with CSF biomarkers assayed by the same Innostest assays (protein Tau, phospho-Tau [pTau], and A β 1-42). We identified 205 patients with protein Tau concentration higher than 1200 pg/mL and final diagnosis.

Results

Among those patients, 105 (51.2%) were suffering from Alzheimer disease, 37 (18%) from sporadic Creutzfeldt-Jakob disease, and 63 (30.7%) from other neurological diseases including paraneoplastic/ central nervous system tumor, frontotemporal dementia, other diagnoses, amyloid angiopathy, Lewy body dementia, and infections of the central nervous system. Phospho-Tau, A β 1-42 and A β 1-42/pTau values differed significantly between the three groups of patients ($p < 0.001$). An A β 1-42/pTau ratio between 4.7 and 9.7 was suggestive of other neurological diseases (threshold in AD: 8.3). CSF 14-3-3 was useful to discriminate Alzheimer disease from Creutzfeldt-Jakob disease in case of A β 1-42 concentrations < 550 pg/mL or pTau > 60 pg/mL.

Conclusion

This work emphasizes the interest of a well-thought-out interpretation of CSF biomarkers in neurological diseases, particularly in the case of high Tau protein concentrations in the CSF.

Keywords

Alzheimer disease; Creutzfeldt-Jakob disease; Cerebrospinal fluid; Tau protein; 14-3-3 protein; dementia

Abbreviations

A β 42: peptide amyloid 1-42

AD: Alzheimer disease

CJD: Creutzfeldt-Jakob disease

CSF: Cerebrospinal fluid

LP: lumbar puncture

pTau: phospho-Tau

1 INTRODUCTION

Most neurodegenerative diseases are associated with the accumulation of abnormal forms of proteins in the brain, leading to neuronal death, and frequently associated with a pathological variation of biomarkers in the cerebrospinal fluid (CSF). These biomarkers have been included in the state-of-the-art differential diagnosis of dementia.

Protein Tau is a soluble protein, which favors the stability of microtubules in the axon and regulates the neuronal cytoskeleton's dynamic [1]. Under pathological conditions, the protein Tau undergoes post-transcriptional phosphorylation processes and aggregates into filamentous brain inclusions, known as neuronal fibrillary tangles [2]. The presence of protein Tau in the CSF has been related to the passive release of the protein by altered neurons [3], or secretion by neurons [4].

In clinical practice, a drastic Tau elevation is challenging to interpret for both the clinical biologists and physicians. Moreover, the medico-economic utility of performing dilution for precise quantification of Tau in this setting is not explicitly stated. However, clinical biologists are mandated to provide counseling regarding the interpretation of biological results according to the International Organization for Standardization (ISO) 15189.

In a multicentric European study including 3,034 patients with various rapidly progressive neurodegenerative diseases, the median CSF protein Tau concentration was 224 pg/mL in all neurodegenerative diseases (Min-Max: 0-38,400) except Creutzfeldt-Jakob disease (CJD), and very high Tau concentrations were a rare pattern observed in only a few percent of the patients (7.6% of patients with Tau>1300 pg/mL and Alzheimer disease -AD-) [5].

We, therefore, aimed to investigate within a retrospective descriptive multicentric study which diagnosis very high CSF Tau concentrations tended to be associated with, and which patterns of biomarkers could provide a practical help to differentiate them.

2 MATERIALS AND METHODS

2.1 Subjects

Patients enrolled in this retrospective study were all coming for exploration in a specialized center of the French ePLM network and had a lumbar puncture (LP) in this setting. The ePLM network [6] includes seven French memory research centers, specialized in the diagnosis, treatment, and follow-up of patients with dementia with suspected dementia and neurodegenerative diseases, using the same diagnostic procedure and criteria. Eligibility criteria's were age over 18 years and cognitive of behavioral disorders.

Patients' familial history of dementia, psychiatric disorders, and neurological diseases were systematically recorded at the initial outpatient consultation. When appropriate, LP and CSF analysis were performed in the routine clinical testing on patients with cognitive or behavioral deficits, in keeping with the local French regulations applying to Memory clinics. For each patient, the diagnosis was made according to validated clinical diagnostic criteria [7][8][9][10][11] and considering CSF biomarkers results. All the patients showing a CSF Tau level above 1200 pg/mL were retrospectively selected.

