P-tau231 as a Diagnostic Biomarker for Alzheimer's Disease and Mild Cognitive Impairment: A Systematic Review and Meta-Analysis

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Abstract

Objective: Some previous studies have shown that cerebrospinal fluid (CSF) levels of p-tau231 were significantly higher in patients with Alzheimer's disease (AD) compared to that in patients with mild cognitive impairment (MCI) and normal control (NC), whereas some other studies did not. Due to contradictory results, we aimed to conduct a systematic review and meta-analysis study on previous investigations to examine the potential role of CSF p-tau231 as a biomarker of AD and MCI. **Method:** PubMed, Scopus, and Web of Science were searched in March 2021 for studies on the CSF level of p-tau231 in AD, MCI, and NC. The statistical analysis was performed via standardized mean difference (SMD) methodology with a 95% confidence interval. **Results:** A total of 10 studies including 1141 subjects were included. The present study showed that CSF level of p-tau231 was significantly higher in AD patients compared to that in MCI patients (SMD = 160.94 [11.11, 310.78], $P = 0.04$) and NC patients (SMD = 436.21 [164.88, 707.54], $P < 0.00$). Moreover, comparison of MCI and NC showed a significantly higher level of CSF p-tau231 in MCI compared to NC (SMD = 341.44 [59.73, 623.14], $P = 0.02$). **Conclusion:** P-tau231 showed to be a valuable biomarker of discrimination AD, MCI, and NC based on our findings. This meta-analysis showed that the CSF p-tau231 can reliably differentiate AD patients from MCI and NC patients. Furthermore, based on our findings the level of CSF p-tau231 was significantly higher in MCI compared to NC. Therefore, p-tau231 can be added to the list of potential biomarkers for the diagnosis of AD and MCI in further studies. However, further investigations are needed to confirm our findings.

Keywords: Alzheimer's disease, biomarker, mild cognitive impairment, p-tau231

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that begins with mild cognitive impairment.[1,2] AD is considered the most common cause of dementia in the elderly.[3] Epidemiological studies demonstrated that the number of individuals who lived with dementia was 43.8 million worldwide by 28.8 million disability-adjusted life-years (DALYs) attributed in 2016 which is going to increase.[4]

AD is characterized by the presence of hyperphosphorylated tau (p-tau) and amyloid beta (Aβ) plaques in the brain.[5] Tau, known as a microtubule-associated protein (MAP), is an essential part of a neuron's stability that helps to maintain the microtubules forming of the neural cytoskeleton.[6,7] In AD, abnormal hyperphosphorylated tau proteins self-aggregate in the neurons and form neurofibrillary tangles that impair axonal transport and lead to synaptic dysfunctions and neuronal death.[7] P-tau as a result of AD progression is released into CSF and blood which can be detected and used for monitoring disease progression and diagnosis.[8]

At the time of clinical diagnosis of AD, neural loss and neuropathological lesions occur earlier in the brain.[9] The critical issue is the early detection of pathological changes and rapid administration of neuroprotective drugs before AD becomes symptomatic.[9,10] Currently, the diagnosis of AD is mainly based on clinical guidelines and exclusion of other causes of dementia.[11] There are different neuropathological changes underlying AD which can be detected by imaging-based and molecular-fluid biomarkers in the cerebrospinal fluid (CSF) or blood.[12] Progress has been made in developing early biomarkers for AD. Recent investigations revealed that Aβ (1–42), total tau (t-tau), and p-tau (p-tau 181, p-tau 217, p-tau231) in CSF are useful biomarkers to distinguish early

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and developed AD from depression, age-associated memory impairment, and some secondary dementias.[13]

CSF p-tau is considered a good prognostic biomarker in AD which can predict progression from cognitively unimpaired to mild cognitive impairment (MCI), and AD dementia.[14–16] Suárez-Calvet *et al*.[8] measured three novel CSF p-tau isoforms including p-tau181, p-tau217, and p-tau231 and demonstrated that increasing these three biomarkers is significant in the preclinical stage of AD and can be utilized in differentiating Aβ positive from Aβ negative individuals. Barthélemy *et al*.[17] demonstrated that based on phosphorylation sites, the p-tau isoform could have different metabolisms. They showed that hyperphosphorylation on threonine 111, 205, S208, 217, and 231 of tau in CSF of the AD patients was increased. Furthermore, some previous studies showed that CSF levels of p-tau231 were significantly higher in patients with AD compared to normal control (NC).[18]

