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REVIEW ARTICLE

Blood phosphorylated Tau181 reliably differentiates amyloid-positive from amyloid-negative subjects in the Alzheimer's disease continuum: A systematic review and meta-analysis

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Abstract

INTRODUCTION: Blood-based biomarkers seem promising for the diagnosis of Alzheimer's disease (AD).

METHODS: We performed a systematic review and meta-analysis on the potential of blood phosphorylated Tau181 (p-tau181) to differentiate amyloid-positive (A+) and amyloid-negative (A−) subjects. Two meta-analyses were conducted, showing the mean p-tau values in blood and cerebrospinal fluid (CSF) in the A+ and A− group, and the second comparing the mean p-tau concentrations in blood and CSF among A+ versus A- participants, by laboratory assessment method.

RESULTS: Eighteen studies (2764 A+ and 5646 A− subjects) were included. The single-group meta-analysis showed mean higher blood p-tau181 values in the A+ than in the A− group. In the head-to-head meta-analysis, blood p-tau reliably differentiated A+ patients from A− participants.

DISCUSSION: Regardless of the laboratory technique, blood p-tau181 reliably differentiates A+ and A− subjects. Therefore, it might have important applications for early diagnosis and inclusion in clinical trials for AD patients.

KEYWORDS
Alzheimer's disease (AD), biomarkers, blood, CSF, meta-analysis, phosphorylated Tau181 (p-tau181)

Highlights

- The role of blood-based biomarkers in discriminating AD patients is still uncertain.
- Blood p-tau181 distinguishes among amyloid-positive and amyloid-negative subjects.
- Blood p-tau181 might allow early diagnosis and inclusion in clinical trials.

Annibale Antonioni, Emanuela Maria Raho, Maria Elena Flacco, and Francesco Di Lorenzo, contributed equally to this work.

1 | INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative dementia worldwide and affected patients are expected to increase exponentially in the next decades.[1] Considering its burden, current efforts are focused on identifying biomarkers that enable a prompt detection.[2] Indeed, early diagnosis is crucial for correcting modifiable risk factors to slow disease progression, as well as for identifying patients eligible for new disease-modifying drugs.[3] Traditionally, the search for AD neuropathological hallmarks, that is, aggregated amyloid-β42 (Aβ42) and phosphorylated Tau protein (p-tau), is performed on cerebrospinal fluid (CSF) and/or by means of nuclear medicine techniques (e.g., positron emission tomography, PET).[4] However, although CSF and PET have proven to be crucial in the diagnostic work-up of AD patients, they are invasive, expansive, seldom available in primary care settings, and require time and expertise.[4] Therefore, in recent years, a great deal of research has been conducted to assess the diagnostic potential of blood-based biomarkers, which are much easier to obtain, less invasive, and they seem very promising in distinguishing, for example, between healthy controls, mild cognitive impairment (MCI), and AD patients.[5,6] Indeed, pathological biomarkers in the CSF can cross the blood-brain barrier (BBB) and are therefore detectable also on blood.[7] Importantly, CSF p-tau is a highly specific AD biomarker and recent studies suggest a potential diagnostic role also for blood p-tau.[8] Based on the protein's phosphorylation site, several isoforms of p-tau can be identified and, at present, the best characterized is p-tau181, although other more recent isoforms, such as p-tau217 and p-tau231, seem promising.[2,9] However, there is often an inconsistency between studies about the ability of blood biomarkers to predict CSF status.[10–12] This disagreement stems from many factors, including the specific p-tau isoform, the laboratory method employed, and the disease's stage. Thus, currently, there is still no consensus about the role of blood biomarkers in AD patients.[13–15] Notably, a recent narrative review showed that it is often possible to highlight a correlation between CSF and blood p-tau measures and higher blood p-tau values in AD than in other neurodegenerative diseases.[12] However, to the best of our knowledge, there are no studies that quantitatively assessed whether blood and CSF p-tau181 reliably differentiate between amyloid-positive and -negative subjects, and to establish whether the former is a reliable measure of the latter in the AD continuum. Additionally, the available evidence is highly fragmented and methodologically heterogeneous, with studies adopting different designs, various comparators, and diverse analytical approaches. As such, the results are complex to interpret by examining single studies. We, thus, performed a single-group meta-analysis, computing the pooled p-tau values in blood and in CSF, separately in the amyloid-positive (A+) and amyloid-negative (A−) group, and a direct-comparison meta-analysis, comparing the mean p-tau concentration in the A+ versus A− subjects, both in blood and in CSF, stratifying by assessment method. We aimed to provide evidence to clinicians and researchers on the actual possibility of using blood p-tau to reliably assess the CSF status. Although recent findings suggest that some p-tau isoforms, particularly p-tau217, show great promise for identifying individuals within the AD spectrum, we focused on p-tau181 due to its well-established diagnostic role, confirmed by the revised criteria for AD diagnosis, and the extensive evidence available in the literature.[16] In contrast, the more recently introduced isoforms are currently limited to research settings.[12] Therefore, providing quantitative data that blood p-tau181, similarly to its CSF counterpart, can reliably categorize A+ and A− individuals could have significant implications for improving AD diagnosis in current clinical practice, especially in settings with limited healthcare and economic resources.

2 | MATERIALS AND METHODS

2.1 | Bibliographic search, data extraction, and quality assessment

This systematic review and meta-analysis was conducted according to the updated version of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Supplementary Material).[17] The study protocol was registered in the "International Prospective Register of Systematic Reviews" (PROSPERO code CRD42023465303). Specifically, the MEDLINE (via PubMed), Scopus (via EBSCO), and Web of Science databases were searched up to August 1, 2023, for studies reporting measurements of p-tau on both blood and CSF in patients in the AD continuum, that is, MCI and AD dementia participants, categorized according to clinical and/or neuropathological criteria. The following search strategy was employed, without language restrictions: ((((p-tau[Title/Abstract]) OR (p-Tau[Title/Abstract])) AND ((cerebrospinal fluid[Title/Abstract]) OR (CSF[Title/Abstract])) AND (blood[Title/Abstract])) AND (Alzheimer[Title/Abstract])). The reference lists of retrieved paper and reviews was also screened for additional suitable papers. Inclusion criteria were: (A) cross-sectional or case series design, either published as primary analyses or as sub-analyses of larger population-based cohort studies; (B) original quantitative data about p-tau values on blood and CSF in patients on the AD continuum. When available, the same measurements were also obtained on comparators, for example, (1) subsets of subjects with different degrees of disease severity (e.g., MCI vs. AD); (2) clinically healthy controls; (3) biomarker-negative subjects; (4) other types of dementia (e.g., frontotemporal dementia, Parkinson's disease, multiple system atrophy, progressive supranuclear palsy/cortico-basal syndrome). Preclinical studies (e.g., on cellular and animal models) and reviews were excluded. Each included article was independently evaluated by two reviewers (A.A. and E.M.R.) who extracted the study characteristics (first author, journal, and year of publication, number of subjects in the A+ and A− groups, diagnostic category(ies)—where possible, differentiating between clinical and/or neuropathological criteria, laboratory technique(s) used), and measures of interest (mean blood and CSF p-tau concentration in the A+ and A− groups, and relative standard deviations). In case of discrepancies in data extraction, a third author was contacted (F.D.L.), and consensus was achieved through discussion. In the first phase of articles selection, less

stringent criteria were adopted by both reviewers, in order to collect the potentially pertinent papers. In the second phase, the pre-defined inclusion criteria were applied to the retrieved articles, which were screened at the full-text level for data extraction. Individual study quality was assessed independently by two authors (A.A. and E.M.R.), using an adapted version of the Newcastle Ottawa Quality Assessment Scale, which evaluates the comparability across groups for confounding factors, the appropriateness of outcome assessment, length of follow-up, and missing data handling and reporting.[18,19] Discrepancies in the quality assessment were solved by a senior author (F.D.L.).