2.2 CSF samples

CSF, obtained by LP using a 25-gauge needle and standardized procedures [12][13][14], was collected in polypropylene tubes [15]. CSF samples were transferred to laboratories within 4h, centrifuged at 1,000g for 10 min at 4°C, aliquoted into 0.5 mL polypropylene tubes, and stored at -80°C for further analysis within two months.

2.3 CSF Biomarkers

CSF Amyloid β 1–42 (A β 1-42), Tau, and Human tau phosphorylated at Thr181 (pTau) were quantified by standard commercially available INNOTEST® sandwich ELISA assays

(INNOTEST_ hTAU Ag, INNOTEST_β- AMYLOID(1–42); INNOTEST_ PHOSPHO-TAU(181P), Fujirebio, Courtaboeuf, France) according to the manufacturer's instructions. The intra-assay variability observed in replicates was less than 10%, or the assay result was excluded and the sample re-assayed. All the laboratories in this study participated in an external quality control assessment organized by the Alzheimer's association's external quality controls program. Internal quality controls were included in each series, and followed with time, to identify a shift of the results. When drastic changes or shifts were observed on controls, a new batch was verified and used.

The 14-3-3 protein detection in the CSF was carried out when CJD was thought to be a possible diagnosis, as recommended in routine clinical practice. A semi-quantitative interpretation was performed by western blot [16]. Results were classified as negative, positive or trace. For further statistical analysis, traces were classified as negative. The ePLM laboratories performing this detection (Montpellier and Paris) participated in national and international external quality assessment schemes.

2.4 Statistical analysis

Descriptive analysis, statistical tests and graphs were performed using the GraphPad Prism 7 (GraphPad, CA, USA), Past (3.2) and Excel software (Microsoft). Kruskal-Wallis tests were realized to compare biomarkers between all groups, and the results were considered significant for $p < 0.05$. Comparison of data between groups was performed by the Mann-Whitney U test for quantitative variables. Tree diagram was conceived in Smartdraw (<https://www.smartdraw.com/>).

3 RESULTS

3.1 Population description

We included 1,743 patients from January 1, 2008, to December 31, 2013. A CSF protein Tau concentration above 1200 pg/mL with a confirmed final diagnosis after one to six years were obtained for 4.5 to 10% of the patients according to the center, leading to a final population of 205 patients.

The patients were mostly female (sex ratio: 0.72). Patients' mean age was 68 years (range: 23-91 years). Final diagnoses are presented in Table 1. One hundred and five patients were diagnosed as suffering from AD (51.2%). Eight presented a combination of AD and vascular dementia. The second most frequent diagnosis in our cohort was CJD (37 patients; 18%). All CJD patients were either certain or probable sporadic cases, according to WHO criteria [11]. The prion protein gene *PRNP* 129 genotype was available in 14 patients (nine patients Met/Met, four Val/Val, and one Met/Val).

Sixty-three patients (30.7 %) exhibited another neurological disease, including frontotemporal dementia, paraneoplastic and CNS tumor, toxic or metabolic dementia, amyloid angiopathy, Lewy body dementia, or infectious diseases. Nine patients were suffering from other neurodegenerative conditions with dementia (two patients with MCI and dementia, one patient with a thalamic hematoma, one patient with a Gayet-Wernicke encephalopathy and acute seizure, one patient with narcolepsy, one patient with normal pressure hydrocephaly, one patient with acute seizures, one patient with multiple system atrophy, and one patient with both alcohol chronic intoxication and possible frontotemporal dementia). The autopsy results obtained for six patients (five CJD and one AD) were by the antemortem "confirmed final diagnosis."

Patients were then divided into three groups AD, CJD, and non-AD non-CJD for further analysis. The time elapsed between the onset of the first symptoms and the LP was available for 82 patients (36 AD, 11 CJD, and 35 non-AD non-CJD patients). It was significantly

different between groups (Figure 1). The lower median was obtained for CJD patients (3 months; range: 1 to 18 months) compared to non-AD non-CJD patients (median: 2 years; range: 1 month to 18 years), and AD patients (median: 3 years; range: 1 month to 10 years).