According to previous studies, the use of p-tau231 may contribute to the earlier and more accurate diagnosis of AD,[19,20] whereas some other studies did not find a significant difference in the level of CSF p-tau231 in AD and MCI patients.[13] Due to contradictory results, we aimed to conduct a systematic review and meta-analysis on previous investigations to examine the potential role of CSF p-tau231 as a biomarker of AD and MCI. In this study, we compared the CSF levels of p-tau231 in patients with NC, MCI, and AD.

Method and Materials

Search strategy and study selection

This systematic review and meta-analysis was performed by following the Preferred Reporting for Systematic Review and Meta-Analysis (PRISMA) consensus statement.[21] PubMed, Scopus, and Web of Science were searched for publications from 1990 to March 2021 using the following non-MeSH terms (p-tau231 or phosphorylated tau at threonine 231 or CSF p-tau231 and Alzheimer's disease or mild cognitive impairments).

Inclusion and exclusion criteria

We included original studies which reported the level of CSF p-tau231 in NC (healthy subjects), and AD or MCI patients. We excluded review and animal studies, case reports, case series, book chapters, editorials, letters, and non-English studies. Also, the eligible studies must define their diagnostic criteria for AD and MCI.

PICO in the present study was defined as follows. Problem or study population (P): Patients with AD or MCI; index test (I): CSF p-tau231; comparison (C): Healthy subjects; outcome (O): The desired outcome was examining the potential role of CSF p-tau231 as a biomarker for AD and MCI.

Study selection

The studies were selected in two steps. At the first, the title and abstracts were screened by two investigators (ER and SS) independently to ensure meeting eligibility criteria. In the next, the same reviewers screened the full text of the remaining articles for final selection. Any disagreement is resolved by a third investigator (FN) consultation at the end of each step.

Data extraction

The following data were manually extracted by two reviewers (ER and SS) using the prepared standard form: First author, year of publication, type of study, follow-up duration, p-tau231 assay method, sample size, age distribution, number of males, number of AD, MCI and NC subjects, the mean level of CSF p-tau231 and standard deviation (SD) in each group.

Quality assessment

To estimate the risk of bias among included studies, the quality assessment of diagnostic accuracy studies (QUADAS-2) criteria[22] was performed by the same reviewers (ER and SS).

Statistical analysis

Statistical analysis was performed via standardized mean difference (SMD) methodology for CSF p-tau231 level among groups (AD vs. MCI vs NC) with a 95% confidence interval on Stata 14.0 statistical software. First, we converted medians and interquartile range to mean and standard deviation based on the method proposed by Hozo *et al*.[23] Cochrane's Q test and I^2 were used for assessing heterogeneity. The I^2 value >75% and *P* value smaller than (<0.10) revealed high heterogeneity among studies. Due to moderate heterogeneity, we applied a random-effects model.

Results

Search results

A total of 150 results were retrieved from PubMed, Scopus, and Web of Science, and one study was added manually. After removing duplicates, 97 studies remained. Among qualified articles for the title and abstract review, 58 studies were excluded according to inclusion and exclusion criteria. The remaining articles were screened carefully via full-text assessments. Finally, a total of 10 studies including 1141 subjects were identified as eligible records for qualitative and quantitative synthesis [Figure 1].

Study characteristics

We included six cross-sectional[18–20,24–26] and four longitudinal studies[27–30] with a total of 1141 subjects (AD = 686, MCI = 260, NC = 195). The full details of included studies are listed in Table 1.

Risk of bias assessments

Visual inspection of the funnel plot revealed probable publication bias [Figure 2]. The results of the QUADAS-2 assessment showed that the risk of bias was high in two studies and unclear in one study [Table 2]. The visual inspection of the funnel plot in all analyses is represented in Figure 2.

CSF p-tau231 in AD vs NC

Nine studies were included in the meta-analysis regarding the comparison of CSF p-tau231 between AD and NC.

