

Blood Biomarkers to Detect Alzheimer Disease in Primary Care and Secondary Care

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IMPORTANCE An accurate blood test for Alzheimer disease (AD) could streamline the diagnostic workup and treatment of AD.

OBJECTIVE To prospectively evaluate a clinically available AD blood test in primary care and secondary care using predefined biomarker cutoff values.

DESIGN, SETTING, AND PARTICIPANTS There were 1213 patients undergoing clinical evaluation due to cognitive symptoms who were examined between February 2020 and January 2024 in Sweden. The biomarker cutoff values had been established in an independent cohort and were applied to a primary care cohort (n = 307) and a secondary care cohort (n = 300); 1 plasma sample per patient was analyzed as part of a single batch for each cohort. The blood test was then evaluated prospectively in the primary care cohort (n = 208) and in the secondary care cohort (n = 398); 1 plasma sample per patient was sent for analysis within 2 weeks of collection.

EXPOSURE Blood tests based on plasma analyses by mass spectrometry to determine the ratio of plasma phosphorylated tau 217 (p-tau217) to non-p-tau217 (expressed as percentage of p-tau217) alone and when combined with the amyloid- β 42 and amyloid- β 40 (A β 42:A β 40) plasma ratio (the amyloid probability score 2 [APS2]).

MAIN OUTCOMES AND MEASURES The primary outcome was AD pathology (determined by abnormal cerebrospinal fluid A β 42:A β 40 ratio and p-tau217). The secondary outcome was clinical AD. The positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy, and area under the curve (AUC) values were calculated.

RESULTS The mean age was 74.2 years (SD, 8.3 years), 48% were women, 23% had subjective cognitive decline, 44% had mild cognitive impairment, and 33% had dementia. In both the primary care and secondary care assessments, 50% of patients had AD pathology. When the plasma samples were analyzed in a single batch in the primary care cohort, the AUC was 0.97 (95% CI, 0.95-0.99) when the APS2 was used, the PPV was 91% (95% CI, 87%-96%), and the NPV was 92% (95% CI, 87%-96%); in the secondary care cohort, the AUC was 0.96 (95% CI, 0.94-0.98) when the APS2 was used, the PPV was 88% (95% CI, 83%-93%), and the NPV was 87% (95% CI, 82%-93%). When the plasma samples were analyzed prospectively (biweekly) in the primary care cohort, the AUC was 0.96 (95% CI, 0.94-0.98) when the APS2 was used, the PPV was 88% (95% CI, 81%-94%), and the NPV was 90% (95% CI, 84%-96%); in the secondary care cohort, the AUC was 0.97 (95% CI, 0.95-0.98) when the APS2 was used, the PPV was 91% (95% CI, 87%-95%), and the NPV was 91% (95% CI, 87%-95%). The diagnostic accuracy was high in the 4 cohorts (range, 88%-92%). Primary care physicians had a diagnostic accuracy of 61% (95% CI, 53%-69%) for identifying clinical AD after clinical examination, cognitive testing, and a computed tomographic scan vs 91% (95% CI, 86%-96%) using the APS2. Dementia specialists had a diagnostic accuracy of 73% (95% CI, 68%-79%) vs 91% (95% CI, 88%-95%) using the APS2. In the overall population, the diagnostic accuracy using the APS2 (90% [95% CI, 88%-92%]) was not different from the diagnostic accuracy using the percentage of p-tau217 alone (90% [95% CI, 88%-91%]).

CONCLUSIONS AND RELEVANCE The APS2 and percentage of p-tau217 alone had high diagnostic accuracy for identifying AD among individuals with cognitive symptoms in primary and secondary care using predefined cutoff values. Future studies should evaluate how the use of blood tests for these biomarkers influences clinical care.

JAMA. 2024;332(15):1245-1257. doi:10.1001/jama.2024.13855
Published online July 28, 2024.