3.2 pTau and A β 1-42/ pTau ratio are useful to discriminate between the three groups of patients

Table 2 summarized the results obtained for biomarkers in the three groups of patients. Figures 2 and 3 presented individual data. The patient's data presented a high degree of overlap. Median pTau, A β 1-42 concentrations, and A β 1-42/pTau ratio differed significantly between the three groups of patients ($p < 0.0001$; Fig 3). CJD patients presented the lowest pTau values, with no patients with pTau higher than 101 pg/mL.

We used A β 1-42/pTau ratio to integrate both beta-amyloid and Tau pathways in a single index.

Similarly to patients with a moderate increase in protein Tau, AD patients mostly presented decreased A β 1-42 concentrations and increased pTau concentrations. Using the following thresholds (550 pg/mL and 60 pg/mL for A β 1-42 and pTau respectively), 25 AD patients were above the threshold for A β 1-42, and 31 below the threshold for pTau. Using both pTau and A β 1-42, 69 patients would have been successfully classified on a total of 105. The A β 1-42/pTau ratio ranged from 0.556 to 22.39 with a median at 2.98. The A β 1-42/pTau ratio threshold for AD would be less than 8.3.

In CJD patients, 28 patients were above 550 pg/mL for A β 1-42 and 24 below 60 pg/mL for pTau. A classification based on these biomarkers would have led to the correct classification of 19 patients. An ambiguous pattern with high pTau and normal A β 1-42 concentration was observed in 11 patients. The A β 1-42/pTau ratio was high (median: 14.85; range 2.96-41).

Finally, in non-AD non-CJD patients, the profile generally showed low or subnormal A β 1-42 concentrations and slightly increased pTau concentrations. Among those 63 patients, 31 were above 550 pg/mL for A β 1-42 and 28 below 60 pg/mL for pTau. A classification based on these biomarkers would have led to an incorrect classification of 18 patients as AD patients.

3.3 The protein 14-3-3 to discriminate between AD and CJD patients

We then analyzed the 14-3-3 detection to evaluate whether this analysis would help for the differential diagnosis. Among the 36 CJD patients with 14-3-3 detection, a positive result would have led to the appropriate classification of 29 patients, 27 when traces was considered as negative.

4 DISCUSSION

The diagnosis of neurodegenerative diseases is challenging in clinical practice, particularly at the onset of the disease, or when dealing with atypical clinical pictures. CSF biomarkers provide useful tools in clinical practice.

Our results confirmed that few patients displayed protein Tau concentration >1200 pg/mL in the CSF (nearly 10% in patients with a final diagnosis).

Very high Tau protein concentrations were first associated with CJD [17] [18]. They have also been described in patients with acute stroke [19], traumatic brain injury [20], herpetic encephalitis [21] and some AD patients [22].

In our cohort, most of the patients with Tau>1200 pg/mL were suffering from AD. According to this result, Van der Vlies et al. described that nearly 10% of AD patients presented very high protein Tau concentrations (mean 1,720 pg/mL) [23], associated with worse performances in memory tests. A protein Tau concentration >900 pg/mL in AD has been associated with earlier nursing home placement [24]. The pathological process leading to the release of a large amount of protein Tau in the CSF in a few AD patients remains to be elucidated. It could be related to the duration of evolution. In our cohort, the recorded times elapsed since the first symptoms obtained for 36 AD patients were heterogeneous, ranging from 2 to 10 years, whereas it ranges from 1.5 to 18 months for CJD (n=11 patients) and 1.5 months and 18 years for non-AD-non-CJD patients (n=35 patients). Most extensive studies should be undertaken to unravel any association between very Tau levels and the duration of the disease in AD.

CJD was the second most frequent diagnosis in our study, although its estimated annual prevalence is around 1.5 per million inhabitants in Europe [25], but according to with the fact that the disease is associated with high Tau concentrations, mainly unphosphorylated Tau [26].

pTau concentrations were generally low, whereas A β 1-42 concentrations were generally high in those patients, differentiating them from AD patients. Indeed, in our cohort, no CJD patient exhibited pTau higher than 101 pg/mL.