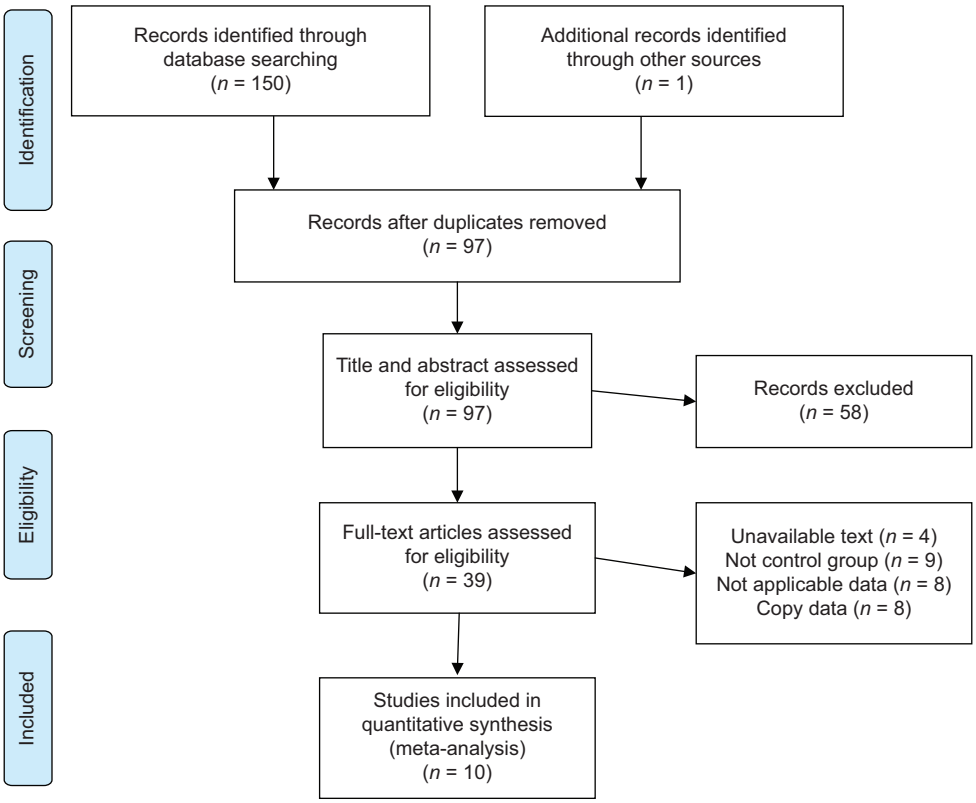

Figure 1: PRISMA diagram of the selection process. PRISMA: Preferred Reported Items for Systematic Reviews and Meta-Analyses

A total of 686 AD and 195 NC subjects were entered. The heterogeneity of the studies was high (Q = 703.18, $P < 0.00$, $I^2 = 98.86\%$) [Figure 3]. Forest plot revealed a significantly higher CSF p-tau231 level in AD patients compared to NC (SMD = 436.21 [164.88, 707.54], $P < 0.00$) [Figure 3].

CSF P-tau231 in AD vs MCI

For the meta-analysis of CSF p-tau231 between AD and MCI subjects, a total of five studies including 372 AD and 247 MCI subjects were entered. The heterogeneity was high (Q = 39.59, $P < 0.00$, $I^2 = 89.90\%$) [Figure 4]. The forest plot demonstrates a significantly higher level of CSF p-tau231 in AD subjects compared to MCI individuals (SMD = 160.94 [11.11, 310.78], $P = 0.04$) [Figure 4].

CSF P-tau231 in MCI vs NC

A total of six studies with 260 MCI and 132 NC individuals were entered for the comparison of CSF p-tau231 between MCI and NC subjects. The heterogeneity of studies was high (Q = 212.65, $P < 0.00$, $I^2 = 97.65\%$) [Figure 5]. The analysis showed that the CSF p-tau231 concentration in MCI patients was significantly higher compared to that in NC subjects (SMD = 341.44 [59.73, 623.14], $P = 0.02$) [Figure 5].