2.2 | Data analysis

First, data from individual studies were combined to estimate the weighted mean concentration (plus 95% CIs) of p-tau181 in the CSF and blood of A+ and A− subjects, using the *metan* package in Stata. In case multiple values of p-tau181 were available for the same study (e.g., assessed by age-class or by AD subgroups), a summary weighted mean was re-computed within the same population. Each analysis was performed separately by assessment method. Second, we performed traditional head-to-head meta-analyses, combining individual study p-tau181 levels of A+ and A− subjects, and comparing (1) CSF and (2) plasma levels of p-tau181 among A+ versus A− participants, stratifying the analyses by laboratory assessment method. We used the random-effect model and computed a summary mean difference, its 95% confidence interval (CI) and the relative intra-study heterogeneity (which was quantified using the I^2 metric). RevMan 5.3 (The Cochrane Collaboration, 2014) and Stata, version 13.1 (Stata Corp. College Station, TX: 2013) were used to analyze the data.

3 | RESULTS

3.1 | Study selection and characteristics

Of the 174 records initially identified through database search, 36 were immediately excluded due to wrong population (preclinical/animal model studies, $n = 11$) or publication type (review articles, $n = 25$). A total of 138 papers were then screened and, after the exclusion of 120 of them not providing extractable data on the concentration of p-tau, 18 studies were included in the systematic review and meta-analysis.[1,2,4,13,15,20–32] Among these, two studies[13,22] were excluded with reason from the head-to-head meta-analyses. Thus, 16 studies were employed for the second part of the analysis. Figure 1 shows a summary of the research process, while Table 1 summarizes the main data extracted from each study (see Figure 1 and Table 1).

Overall, we included 2764 participants categorized as A+ and 5646 subjects classified as A− according to clinical[1,20,21,23,25,26,28,30,32], biomarker[2,15,24,27,29] or both criteria.[4,13,22,31] Regarding the laboratory methods used to assess p-tau levels, the Single Molecule Array for Protein Detection (Simoa) was the most widely used[1,2,4,13,15,21,23–32], while a smaller number of studies used the Meso Scale Discovery platform (MSD)[2,4,21,22] or other immunoassays-based technologies.[22] Only two studies employed an original technique, characterized by an immunoprecipitation technique alone[20] or in combination with mass spectrometry (IP-MS)[13], respectively. The methodological characteristics of the included cohort and cross-sectional studies are summarized in the Supplementary Material. Among the cohort studies, the patients' selection, the outcome(s) ascertainment, and the evaluation of the comparability of subjects were optimal/adequate in almost all studies. Of the seven cross-sectional studies included, the items pertaining to outcome assessment and patients' selection were adequately addressed in six and five studies, respectively, while the ascertainment of the outcome was optimal or adequate in all studies.

3.2 | Single group meta-analysis

Table 2 summarizes the results of the 18 studies included in the single-group meta-analysis (see Table 2).

In reference to Simoa, that is, the most widely employed assessment method (19 studies on CSF and 18 on blood, respectively), p-tau values were highest in CSF (weighted mean [WM], 177.2; 95% CI, 133.9 to 220.4) and, to a lesser extent, in blood (WM, 13.5; 95% CI, 16.8 to 19.2) in the A+ group, while the A− group showed markedly lower values in both CSF (WM, 60.8; 95% CI, 59.5 to 69.1) and, especially, blood (WM, 8.89; 95% CI, 6.82 to 10.9). Regarding MSD, that is, the second most used technique (five studies), the analysis showed results with a distribution overlapping with that of the evaluations with Simoa, that is, higher in the A+ group in CSF (WM, 103.5; 95% CI, 74.5 to 132.5) and, to a lesser extent, on blood (WM, 2.00; 95% CI, 1.56 to 2.44), and lower in the A− group with, however, higher values on CSF (WM, 47.2; 95% CI, 30.3 to 64.1) than on blood (WM, 1.03; 95% CI, 0.77 to 1.29). One study[20] evaluated in two separate cohorts an original immunoprecipitation method that documented similar trends, that is, the A+ showed higher values in CSF (WM, 567.4; 95% CI, 407.7 to 727.1) than in blood (WM, 3.26; 95% CI, 2.53 to 3.98), while the A− subjects documented lower values overall than the previous group and, however, higher values in CSF (WM, 307.5; 95% CI, 268.6 to 346.4) than in blood (WM, 1.89; 95% CI, 1.43 to 2.36). Finally, three studies, which employed an immunoassay[22], an IP-MS,[13] and a specific enzymatic method[13], respectively, documented trends overlapping with the previous ones. In particular, CSF (first study: WM, 39.1; 95% CI, -291.8 to 370.1; second study: WM, 31.4; 95% CI, 30.5 to 32.4; third study: WM, 64.1; 95% CI, 58.5 to 69.7) and blood (first study: WM, 0.540; 95% CI, 0.501 to 0.579; second study: WM, 27.4; 95% CI, 26.6 to 28.2; third study: WM, 5.38; 95% CI, 5.19 to 5.57) from group A+ showed higher values than their counterparts from group A−, which were characterized, once again, by higher values in CSF (first study: WM, 15.9; 95% CI, 14.8 to 16.9; second study: WM, 21.5; 95% CI, 21.1 to 21.9; third study: WM, 36.0; 95% CI, 33.4 to 38.6) than in blood (first study: WM, 0.346; 95% CI, 0.315 to 0.377; second study: WM, 20.3; 95% CI, 19.7 to 20.9; third study: WM, 3.83; 95% CI, 3.56 to 4.10). Thus, overall, all methods document higher mean values in the CSF (i.e., in the central nervous system, CNS, compartment) and, importantly, the values in the

TABLE 1 Selected characteristics of the included studies.