In most cases, our patients benefited of a protein 14-3-3 detection, that was positive in 73%, and ambiguous in 19%. The detection of the 14-3-3 protein in the CSF is currently used in the pre-mortem diagnosis of sporadic Creutzfeldt Jakob disease (CJD)[27]. The detection of the pathologic prion protein by real-time *in vitro* protein amplification is however quite promising in this setting [28] [29].

Finally, the third group of patients displayed neurodegenerative dementia (frontotemporal dementia, Lewy body dementia), but also toxic, metabolic, neoplastic, infectious, and vascular neurological diseases. This subgroup could be underestimated since we retained for our study only patients with an *ante-mortem* final diagnosis after one to six years after LP. This group was highly heterogeneous in term of etiology, duration and biomarkers' patterns. Our secondary objective was to determine which biomarkers were the most relevant to differentiate these groups biologically.

First, there was a significant overlap of biomarkers in the three patients groups, underlying the difficulty of differential diagnosis. As could be expected, CJD and AD patients were the most straightforward groups to differentiate, both using biomarkers and the duration of the disease. A very high Tau concentration with a low A β 1-42 and a high pTau first evoke AD, particularly after several months of evolution.

However, low A β 1-42 was not a significant reason to rule out the diagnosis of CJD, since eight patients finally diagnosed as CJD presented A β 1-42 lower than 550 pg/mL. In this setting, the diagnostic of CJD could be documented by 14-3-3 detection.

We finally evidenced that a ratio A β 1-42/pTau comprised between 4.5 (Upper 95 percentile of CI of mean for AD group) and 12.6 (Lower 95 percentile of CI of mean for CJD group)

was suggestive of non-AD non-CJD diagnosis in patients with protein Tau concentration >1200 pg/mL. Typical patterns of biomarkers in the three groups are presented in Table 3. A tree diagram is proposed in Figure 4.

The main limitation of our study is its retrospective setting, which is a limitation for evaluating a personalized approach in the diagnosis. Moreover, biomarkers were used in the diagnosis which may generate a bias.

In conclusion, this study gave some avenues for interpreting biomarkers for patients with Tau>1200 pg/mL.

Using A β 1-42/1-40 ratio, instead of CSF A β 1-42 alone, is also known to reduce the number of discordant cases. Moreover, a combination of biological fluids and imaging biomarkers such as PET, is probably the best way to capture all the aspects of the Tau pathology, as previously published [30].

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TABLE LEGENDS

Table 1: Diagnosis spectrum of Tau>1200 pg/mL in CSF from patients with final diagnosis

Table 2: Concentrations of CSF biomarkers in the different groups of patients. IQ : interquartile

Table 3: Typical Biomarkers' patterns compared to threshold in the three groups of patients with Tau higher than 1200 pg/mL

FIGURE LEGENDS

Figure 1: Comparison of the duration between the first recorded symptoms and LP in the three sub-groups of patients.

Median and interquartile were represented. Kruskal Wallis test was used to determine the significance of the difference between the three groups, and the Mann Whitney test was used to comparing each pair (full line).

Figure 2: XY plot of the studied population

All patients' results were plotted according to their respective values of pTau and A β 1-42 in pg/mL. The lower thresholds for AD diagnosis in our network (A β 1-42 < 550 pg/mL and pTau > 60 pg/mL) were indicated by the dotted arrows.

Figure 3: Scatter dot plot (Median with IQ range) of CSF biomarkers in patients divided into three sub-groups AD, CJD, non-AD non-CJD

Kruskal Wallis test was used to determine the significance of the difference between the three groups, whereas Mann Whitney was used to comparing each pair (full line).

A- Scatter dot plot of A β 1-42

B- Scatter dot of pTau

C- Scatter dot of A β 1-42/ pTau ratio

Figure 4 : Tree diagram for diagnosis of patients with Tau higher than 1200 pg/mL

*For AD diagnosis, low A β 1-42 and high pTau refer to values above 550 pg/mL for A β 1-42 and below 60 pg/mL for pTau.