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One in 5 women and 1 in 10 men develop dementia due to Alzheimer disease.¹ Individuals with cognitive symptoms are first seen in primary care, with a minority being referred to secondary care.² Symptomatic Alzheimer disease is misdiagnosed in 25% to 35% of patients treated at specialized clinics and likely even more patients treated in primary care.^{3,4} Tests sometimes available only at specialized clinics, such as positron emission tomography (PET) or the collection of cerebrospinal fluid to assess Alzheimer disease biomarkers, reduce the rate of misdiagnosis.^{3,4}

Two anti-amyloid immunotherapies have been approved for the treatment of patients with early symptomatic Alzheimer disease,^{5,6} and other treatments are likely to follow. Initiation of treatment requires biomarker-positive test results for Alzheimer disease, leading to increased need for biomarker testing. However, primary care physicians lack accessible and reliable biomarker tools to diagnose Alzheimer disease. Even in secondary care, there is limited availability of cerebrospinal fluid and PET examinations. The lack of accessible testing methods for Alzheimer disease biomarkers is a substantial obstacle to the initiation and effective use of anti-amyloid immunotherapies to treat patients with Alzheimer disease.^{7,8}

These issues have driven the development of Alzheimer disease blood biomarker tests with potential for high accessibility in both primary and secondary care.⁹ The most promising is plasma phosphorylated tau 217 (p-tau217), which is strongly associated with Alzheimer disease pathology in cerebrospinal fluid and in Alzheimer disease biomarkers measured by PET, in addition to neuropathological changes in patients with Alzheimer disease.⁹⁻¹¹ A blood test based on the ratio of p-tau217 to non-p-tau217 (expressed as percentage of p-tau217) can be used to account for the influence of non-Alzheimer disease-related factors on plasma p-tau217 concentrations.¹² The diagnostic accuracy of p-tau217 can improve when combined with the amyloid- β 42 and amyloid- β 40 (A β 42:A β 40) plasma ratio.^{13,14}

The percentage of plasma p-tau217 has recently been shown to provide comparable diagnostic accuracy (90%) as clinically approved cerebrospinal fluid biomarkers (vs 91% using the p-tau to A β 42 ratio and 87% using the A β 42:A β 40 ratio) in individuals with cognitive impairment assessed in secondary care using A β PET.¹⁵ Although assessments using these blood biomarkers are promising, several knowledge gaps hinder their clinical implementation.¹⁶ The biomarkers must be validated in primary care and compared with standard clinical assessments in terms of diagnostic accuracy.¹⁶ The biomarkers must also be accurate when using predefined cutoff values and continuous analysis of samples (similar to clinical practice).

In both the primary and secondary care cohorts, we aimed to (1) examine the ability of plasma percentage of p-tau217 alone and when combined with the A β 42:A β 40 plasma ratio (the amyloid probability score 2 [APS2]) to detect Alzheimer disease pathology or clinical Alzheimer disease in patients with cognitive symptoms using predefined cutoff values; (2) evaluate the diagnostic accuracy of blood biomarkers when analyzed in batches prospectively (biweekly); and (3) compare the diagnostic accuracy of

Key Points

Question Can a blood test based on the ratio of plasma phosphorylated tau 217 (p-tau217) relative to non-p-tau217 (expressed as percentage of p-tau217) combined with the amyloid- β 42 and amyloid- β 40 plasma ratio (the amyloid probability score 2 [APS2]) accurately identify Alzheimer disease in primary care and secondary care when prospectively applying predefined biomarker cutoff values?

Findings There were 1213 patients undergoing cognitive evaluation in primary or secondary care. The APS2 had high diagnostic accuracy (range, 88%-92%) for detecting Alzheimer disease pathology in both primary and secondary care. Dementia specialists identified clinical Alzheimer disease with a diagnostic accuracy of 73% vs 91% using the APS2 and primary care physicians had a diagnostic accuracy of 61% vs 91% using the APS2.

Meaning This blood test (the APS2) had high diagnostic accuracy for identifying Alzheimer disease among individuals with cognitive symptoms in primary and secondary care, providing superior performance compared with the diagnostic accuracy after standard clinical evaluation (not using Alzheimer disease biomarkers).

blood biomarkers with the diagnostic accuracy of primary care physicians or dementia specialists. Secondary objectives were to (1) examine the performance at different cognitive stages and (2) compare different cutoff value approaches for the blood test.

Methods

Participants

All participants provided written informed consent and the study was approved by the Swedish Ethical Review Authority. The study report adheres to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) recommendations. The study included 2 cohorts from primary and secondary care clinics at which the plasma samples were analyzed together at 1 time point in a single batch. The study also included 2 cohorts from primary and secondary care clinics at which the plasma samples were analyzed prospectively in batches biweekly (ie, twice monthly) throughout the enrollment period, which is more similar to clinical practice. Only 1 plasma sample per patient was analyzed.