First author (Ref)	Year	Assessment method	*No.* A+ pts	Mean CSF A+	SD CSF A+	Mean plasma A+	SD plasma A+	*No.* A− pts	Mean CSF A−	SD CSF A−	Mean plasma A−	SD plasma A−
Barthélemy (a)[20]	2020	Immunoprec. (original method)	5 (preclinical AD); 8 (AD-MCI); 2 (AD moderate)	438; 501; 681	119; 93; 263	3.4; 4.3; 5.5	0.9; 2.3; 2	9 (young CN); 8 (aged CN); 2 (non AD-MCI)	286; 295; 281	33; 30; 44	1.6; 2; 1.6	0.6; 0.4; 0.1
Barthélemy (b)[20]	2020	Immunoprec. (original method)	20 (preclinical AD); 24 (AD-MCI); 6 (AD moderate)	574; 688; 777	282; 241; 420	2.7; 2.9; 3.4	1.2; 1; 1.5	31 (aged CN); 11 (non AD-MCI)	339; 310	105; 96	2.1; 2.3	0.7; 0.9
Janelidze (a)[4]	2020	ThermoFisher (Simoa) +Lilly (MSD)	38 (A+ AD); 38 (CN A+); 28 (MCI A+)	390; 170; 339	166; 94.1; 201	4.4; 1.9; 3.8	2.29; 1.04; 2.37	26 (CN A−)	93	43.7	1.3	1.1
Janelidze (b)[4]	2020	ThermoFisher (Simoa) +Lilly (MSD)	81 (A+ MCI); 93 (CN A+)	266; 122	173; 124	2.8; 2.2	2.07; 1.26	126 (CN A−); 44 (MCI A−)	94; 86	25.9; 15.6	1.2; 1.3	0.59; 0.59
Karikari (a)[23]	2020	Quanterix (Simoa)	33 (AD)	96.6	51.4	24.9	7.8	27 (young CN); 113 (old CN); 45 MCI; 8 FTD	20.8; 40.5; 71.4; 25.8	7.5; 19.3; 57; 9.4	7.9; 10; 14.8; 6.9	2.6; 3.3; 6.7; 2.1
Karikari (b)[23]	2020	Simoa	126 (AD)	86.9	35.7	19.2	9.4	337 (old CN); 191 (MCI); 18 bvFTD/PPA; 36 PD/MSA; 12 vascular dementia; 21 PSP/CBS	45; 55.5; 38.9; 40.1; 36.7; 31.0	18.2; 25.8; 12.8; 17.1; 13.4; 13.0	9.4; 12.5; 11.2; 11.9; 9.9; 9.9	6; 8.6; 7.4; 9.3; 6.0; 3.8
Suárez-Calvet[24]	2020	Quanterix (Simoa)	131 (A+)	477	304	10.9	4.57	250 (A−)	278	97.7	8.83	3.21
Chen[25]	2021	Quanterix (Simoa)	221 (AD)	36.8	16.1	23.7	8.85	403 (CN); 560 (MCI)	22.3; 26.2	9.42; 14.2	15.4; 18.4	10.5; 11.1
Moscoso[26]	2021	Quanterix (Simoa)	186 (AD)	33.4	58.7	23.2	42.2	359 (CN); 518 (MCI)	19.6; 22.8	38.5; 61.6	13.6; 15.8	52.9; 50.4
Rauchmann[27]	2021	Quanterix (Simoa)	300 (A+TN+); 173 (A+TN-)	39; 17	13; 5	24; 17	11; 10	260 (A−TN-); 132 (A−TN+)	17; 32	4; 11	13; 20	9; 39

(Continues)

TABLE 1 (Continued)

First author (Ref)	Year	Assessment method	*No.* A+ pts	Mean CSF A+	SD CSF A+	Mean plasma A+	SD plasma A+	*No.* A− pts	Mean CSF A−	SD CSF A−	Mean plasma A−	SD plasma A−
Thijssen[21]	2021	Thermo Scientific (Simoa) or Lilly (MSD)	58 (AD)	39.9	18	2.3	1	CN (118); MCI (99)	20.9; 28.7	8; 12	0.9; 1.2	1; 1
Gao[1]	2022	Quanterix (Simoa)	107 EOAD; 66 LOAD	141 (E); 123 (L)	100 (E); 61 (L)	6.2 (E); 5.8 (L)	3.0 (E); 2.7 (L)	96 CN; 94 MCI	38; 75	15; 60	2,7; 3.6	1,9; 2.4
Janelidze (a)[13]	2023	ADx (Simoa)	45 (MCI-ADD); 26 MCI non progr. A+	2.11; 1.1	0.71; 0.53	46.3; 30.0	18.4; 16.4	64 MCI non progr. A−	0.659	0.23	19.5	12.5
Janelidze (b)[13]	2023	WashU (%) (IP-MS)	45 (MCI-ADD); 26 MCI non progr. A+	32.6; 28.9	3.85; 4.30	28.4; 24.5	4.74; 6.15	64 MCI non progr. A−	21.5	2.22	20.3	3.33
Janelidze (c)[13]	2023	UgoT (Simoa)	45 (MCI-ADD); 26 MCI non progr. A+	93.2; 81.4	97.18; 58.15	3.38; 2.43	1.1; 1.16	64 MCI non progr. A−	25.6	18.4	1.88	0.81
Janelidze (d)[13]	2023	Fuji	45 (MCI-ADD); 26 MCI non progr. A+	83.5; 48.8	35.11; 22.9	5.61; 4.73	1.09; 1.36	64 MCI non progr. A−	36	15.3	3.83	1.58
Kac (c)[15]	2022	ThermoFisher (Simoa)	19 (biomarker+)	88.9 (Innotest); 895.8 (Simoa)	23.7 (Innotest); 350.9 (Simoa)	10.1 (s)	10.4 (s)	16 (biomarker-)	28.4 (Innotest); 176.4 (Simoa)	8.0 (Innotest); 57.3 (Simoa)	2.5 (s)	2.2 (s)
Kac (a)[15]	2022	ThermoFisher (Simoa)	18 (biomarker+)	91.2	26.2	10.4 (p); 9.3 (s)	4.1 (p); 3.4 (s)	15 (biomarker-)	42.5	12.7	5.8 (p); 4.5 (s)	2.8 (p); 2 (s)
Moscoso (a)[28]	2022	Quanterix (Simoa)	70 (MCI+AD)	25.8	65.9	NA	NA	93 (CN)	22.5	46.1	NA	NA
Palmqvist (a)[22]	2022	Roche immunoassay	68 (AD A+); 4 (CN A+); 38 (MCI A+)	38.4; 46.7; 38.6	16.5; 11.9; 22.8	0.571; 0.48; 0.485	0.211; 0.158; 0.225	28 (CN A−); 68 (MCI A−)	15.7; 16	5.28; 4.66	0.327; 0.357	0.145; 0.167
Palmqvist (b)[22]	2022	Lilly (MSD)	144 (MCI A+); 146 (CN A+)	30.9; 28.2	14.6; 12.4	1.47; 1.26	0.711; 0.605	315 (CN A−); 88 (MCI A−)	17.5; 16.7	5.16; 6	0.895; 0.936	0.489; 0.393

(Continues)

TABLE 1 (Continued)