Table 1

Diagnosis	Number of patients (%)
Alzheimer Disease	105 (51.2)
Isolated	97 (47.3)
Mixed/combined	8 (3.9)
Sporadic Creutzfeldt Jakob Disease	37 (18)
Other	63 (30.7)
Paraneoplastic/CNS Tumour	8 (3.9)
Frontotemporal dementia	7 (3.4)
Vascular	5 (2.4)
Toxic and metabolic dementia	4 (2)
Amyloid angiopathy	3 (1.5)
Lewy body dementia	3 (1.5)
Infectious	2 (1)
Other dementia or neurological diseases	9 (4.4)
Total	205 (100)

Table 2

Patients subgroup	AD	CJD	Non-AD non-CJD
Number of patients	105	37	63
Aβ1-42 concentration			
Median	433	734	500
IQ range	355-543	479-956	346-767
Minimum-Maximum	125-1030	195-1493	125-2021
pTau concentration			
Median	146	57	85
IQ range	119-176	39-75	45-138
Minimum-Maximum	28-252	15-101	16-227
Aβ1-42/pTau			
Median	3.98	14.85	6.11
IQ range	2.40-4.01	9.70-19.57	3.53-14.7
Minimum-Maximum	0.56-22.39	2.96-41	1.59-36.11

Table 3

Biomarker/Diagnosis	AD	CJD	Non AD non CJD
A β 1-42	↓ or ↓↓	N	N or ↓
pTau	↑↑	N	↑
A β 1-42/pTau	Low	High	In-between
14-3-3	Negative	Positive	Negative, Ambiguous or Positive