Patients in the 2 primary care cohorts were recruited at 17 primary care centers in southern Sweden (12 public and 5 private primary care centers) from February 2020 to October 2022 (single-batch analysis) and from October 2022 to October 2023 (prospective, biweekly batch analysis) as part of the prospective BioFINDER Primary Care study (NCT06120361), which consecutively includes patients undergoing investigation for a dementia diagnosis in primary care. Patients in the single-batch analysis in secondary care were recruited at the Memory Clinic of Skåne University Hospital or the Memory Clinic of Ängelholm Hospital in Sweden as part of the BioFINDER 2 study¹⁰ (NCT03174938) from January 2019 to November 2023.

For the prospective, biweekly analysis in secondary care, patients were recruited as part of the BioFINDER Memory Clinic study (NCT06122415) from December 2022 to January 2024 at the Memory Clinic of Skåne University Hospital. Further cohort details appear in the eMethods in [Supplement 1](#).

Patients were classified as having subjective cognitive decline, mild cognitive impairment, or dementia based on cognitive test results and clinical assessments, independent of underlying etiology and Alzheimer disease biomarker results (described in the eMethods in [Supplement 1](#)).

Physician Assessment of Alzheimer Disease

In the prospectively analyzed primary and secondary care cohorts, primary care physicians and dementia specialists documented whether they thought their patients had Alzheimer disease pathology. The physicians based their diagnoses of Alzheimer disease on the standard evaluation (clinical examination, cognitive testing, and a computed tomographic scan) prior to seeing any Alzheimer disease biomarker results. The certainty of the presence of Alzheimer disease pathology was reported on a scale from 0 (not at all certain) to 10 (completely certain). In a secondary analysis, physicians were also asked if they thought their patients had clinical Alzheimer disease (ie, symptoms caused by Alzheimer disease pathology). Additional details appear in the eMethods in [Supplement 1](#).

Plasma Sampling and Analysis

Plasma handling procedures are described in the eMethods in [Supplement 1](#). The plasma analyses were performed while all personnel analyzing the samples were blinded to all clinical or biomarker data. Mass spectrometry assays (C2N Diagnostics) were used to analyze the following biomarkers: A β 42, A β 40, p-tau217, and non-p-tau217 (eMethods in [Supplement 1](#)).^{14,17}

The PrecivityAD2 blood test algorithm is a logistic regression model trained in an independent cohort to estimate amyloid positivity using a combination of plasma A β 42:A β 40 ratio and percentage of p-tau217.¹⁸ The probability output (score range, 0-100) of the logistic regression model is referred to as the APS2 (eMethods in [Supplement 1](#)).

Cerebrospinal Fluid Sampling and Analysis

Cerebrospinal fluid was collected and handled according to a standardized protocol,¹⁹ and the biomarkers A β 42 and A β 40 were analyzed at the Sahlgrenska University Hospital clinical chemistry laboratory using the Lumipulse assays, which have been approved for use by the US Food and Drug Administration (FDA).²⁰ The biomarker p-tau217 was analyzed on the MesoScale Discovery platform using an assay developed by Lilly.^{10,11,21}

The 82 participants who did not undergo cerebrospinal fluid sampling (because they were unable to undergo lumbar puncture) in the primary care cohorts instead underwent [¹⁸F]flutemetamol PET imaging (described in the eMethods in [Supplement 1](#)).

Outcomes

The primary outcome was presence of Alzheimer disease pathology, which was defined according to the 2018 National

Institute on Aging and the Alzheimer's Association criteria as A β and tau positivity.²² A positive finding of the A β biomarker was defined according to the FDA-approved cutoff value (≤ 0.072) using the Lumipulse assay for A β 42:A β 40 ratio based on cerebrospinal fluid.²⁰ A positive finding of the tau biomarker was defined as a p-tau217 level greater than 11.42 pg/mL in cerebrospinal fluid.²³ A positive visual read of the [¹⁸F]flutemetamol PET scan for A β was used to define the presence of Alzheimer disease pathology in the primary care cohorts for the participants in whom lumbar puncture could not be performed.

Clinical Alzheimer disease was used as a secondary outcome and was defined according to criteria from the International Working Group,²⁴ which include a typical presentation of the clinical syndrome of Alzheimer disease and confirmation with an Alzheimer disease biomarker (eMethods in [Supplement 1](#)). The analyses using clinical Alzheimer disease vs non-Alzheimer disease as an outcome were only performed in patients with mild cognitive impairment or dementia because a clinical diagnosis (for Alzheimer disease and other types of dementia) only can be established at these stages according to current clinical criteria.²⁴⁻²⁹ In addition, results from the [¹⁸F]flutemetamol PET scan (positivity was defined as a standardized uptake value ratio > 1.033) were used as a secondary outcome in a subsample of primary and secondary care participants (eMethods in [Supplement 1](#)).³⁰ Additional secondary outcomes were the cerebrospinal fluid A β 42:A β 40 ratio alone (≤ 0.072 for positivity) and the cerebrospinal fluid A β 42 to p-tau181 ratio (< 15 for positivity) (eMethods in [Supplement 1](#)).