Discussion

In this study, we compared the CSF level of p-tau231 between subjects with AD, MCI, and NC to assess the possible role of p-tau231 in distinguishing AD and MCI from normal people. This meta-analysis gave evidence that CSF p-tau231 levels in AD patients were higher than in MCI patients and NC. Additionally, CSF p-tau231 levels were significantly higher in MCI patients compared to NC. Our results showed that p-tau231 may be a reliable biomarker for differential diagnosis of AD and MCI. As far as we know, this is the first meta-analysis study on the use of CSF p-tau231 for distinguishing MCI and AD.

Several isoforms of p-tau have been investigated in the CSF of AD patients. The most common form is p-tau181, which showed promising results in differentiating AD from MCI or NC.[8] Also, several other isoforms of p-tau such as p-tau217 and p-tau231 showed considerable results in distinguishing between AD and MCI patients.[16,19] A study by Spiegel *et al*.[31] reported better performance of p-tau231 than p-tau181 in the separation of AD from normal people. Additionally, the level of CSF p-tau231 was reported to be correlated with neocortical neurofibrillary pathology in post-mortem studies whereas there was no correlation for p-tau181.[32,33]

There is limited evidence regarding the use of CSF p-tau231 as a biomarker for AD while some previous investigations represented this biomarker as a good diagnostic tool. Suárez-Calvet *et al*.'s[8] study showed that CSF p-tau231 was elevated in the preclinical stages of AD (Aβ positive). Hampel *et al*.[34] demonstrated that p-tau231 gave better results compared to t-tau in the early detection of AD. The high level of CSF p-tau231 is the result of the specific involvement

Table 1: Characteristics of included studies

Author	Year of publication	Country	Study type	Follow-up duration	P-tau231 assay methods	Age (mean±SD)	Sample size (*n*)	Males (*n*)	AD subjects (*n*)	P-tau231 AD group	MCI subjects (*n*)	P-tau231 MCI]	Control subjects (*n*)	P-tau231 CN
Brys	2007	USA	Cross-sectional and longitudinal study	2 years	ELISA	70.7±8.8	86	41	22	51.53 pg/ml	43	20.4 pg/ml	21	14.6 pg/ml
Buerger	2004	Germany	Cross-sectional	–	ELISA	67.5±8.4	47	14	37	710 pg/ml	–	–	10	78 pg/ml
Buerger	2002	Germany	Cross-sectional	–	ELISA	67.9±8.2	162	78	55	613.6 pg/ml	77	501 pg/ml	30	92.9 pg/ml
Buerger	2005	Germany	Cross-sectional	–	ELISA	71.9±7.9	131	NR	71	714.75 pg/ml	31	547.75 pg/ml	29	141.25 pg/ml
Hampel	2001	Germany	Longitudinal	1.5 years	ELISA	65.8±9.1	29	9	17	496.2 pg/ml	–	–	12	312.2 pg/ml
Hampel	2004	Germany	Cross-sectional	–	ELISA	66.3±7.9	131	52	108	650.8 pg/ml	–	–	23	39.5 pg/ml
Kidmet-Piskac	2018	Croatia	Cross-sectional	–	ELISA	65±11.4	198	79	152	532 pg/ml	–	–	18	271 pg/ml
Leko	2016	Croatia	Longitudinal	2.6 years	ELISA	61±9	118	52	109	6955 pg/ml	43	3070 pg/ml	9	606 pg/ml
Leko	2020	Croatia	Cross-sectional	–	ELISA	66	179	83	115	3900 pg/ml	53	1800 pg/ml	11	1100 pg/ml
Leon	2004	USA	Longitudinal	4 years	ELISA	70.9±4.6	45	20	–	–	13	563.9 pg/ml	32	111.4 pg/ml

NR: Not reported, AD: Alzheimer's disease, MCI: Mild cognitive impairment

Figure 2: Funnel plots of CSF p-tau231 in AD compared to CN (a), AD compared to MCI (b), MCI compared to CN (c)

of the threonine 231 epitope in the pathology of AD and MCI. Furthermore, existing evidence demonstrated that CSF p-tau231 levels might predict the degree of neuronal damage and atrophy in AD patients.