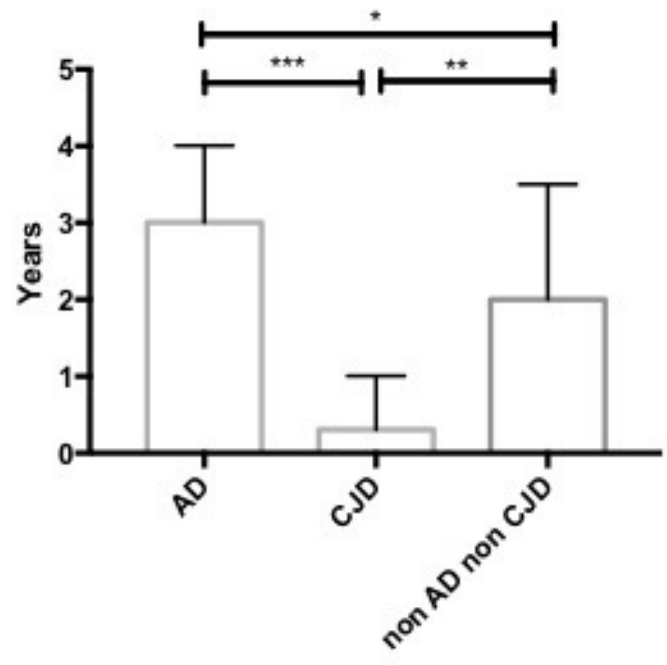


Figure 1

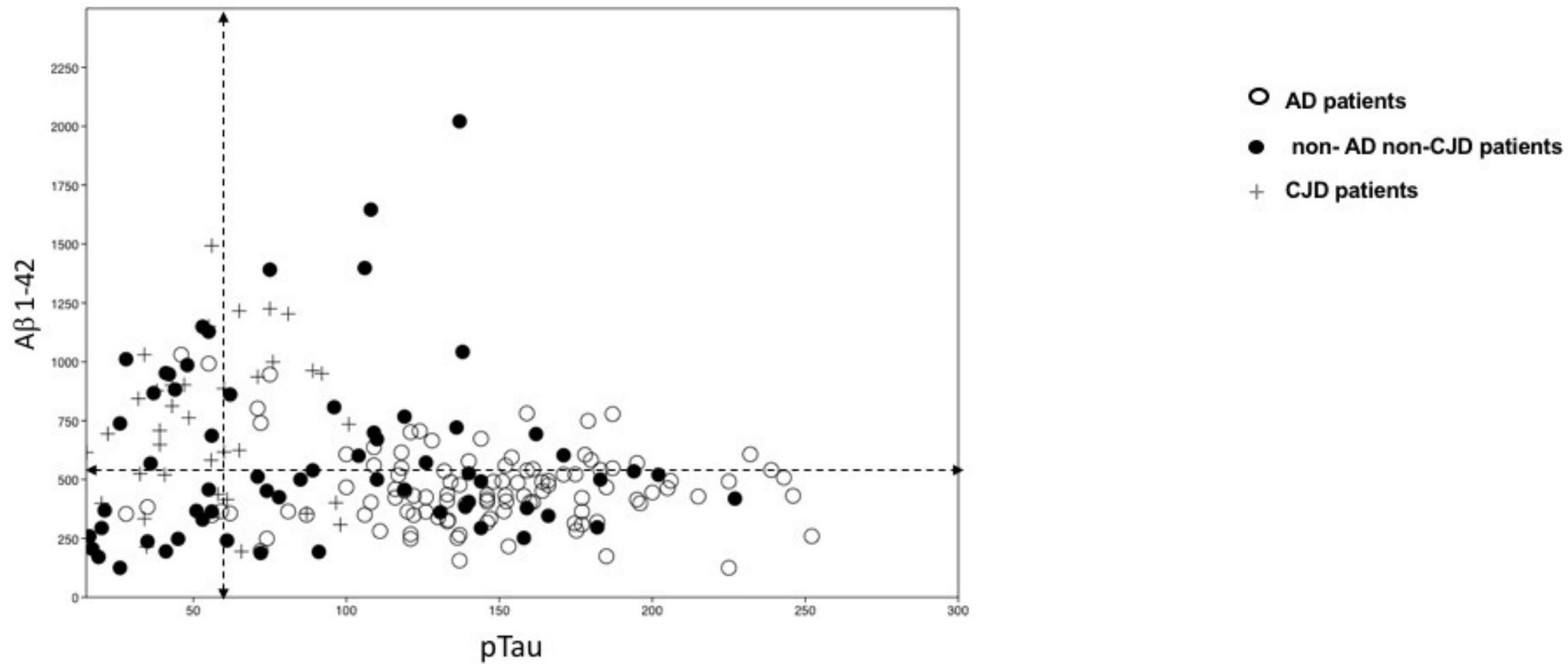


Figure 2