First author (Ref)	Year	Assessment method	*No.* A+ pts	Mean CSF A+	SD CSF A+	Mean plasma A+	SD plasma A+	*No.* A− pts	Mean CSF A−	SD CSF A−	Mean plasma A−	SD plasma A−
Thanapornsangsuth[29]	2022	Quanterix (Simoa)	4 (AD A+T+); 10 (AD continuum A+)	95.25; 47.65	15.13; 45.96	13.18; 5.11	12.9; 7.41	59 (Non AD A−T-)	21	9.89	2.25	50.8
Tissot[30]	2022	Quanterix (Simoa)	32 (AD)	110.6	63.4	26.8	12.9	30 young CN, 162 CN, 60 MCI	22.4; 43.2; 76.8	7.5; 25.2; 50.2	8; 11.3; 16.1	3.6; 6.9; 8.6
Ashton (a)[2]	2023	Quanterix (Simoa)	127	168.8	2.67	4.4	1.62	70	33.6	22.07	2.65	1.35
Ashton (b)[2]	2023	ADx (Simoa)	127	1095	508	27.1	12.7	70	229.5	146.1	7.78	5.81
Ashton (c)[2]	2023	Lilly (MSD)	127	66.8	31.5	1.58	0.726	70	20.3	12.7	0.61	0.25
Ashton (d)[2]	2023	UGoT (Simoa)	127	801.3	227.9	15.5	3.48	70	381.4	96.5	11.2	3.04
Cai[31]	2023	Quanterix (Simoa)	85 (MCI converted to AD A+); 11 (CN converted to MCI A+)	63.9; 45.3	25.7; 18.4	26.4; 18.8	15.2; 9.1	215 (CN A−/not converted to MCI); 278 (MCI A−/not converted to AD)	34.2; 35.5	17.8; 20.7	14.8; 16	10.3; 8.8
Shang[32]	2023	Quanterix (Simoa)	169 (AD continuum, including 120 AD)	84.7; 70.6	31; 34.5	4.9; 4.8	1.9; 2.2	128 (non AD)	40.2	18.8	2.7	1.4

Abbreviations: AD, Alzheimer disease; ADD, Alzheimer's disease dementia; A+, amyloid-positive; A−, amyloid-negative; bvFTD/PPA, behavioural variant FTD/primary progressive aphasias; CN, cognitively normal; CSF, cerebrospinal fluid; E, Early; EOAD, early-onset AD; FTD, fronto-temporal dementia; L, late; LOAD, late-onset AD; MCI, mild cognitive impairment; N, number of subjects; NA, not available; p, plasma; PD/MSA: Parkinson's disease/multiple system atrophy; progr, progressors; PSP/CBS: progressive supranueclar palsy/cortico-basal syndrome; pts, patients; s, serum; T, Tau pattern.

TABLE 2 Cerebrospinal fluid (CSF) and plasma p-tau181 levels in amyloid-positive (A+) and amyloid-negative (A−) patients, stratified by assessment method

	A+ patients				A− patients			
	Cerebrospinal fluid[a]		Plasma[a]		Cerebrospinal fluid[a]		Plasma[a]	
Assessment method:	*N* studies (sample)	Weighted mean (95% CI)	*N* studies (sample)	Weighted mean (95% CI)	*N* studies (sample)	Weighted mean (95% CI)	*N* studies (sample)	Weighted mean (95% CI)
– Simoa[b]	19 (2427)	177.2 (133.9–220.4)	18 (2336)	13.5 (16.8–19.2)	19 (4879)	60.8 (59.5–69.1)	18 (4783)	8.89 (6.82–10.9)
– Lilly (MSD)	5 (753)	103.5 (74.5–132.5)	5 (753)	2.00 (1.56–2.44)	5 (886)	47.2 (30.3–64.1)	5 (886)	1.03 (0.77–1.29)
– Immunoprecipitation	2 (65)	567.4 (407.7–727.1)	2 (65)	3.26 (2.53–3.98)	2 (61)	307.5 (268.6–346.4)	2 (61)	1.89 (1.43–2.36)
– Immunoassay	1 (104)	39.1 (-291.8; 370.1)	1 (110)	0.540 (0.501–0.579)	1 (96)	15.9 (14.8–16.9)	1 (96)	0.346 (0.315–0.377)
– WashU (IP-MS)	1 (71)	31.4 (30.5–32.4)	1 (71)	27.4 (26.6–28.2)	1 (64)	21.5 (21.1–21.9)	1 (64)	20.3 (19.7–20.9)
– Fuji	1 (71)	64.1 (58.5–69.7)	1 (71)	5.38 (5.19–5.57)	1 (64)	36.0 (33.4–38.6)	1 (64)	3.83 (3.56–4.10)

Note:Weighted means were obtained combining data from individual studies to perform meta-analyses of single-group continuous data.

Abbreviation:CI, confidence interval; IP-MS, immunoprecipitation technique in combination with mass spectrometry; MSD, Meso Scale Discovery platform.

[a] Plasma levels expressed as pg/mL; CSF levels expressed as ng/mL.

[b] Including the following: Quanterix, ADx, Ugot, Janssen, Thermo Fisher/Thermo Scientific.