Based on several previous studies, p-tau231 is a very specific marker for AD diagnosis.[19,28,35] However, another study demonstrated that CSF p-tau231 did not differentiate AD from vascular dementia (VaD) while there was another study showed the opposite result.[18] Consequently, there is limited data on the

Table 2: Risk of bias assessment among included studies

Study	Risk of Bias				Applicability Concerns		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
Brys *et al*., 2007	☺	☺	☺	☺	☺	☺	☺
Buerger *et al*., 2002	☺	?	☺	☺	☺	☺	☺
Buerger *et al*., 2004	☹	☺	☺	☺	☺	☺	☺
Buerger *et al*., 2005	☺	☺	☺	☺	☺	☺	☺
Hampel *et al*., 2001	☺	☺	☺	☺	☺	☺	☺
Hampel *et al*., 2004	☺	☺	☺	☺	☺	☺	☺
Kidmet-Piskac *et al*., 2018	☺	☺	☺	☺	☺	☺	☺
Leko *et al*., 2016	☺	☺	☺	☺	☺	☺	☺
Leko *et al*., 2020	☺	☺	☺	☺	☺	☺	☺
Leon *et al*., 2004	☺	☺	☹	☺	☺	☺	☹

? Unclear Risk ☹ High Risk ☺ Low Risk

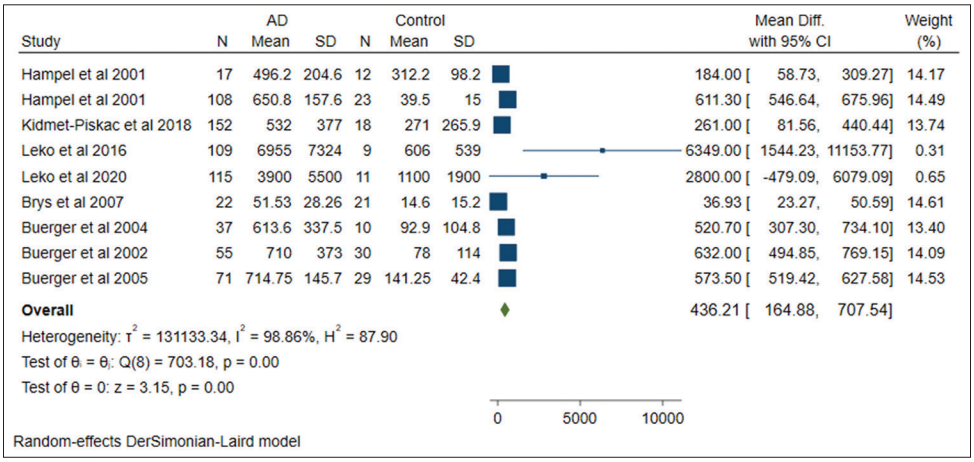

Figure 3: Forest plot of CSF p-tau231 levels in AD compared to CN

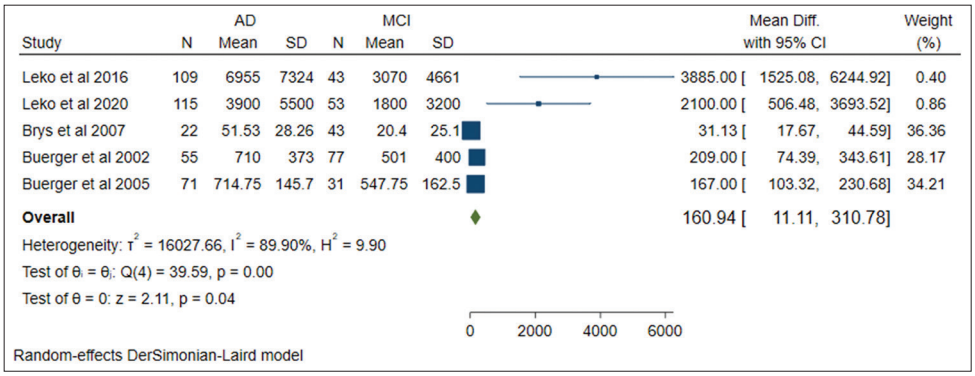

Figure 4: Forest plot of CSF p-tau231 levels in AD compared to MCI

use of CSF p-tau231 to differentiate AD dementia from non-AD dementia which should be considered in further studies.