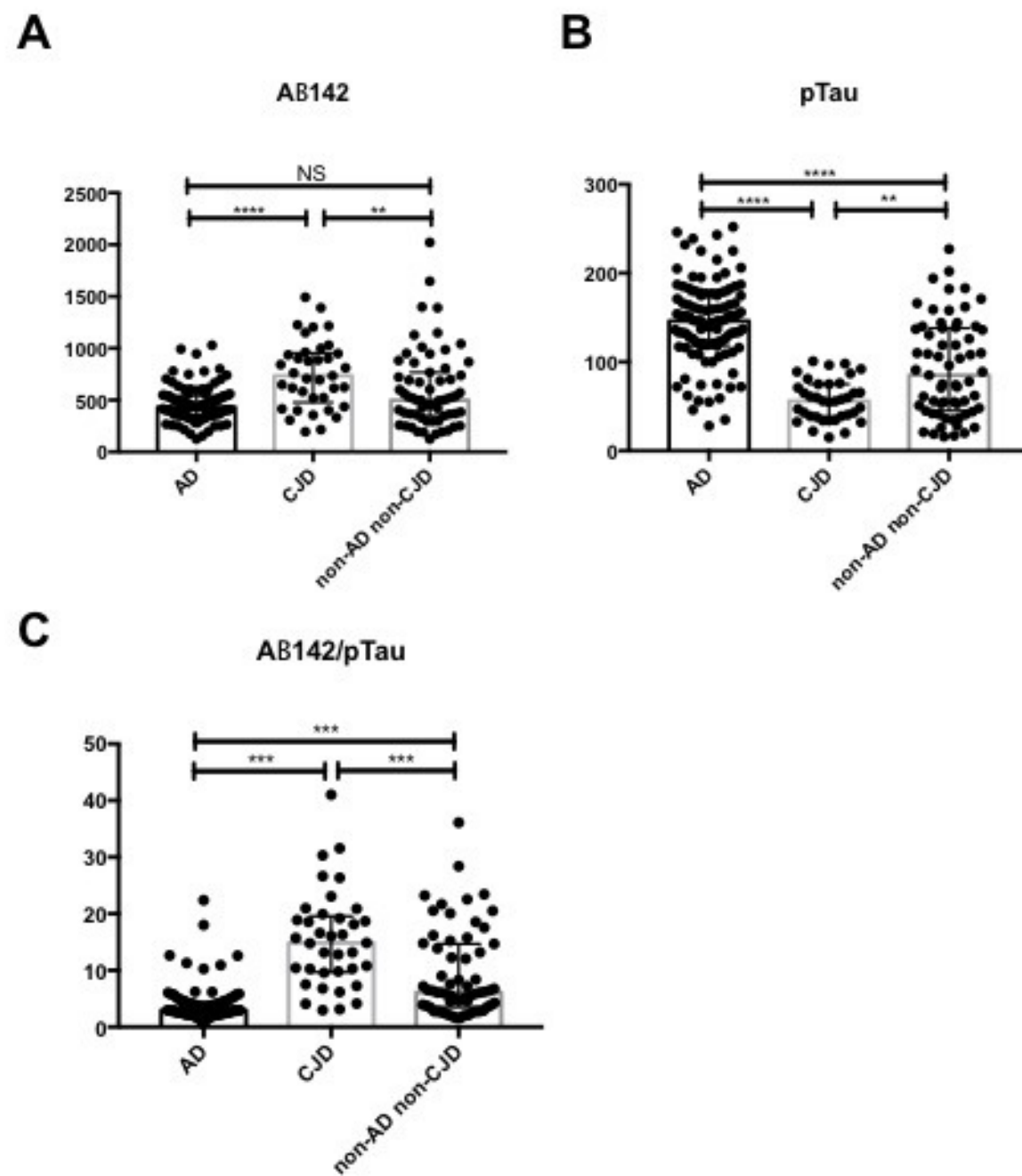


Figure 3

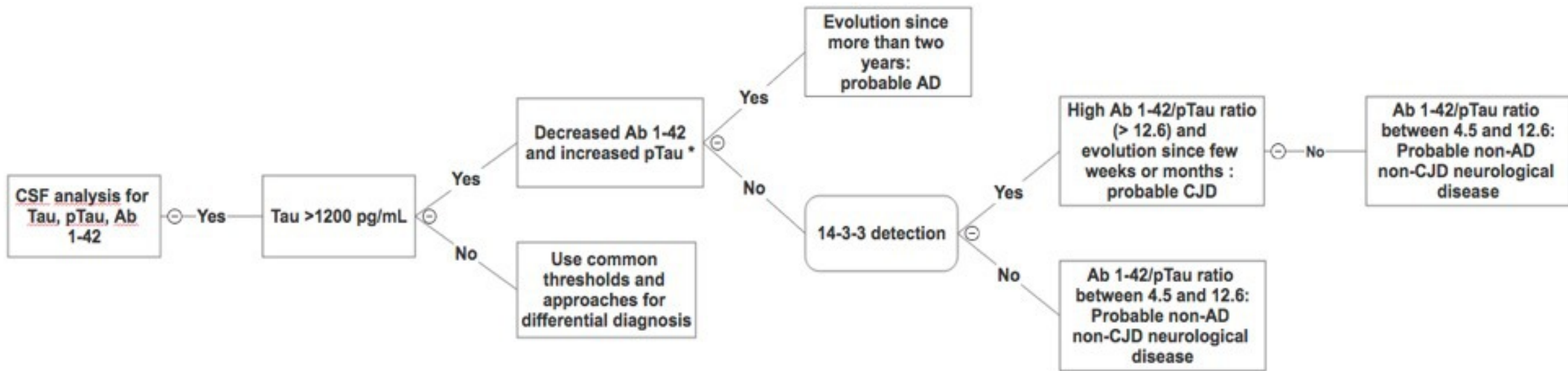


Figure 4