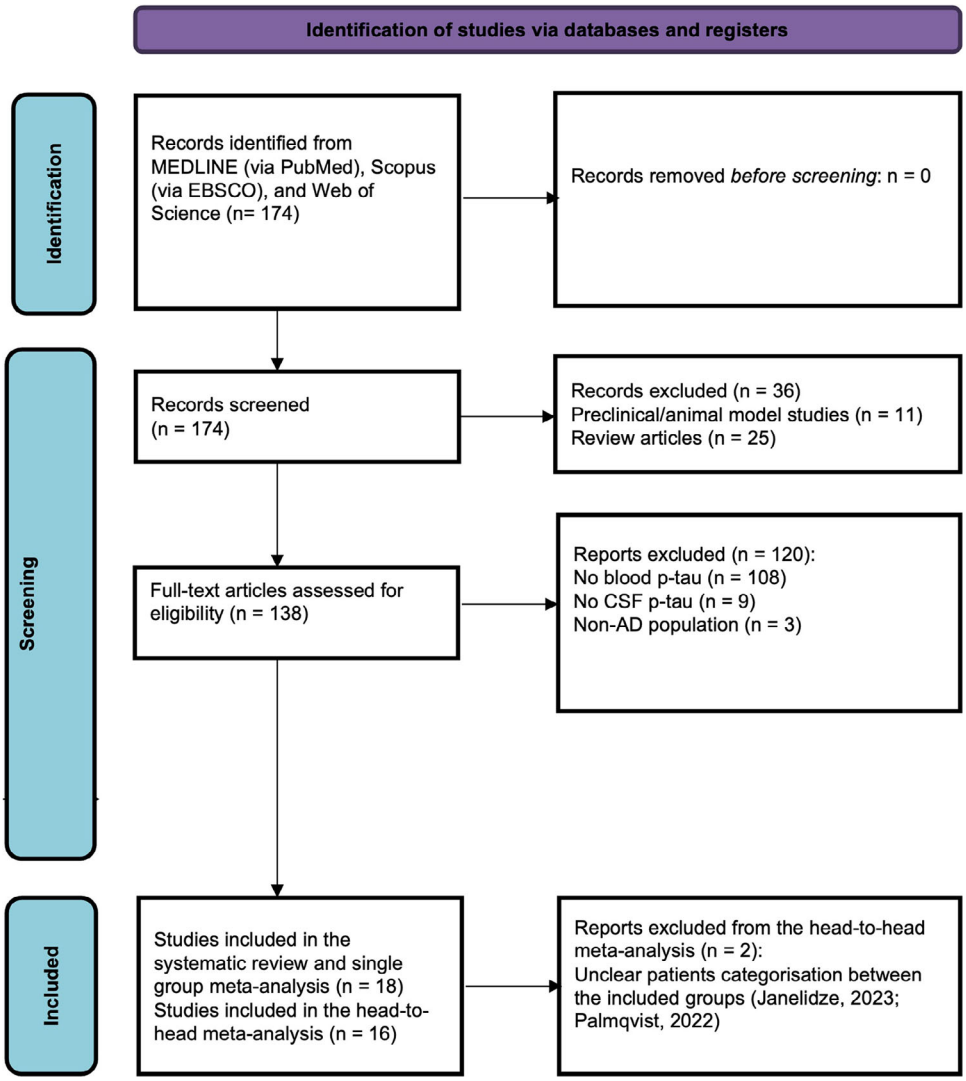

FIGURE 1 PRISMA flow diagram outlining literature review and study selection. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

A+ group are always higher than those in the A− group, irrespective of the biological substrate (i.e., CSF or blood) investigated. Specifically, the highest p-tau values on CSF were documented by the immunoprecipitation method in both the A+ and A− groups[20], while IP-MS showed the highest plasma values in both groups.[13]

3.3 | Head-to-head meta-analysis

Whereas the previous meta-analysis only described the mean p-tau181 values on CSF and blood, stratified by assessment method, in the A+ and A− groups, in this section a direct comparison of the measurements in these groups will be presented in order to assess whether the p-tau181 values reliably differentiate the subjects in the two groups. Since two studies were excluded[13,22], 16 studies have been used for the head-to-head meta-analysis. Table 3 and Figures 2 and 3 summaries the results of the studies included in the head-to-head meta-analysis, stratified by assessment method (see Table 3 and Figure 2 and 3).

3.3.1 | CSF levels of p-tau181 among A+ versus A− participants

Table 3 (first section) summarizes the results about CSF p-tau181 values in A+ and A− groups (see Table 3). Direct comparison of p-tau181 values on CSF shows that participants in the A+ group are characterized, in a statistically significant way, by higher values than those in the A− group, irrespective of the method used. Specifically, using the Simoa technique, mean p-tau181 values were significantly higher in the A+ group than in the A− group (mean difference [MD], 63.76; 95% CI, 54.93 to 72.59; $p < 0.001$) (see Figure 2, section C). Similarly, MSD also showed higher p-tau levels in A+ subjects than in A− ones (MD, 52.03; 95% CI, 32.70 to 71.36, $p < 0.001$) (see Figure 2, section B). Finally,

TABLE 3 Results of the meta-analyses comparing the cerebrospinal fluid (CSF) and plasma levels of p-tau181 among amyloid-positive (A+) versus amyloid-negative (A−) patients, stratified by assessment method (see also Figures 1, 2)

Parameter	*No.* studies (total sample)	N / N	Mean difference (95% CI)	*p*-Value	I^2, %
1. p-tau181 CSF levels (in ng/mL)					
– Immunoprecipitation	2 (126)	65 / 61	255.9 (135.6; 376.3)	<0.001	83
– MSD	5 (1639)	753 / 886	52.03 (32.70; 71.36)	<0.001	98
– Simoa[a]	19 (7306)	2427 / 4879	63.76 (54.93; 72.59)	<0.001	99
2. p-tau181 plasma levels (in pg/mL)					
– Immunoprecipitation	2 (126)	65 / 61	1.38 (0.18; 2.57)	0.02	88
– MSD	5 (1639)	753 / 886	0.94 (0.59; 1.29)	<0.001	94
– Simoa[a]	18 (7119)	2336 / 4783	7.39 (5.99; 8.80)	<0.001	97

Abbreviations: CI, confidence interval; CSF, cerebrospinal fluid; MSD, Meso Scale Discovery platform; N/N, Total no. of subjects in the A+ group / Total n. of subjects in the A− group.
[a]Including the following: Quanterix, ADx, Ugot, Janssen, Thermo Fisher/ Thermo Scientific.

although it was evaluated in only two cohorts of patients, immunoprecipitation was also shown to discriminate A+ versus A− participants, reporting significantly higher values in the former than in the latter (MD, 255.9; 95% CI, 135.6 to 376.3; $p < 0.001$) (see Figure 2, section A). Taken together, these results demonstrate that the assessment of p-tau181 on CSF strongly and reliably distinguishes patients with amyloid pathology from those without this neruopathological feature.

3.3.2 | Blood levels of p-tau181 among A+ versus A− participants

Table 3 (second section) summarizes the results about blood p-tau181 values in A+ and A− groups (see Table 3). Similar to the analysis on CSF, p-tau assessments on blood, regardless of the laboratory technique used, have proved that they can reliably differentiate A+ from A− participants in a statistically significant way. In particular, the Simoa technique showed that A+ subjects are characterized by higher values than A− ones (MD, 7.39; 95% CI, 5.99 to 8.80; $p < 0.001$) (see Figure 3, section C), and the same was demonstrated for MSD (MD, 0.94; 95% CI, 0.59 to 1.29; $p < 0.001$) (see Figure 3, section B). Finally, even in this case, immunoprecipitation showed statistically significant difference between A+ and A− participants (MD, 1.38; 95% CI, 0.18 to 2.57; $p = 0.02$) (see Figure 3, section A). Therefore, crucially, blood p-tau measurements also proved to be able to differentiate between A+ and A− subjects, confirming the already established finding on CSF.

4 | DISCUSSION

Here, as far as we know for the first time, we have gathered the currently available evidence that allows a direct comparison between p-tau values on CSF and blood. Specifically, the two most relevant aspects that arise from our systematic review and meta-analysis of the literature are the following: firstly, the mean p-tau values confirm a gradient, that is, they are higher on CSF than on blood, and, above all, they show higher values in A+ than in A− individuals, regardless of the laboratory method considered. Secondly, and most importantly, we have shown that all the examined laboratory methods employed for the assessment of p-tau, that is, Simoa, MSD, and immunoprecipitation, not only on CSF, but also on blood, are able to reliably differentiate amyloid subjects from those who are A−. Previous work by our group had already highlighted that most of the individual studies evaluating p-tau on both CSF and blood identified a correlation (of varying magnitude) between these measures.[12] However, as this was a qualitative synthesis of the evidence, it did not allow us to make quantitative considerations about either the mean p-tau values or their possible role in order to differentiate A+ and A− participants. This systematic review and meta-analysis allowed us to address these challenges and provided a robust and reliable quantitative demonstration that, regardless of the laboratory technique used, blood p-tau181 consistently distinguishes between A+ and A− individuals across the AD continuum. This finding holds significant potential for both diagnostic and therapeutic applications. Indeed, it is now established that AD symptoms manifest even decades after the onset of neuropathological process, when the brain damage is already marked and available therapies can just slow down cognitive decline, but they are unable to halt or reverse the neurodegenerative process.[33] Notably, in recent years, a great deal of research focused on disease-modifying drugs, which have higher efficacy in patients in the early disease stages, that is, MCI, before the pathological protein load becomes too severe.[34] Therefore, currently, it is fundamental to get tools to detect subjects characterized by amyloid pathology at an early stage in a simple, safe, and non-invasive as possible way, in order to promptly identify, among at-risk populations, the most suitable patients for these innovative therapies. In this context, blood-based assessments are a growing research interest, as they offer a safe and viable alternative to lumbar puncture and PET scans, which are invasive, expensive, and not easily accessible.[35]