We found that CSF p-tau231 levels were higher in AD patients compared to NC. However, our results had high heterogeneity and our sample size was small. Therefore, these findings should be interpreted carefully. Additionally, AD patients are more likely to be older than MCI and NC individuals which might affect the results, and further study by controlling the effect of normal aging should confirm our findings.

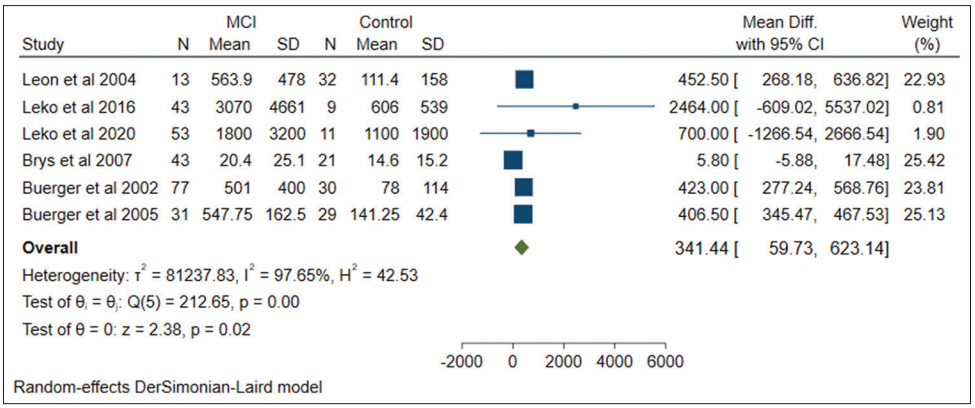

Figure 5: Forest plot of CSF p-tau231 levels in MCI compared to CN

Hampel *et al*.[36] also suggested that p-tau231 could be utilized as a biomarker to monitor AD progression and showed good discriminating power in comparing AD to frontotemporal dementia. The concentration of CSF p-tau231 alone also showed a good correlation with disease progression in patients with AD.[19,20,35] In this meta-analysis, we showed that CSF p-tau231 levels were significantly higher in MCI patients compared to NC subjects. Similarly, Buerger *et al*. also showed that p-tau231 levels were higher in MCI patients and were also negatively associated with their Mini-Mental State Examination (MMSE) score, which indicated that p-tau231 may be a good biomarker for screening cognitive decline.[35]

Currently, clinical and experimental findings support that the core AD biomarkers including CSF Aβ, T-tau, and p-tau can reflect AD's key pathophysiological elements and provide diagnostically relevant information in the early stages of AD.[37] However, due to heterogeneity in the pathology of AD, there is a need for expansion of CSF and other types of biomarkers.

Limitations

Our study had limitations such as the laboratory variability (ELISA kits) among studies in CSF p-tau231 measurement that increased the heterogeneity and limited us to defining cutoff values. Also, there was a limited number of studies with a small sample size that evaluated CSF p-tau231 in AD and MCI. Another limitation was the similarity in the authors of the included studies. However, we included studies with different participants based on reference hospitals. Furthermore, there was variability in genetic background, change of diagnostic criteria for the MCI and AD over time, disease status, and presence of other comorbidities in participants. Another limitation that should be mentioned is the heterogeneity in MCI subjects while our entered studies mostly included all MCI subjects. MCI individuals without underlying AD pathology (non-amnestic MCI) would not have high CSF AD biomarkers, and thus, this biomarker may not be helpful in differentiating non-amnestic MCI from CN and AD dementia cohort.

Conclusion

P-tau231 was observed to be a valuable biomarker for discrimination of AD, MCI, and NC based on our findings. This meta-analysis showed that the CSF p-tau231 can reliably differentiate AD patients from MCI and NC. Furthermore, based on our findings the CSF p-tau231 can differentiate MCI from NC. Our findings showed a reliable result for p-tau231 as a biomarker for AD and MCI, and we believe that it can be added to the list of potential biomarkers for the diagnosis of AD and MCI in further studies. However, further longitudinal investigations that include CSF p-tau231 and other accepted biomarkers are needed to confirm our findings in comparing the discriminating power of these biomarkers at the early stages of AD.

Data availability statement

The data used in this manuscript is openly available.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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