This is particularly significant when considering that the current diagnostic standard, indeed based on lumbar puncture and nuclear

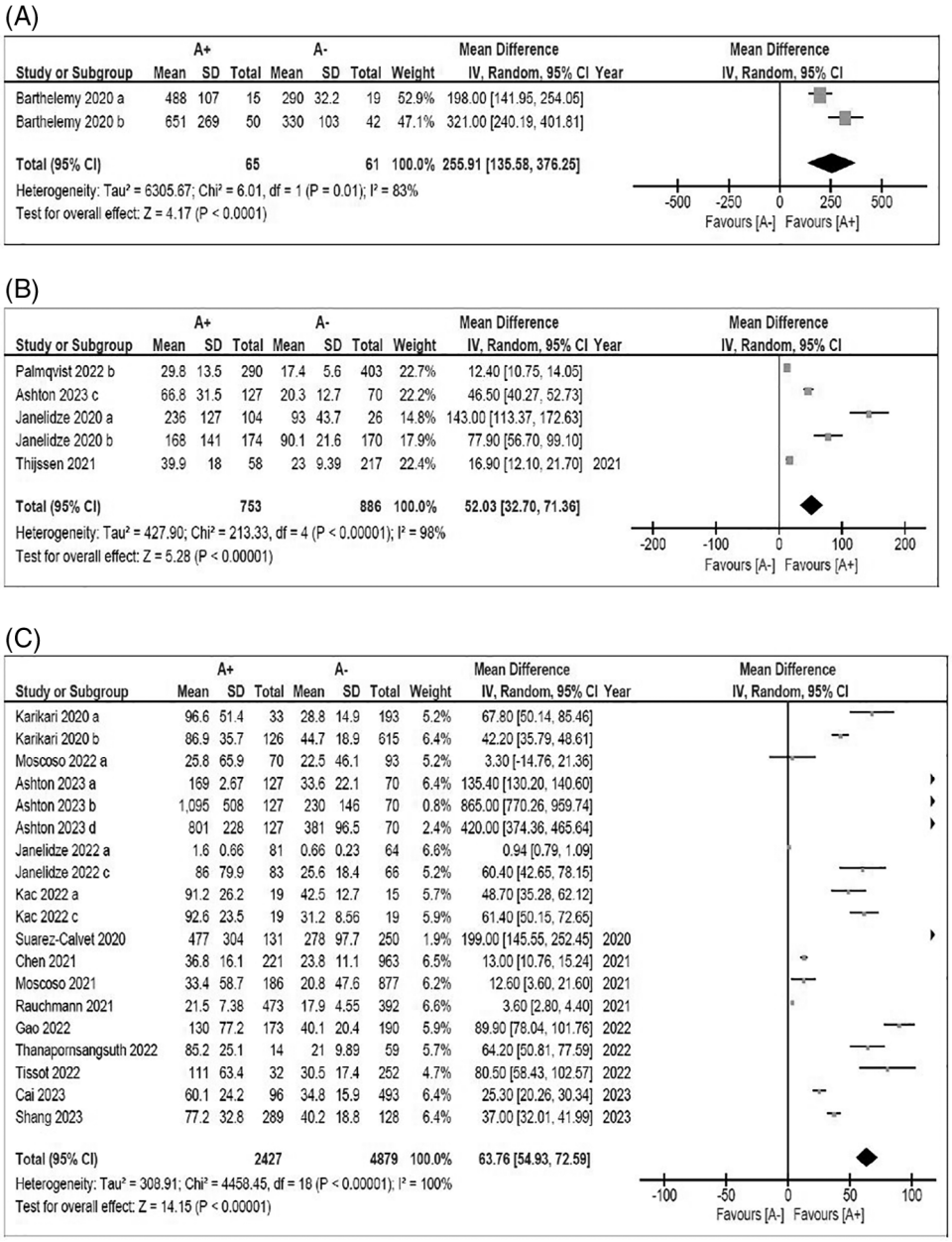

FIGURE 2 Results of the meta-analyses comparing the mean CSF levels (in ng/mL) among A+ versus A− subjects, stratified by assessment method (A: Immunoprecipitation; B: Lilly [MSD]; C: Simoa). A+, amyloid-positive; A−, amyloid-negative; CSF, cerebrospinal fluid; MSD, Meso Scale Discovery platform.

medicine techniques, restricts access to diagnostic measures to major centers in North America, Europe, and Australia. This effectively excludes about 80% of the global population from essential diagnostic tools.[36] Furthermore, even in these regions, as mentioned, access is limited by various factors, such as the availability of clinicians skilled in performing and interpreting these techniques, which are invasive, costly, and conducted only in high-level centers.[8] Given these challenges, it becomes evident how blood-based measures can offer a potentially viable alternative capable of providing access to diagnostic facilities to a much larger portion of the population, including in economically disadvantaged countries. Indeed, pathological brain proteins can cross the BBB, moving from the CSF to the bloodstream, particularly as the BBB undergoes more damage and dysfunction.[37] Furthermore, interestingly, some evidence suggests that MCI patients, as compared to overt dementia, are those in whom the correlation between blood biomarkers and CSF reaches the highest values, reasonably because blood levels reach a plateau when neuropathological damage is advanced.[38] Therefore, MCI patients are not only the target population to be identified for new drug trials, but they are also characterized by the strongest and most reliable correlation between blood and CSF values.[12] However, despite these encouraging premises, the identification of suitable blood biomarkers turned out to be considerably complex. For example, neurodegeneration biomarkers, for example, neurofilament light chains and total Tau protein, are non-

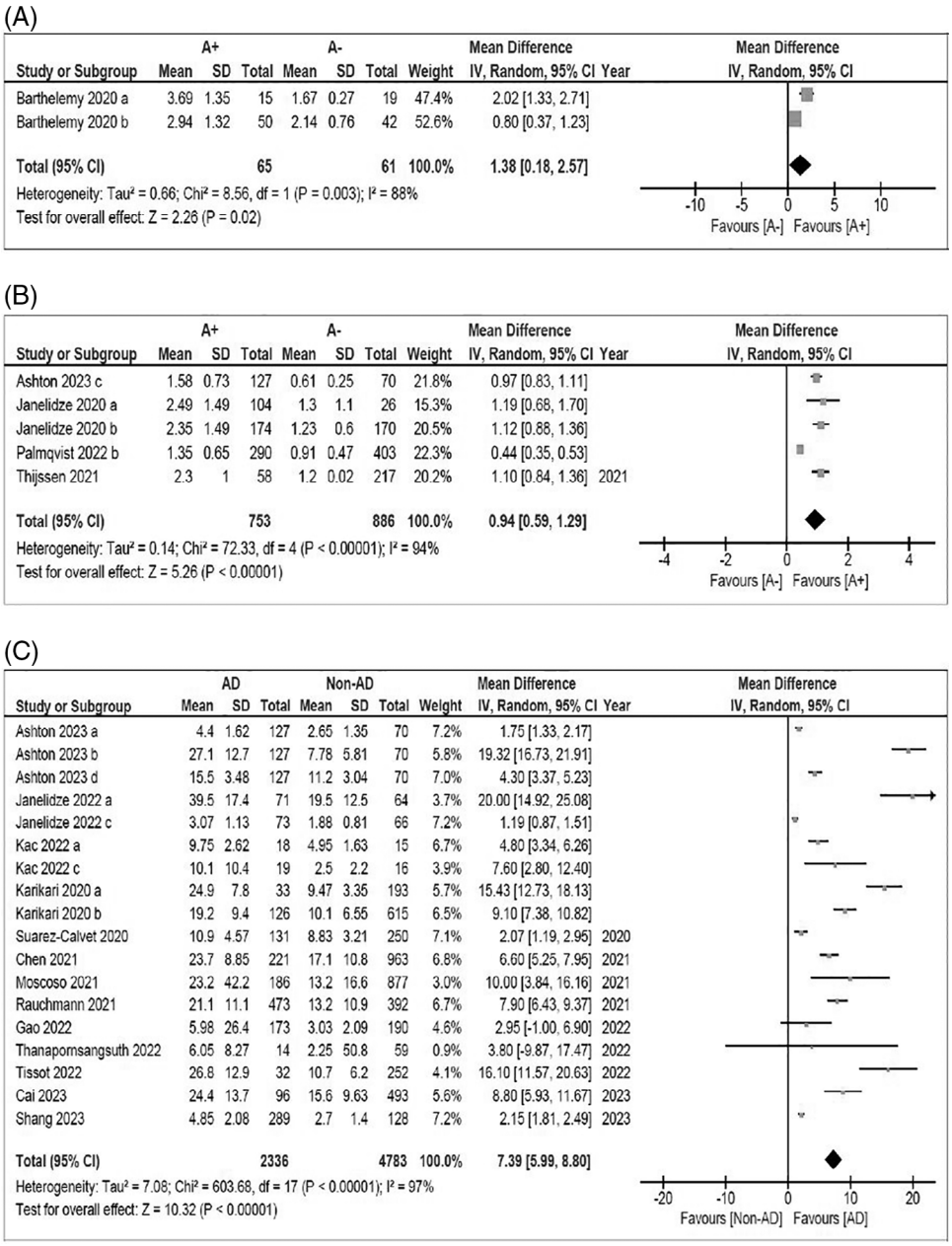

FIGURE 3 Results of the meta-analyses comparing the mean plasma levels (in pg/mL) among A+ versus A− subjects, stratified by assessment method (A: Immunoprecipitation; B: Lilly [MSD]; C: Simoa). A+, amyloid-positive; A−, amyloid-negative; MSD, Meso Scale Discovery platform.

specific markers of neuronal damage that can result from a wide number of causes (e.g., multiple sclerosis, neurodegeneration, stroke, and traumatic brain injury); therefore, they are not useful, on their own, to reliably identify the AD-related neuropathological process.[39,40] Therefore, the main research interest is focused on specific blood AD biomarkers and, in particular, amyloid is the most characteristic neuropathological signature in AD patients.[41] Consistently, there were considerable expectations regarding mass spectrometry-based assays for plasma Aβ, indicative of cerebral amyloid load. However, its utility in distinguishing between AD patients and healthy controls was hampered not only due to the marked overlap in its concentration between Aβ PET-positive and PET-negative individuals, but also because a significant blood amount derives from its peripheral expression (i.e., outside the CNS), and not from crossing the BBB.[42] Furthermore, importantly, a review highlighted that blood Aβ assessment is still characterized by persistent constraints even with novel immunoassay-based techniques, since also these methods are susceptible to blood interferences and only allow an indirect detection of Aβ.[43] However, of note, also p-tau represents a specific AD biomarker, whose formation depends on the abnormal Aβ accumulation, which leads to the activation of intracellular kinases that hyperphosphorylate Tau, altering its physiological role and its normal conformation and promoting its accumulation in neurofibrillary tangles.[44] Despite its presence in peripheral tissues like the kidney, skeletal muscle, and breast, evidence

highlights that blood p-tau could represent a relevant biomarker for distinguishing between AD, different neurodegenerative diseases, and healthy individuals.[12] Indeed, the neuropathological damage progressively compromises the BBB, resulting in elevated blood p-tau levels parallel to CNS damage.[45] Consistently, some research has shown that levels of the plasma p-tau isoforms correlate with age and, indeed, are lower in young people compared to cognitively normal elderly subjects.[46] Importantly, our first meta-analysis (single group, see Section 3.1) supports these literature data, as the mean p-tau values are consistently higher in CSF (showing that its peripheral expression is, coherently, overall limited) than in blood, and especially in the A+ group compared to the A− group. This finding is relevant, as it confirms the specificity of p-tau as a biomarker of the AD continuum. However, our most significant finding is the one provided by the second (head-to-head, section 3.2) meta-analysis, which shows that blood p-tau is able to discriminate, in a consistently statistically significant way regardless of the laboratory method employed, subjects on the AD continuum from healthy controls or different disorders. These findings confirm and further strengthen evidence suggesting that blood p-tau shows a strong correlation with cognitive performance and, in some instances, appears to predict the progression of cognitive decline by mirroring its counterpart in the CSF.[47] Among the laboratory techniques considered, Simoa is certainly the most widely used, as demonstrated by the large number of studies that have employed it. Indeed, it is a relatively simple and reliable method and recent studies have confirmed that it allows plasma biomarkers (including p-tau) to be measured, showing higher values in AD patients and good correlation between blood and CSF measures.[48] Previous work, indeed, pointed out that Simoa, in particular the Quanterix kit, was the most widely used method in this context and 59% of the considered studies documented a correlation between CSF and blood p-tau measurements.[12] However, even though the number of studies is very limited and further research will be needed to confirm its potential, our analysis suggests that immunoprecipitation-based methods also seem promising, as they have been able to detect the highest p-tau values (i.e., reasonably with the highest sensitivity) both on CSF[20] and, when combined with mass spectrometry, on blood.[13] Finally, even if the majority of included studies categorized patients based on biomarkers, some classified participants from a clinical perspective. Therefore, our results suggest the potential role of these blood biomarkers in pre-clinical contexts, such as screening tools for individuals experiencing cognitive complaints in order to promptly identify A+ individuals.

Although we performed an extensive literature search and we included, using rigorous selection criteria, 2764 A+ and 5646 A− subjects, our systematic review and meta-analysis acknowledge several limitations. First of all, the included studies categorized participants with very heterogeneous criteria from both a clinical and biomarker perspective. This entails limitations on the possibility of confidently generalizing the results since, on the one hand, clinical criteria are not invariably sufficient to identify subjects with AD neuropathology, especially considering that they often lack sensitivity and specificity, are heavily influenced by the experience of the clinicians applying them, and often result inadequate for correctly identifying patients with unusual clinical manifestations (e.g., atypical onset, overlap with different dementias).[49,50] On the other hand, positive biomarkers for AD do not necessarily imply the development of a clinical disease, given its complex and multifactorial nature.[49,51] In this context, it is reasonable to hypothesize that the availability of blood tests, ideally performed with standardized, low-cost methods, and good reproducibility across different centers, will enable a simpler and more accessible categorization of subjects based on biomarkers.[52] Indeed, while clinical and neuropsychological assessments are relatively low-cost and widely available, the current neuropathological evaluation is more challenging to obtain for the previously discussed reasons.[53] Hopefully, the systematic introduction of blood-based biomarker evaluations will not only facilitate a more consistent diagnostic categorization of participants but also enhance access to international clinical trials in countries where limited resources currently preclude the essential neurobiological classification of included patients. However, it is also important to consider that, while our study categorized participants as A+ or A−, future research, particularly when validated and standardized clinical and neuropsychological assessment tools become available—currently lacking or highly heterogeneous and, as previously mentioned, often insufficiently sensitive or specific—could evaluate whether blood p-tau can reliably stratify individuals according to the cognitive status as well. This would enable a biomarker-based classification to complement a clinically-based stratification. Furthermore, it is important to note that the vast majority of studies in the literature focus, because of economic reasons, invasiveness, and analytical complexity, only on blood or CSF p-tau measurements, and this hampers to ascertain whether there is a correlation between peripheral and CSF status (i.e., whether blood p-tau truly reflects AD neuropathology in the CNS). Additionally, it also confounds the extent to which CSF changes are detectable and measurable in the blood.[54] This, therefore, limited the number of included studies and, consequently, the sample size included in this meta-analysis, which nevertheless allowed us to obtain a robust statistic favoring the ability of blood p-tau to distinguish between A+ and A−. Ideally, future studies on large populations will assess p-tau levels in both blood and CSF to further support the ability of the former to correctly categories individuals within the AD spectrum, leading to a scenario where lumbar punctures are no longer essential. This is particularly important as studies have shown that p-tau181 has significant within-subject variation, highlighting the need for large participant samples to achieve reliable measures in quantitative analyses.[55] Another very relevant limitation is that no normative cutoff values of blood p-tau to distinguish A+ and A− individuals are currently available. Indeed, these measures are strongly dependent on the methodology used by each laboratory; therefore, discrimination at present is only possible by direct comparison with (reasonably) healthy populations.[5] The mean values provided by this meta-analysis, obtained by a large number of patients from both groups, could be a relevant starting point for identifying these cutoff values, which are crucial for the correct diagnosis of patients. Indeed, importantly, in each included study, the possibility of counting the same participants multiple times was minimized by stratifying the results according to the laboratory technique used, with no overall concentration provided.

Furthermore, while some studies may have drawn from the same large international cohorts, differences in selection criteria, subject groups, and analytical methods made overlap unlikely. A further limitation, which could not be investigated in this meta-analysis as it was not covered in depth by many of the included studies, stems from the fact that the permeability of the BBB (which critically affects blood p-tau measurements)[56] does not only depend on (possible) neuropathological damage, but also on numerous other factors, for example, hypertension, age, diabetes mellitus, and kidney disease.[57] This may have influenced the measurements obtained for factors other than AD pathology (or not) alone, prompting caution on the interpretation of our results. However, since most of the participants included in the two groups (i.e., A+ and A−) are generally of advanced age, statistically, a similar distribution of these co-diseases can be assumed, which should, reasonably, mitigate their possible effect on our analyses.[58] Also, importantly, some studies suggested that BBB permeability alterations do not consistently influence p-tau values, leading us to hypothesize a role, currently poorly understood, of other protein clearance pathways, for example, interstitial fluid bulk flow, lysosomal degradation, and CSF absorption.[10] It is hoped that future studies will systematically take these aspects into account to assess their influence on the correlation between blood and CSF p-tau. Finally, we chose to focus on p-tau181 because it is the most investigated one in the literature and it is considered the most AD specific.[59] However, it is currently known that the other p-tau isoforms, for example, p-tau217 and 231, which differ in their phosphorylation site, seem to reflect different processes and/or stages of AD neuropathology, and it is not yet clear which is the most early and reliable marker among those examined.[12] Importantly, a recent study highlighted that blood p-tau217 exhibits clinical performance equivalent to or better than CSF tests in detecting AD pathology.[60] This allows us to speculate that these isoforms will also be pivotal in this context. In the coming years, it will be crucial to quantitative analyze these isoforms as well, possibly differentiating patients according to stage of disease, so as to assess their different diagnostic usefulness depending on the stage of disease.

5 | CONCLUSION AND FUTURE DIRECTIONS

In conclusion, the data from our meta-analyses confirm that p-tau is a specific biomarker of the AD continuum whose values are systematically higher in the CSF and, to a lesser extent, in the blood of patients with AD neuropathology, regardless of the laboratory method employed. Moreover, crucially, blood p-tau has been shown to discriminate A+ from A− subjects in a statistically strong and reliable way, raising important implications from an early diagnosis and inclusion in clinical trials perspectives. Hopefully, these results will promote the use of blood-based biomarkers in the clinical practice of AD spectrum patients, a crucial aspect to reduce the reliance on expensive and invasive diagnostic tools. Finally, importantly, this will also ensure adequate access to diagnostic routes also for countries with fewer economic resources, a key aim to reduce the inequality in access to care that characterizes today's healthcare landscape.

AUTHOR CONTRIBUTIONS

Conceptualization, Annibale Antonioni, Emanuela Maria Raho, and Francesco Di Lorenzo; methodology, Annibale Antonioni, Emanuela Maria Raho, Maria Elena Flacco, and Francesco Di Lorenzo; data extraction, Annibale Antonioni, Emanuela Maria Raho, and Francesco Di Lorenzo; data curation, Annibale Antonioni, Emanuela Maria Raho, Maria Elena Flacco, and Francesco Di Lorenzo; formal analysis: Lamberto Manzoli and Maria Elena Flacco; writing—original draft preparation, Annibale Antonioni and Emanuela Maria Raho; writing—review and editing, Annibale Antonioni, Emanuela Maria Raho, Lamberto Manzoli, Giacomo Koch, Maria Elena Flacco, and Francesco Di Lorenzo; supervision, Lamberto Manzoli, Giacomo Koch, Maria Elena Flacco, and Francesco Di Lorenzo. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

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INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The dataset created to perform the analysis is available, upon reasonable request, to Maria Elena Flacco and Francesco Di Lorenzo.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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