Blood Biomarker Cutoff Values

The blood biomarker cutoff values were established in an independent cohort (eMethods in [Supplement 1](#)). The cutoff value was set at 90% specificity for Alzheimer disease pathology (the 1 cutoff-value approach). In addition, a 2 cutoff-value approach (using 1 upper and 1 lower cutoff value) was also established. This 2 cutoff-value approach is similar to the FDA-cleared approach for A β 42:A β 40 ratio in Lumipulse assays based on cerebrospinal fluid,²⁰ and according to appropriate use recommendations for Alzheimer disease blood biomarkers.¹⁶ The 2 cutoff values corresponded to 95% sensitivity and 95% specificity in the independent cohort. Any results between these 2 cutoff values were termed *intermediate*. The selection of 1 cutoff value at 90% specificity and 2 cutoff values at 95% sensitivity and 95% specificity followed a previously published design.¹⁵ The rationale for this approach appears in other publications^{9,16,31,32} and in the eMethods in [Supplement 1](#).

Statistical Analysis

There were no missing blood biomarker values or missing data for the primary outcome. The binary variables were compared using χ^2 tests and the continuous variables were compared using the Mann-Whitney test. The receiver operating characteristic curves were used to calculate the area under the curve (AUC) values. Significant differences between the AUC values were tested using DeLong statistics.

The predefined cutoff values were used to calculate diagnostic accuracy (percentage of correctly classified cases of Alzheimer disease), positive predictive value, and negative predictive value for both the APS2 and the percentage of p-tau217 alone. In the 2 cutoff-value approach, the participants with an intermediate test result were not considered when calculating these measures. The 95% CIs were calculated using bootstrapping ($n = 5000$ resamples with replacement) and the differences in test metrics (eg, diagnostic accuracy) were calculated using the distribution of the bootstrapped differences.

A 2-sided P value less than .05 indicated statistical significance. Version 4.3 of R programming language (R Foundation for Statistical Computing) was used for all statistical analyses.

Results

Participants and Biomarker Characteristics

There were 1213 patients (515 from primary care and 698 from secondary care) with cognitive symptoms who participated in the study (eFigure 1 in Supplement 1). The mean age was 74.2 years (SD, 8.3 years) and 581 (48%) were women (Table and eTables 1-3 in Supplement 1). Compared with patients in the secondary care cohort, patients in the primary care cohort were older; had fewer years of education; had a higher prevalence of cardiovascular disease, hyperlipidemia, chronic kidney disease, and diabetes; and had a lower prevalence of dementia (Table). There was no difference in the prevalence of Alzheimer disease pathology (49.9% in the primary care cohort vs 49.7% in the secondary care cohort; standardized between-group difference, -0.4% [95% CI, -11.5% to 10.8%]). The box plots for APS2, the percentage of p-tau217, and the A β 42:A β 40 ratio in plasma (with Alzheimer disease pathology as a grouping variable) appear in eFigure 2 in Supplement 1.

Diagnostic Performance of the APS2 vs P-Tau217 Alone in the Secondary Care Cohort Using the Single-Batch Plasma Analysis

When the predefined single cutoff values were applied to the secondary care cohort (single-batch analysis; $n = 300$), there was a diagnostic accuracy of 88% (95% CI, 84%-91%) with the APS2 compared with a diagnostic accuracy of 91% (95% CI, 87%-94%) with the percentage of p-tau217 alone. When the APS2 was used, the positive predictive value was 88% (95% CI, 83%-93%), the negative predictive value was 87% (95% CI, 82%-93%), and the AUC was 0.96 (95% CI, 0.94-0.98). When the percentage of p-tau217 alone was used, the positive predictive value was 89% (95% CI, 84%-94%), the negative predictive value was 92% (95% CI, 88%-97%), and the AUC was 0.97 (95% CI, 0.95-0.99) (Figure 1A).

With the 2 cutoff-value approach, use of the APS2 resulted in a diagnostic accuracy of 93% (95% CI, 90%-96%), a positive predictive value of 97% (95% CI, 95%-100%), and a negative predictive value of 89% (95% CI, 84%-94%); however, 12% (95% CI, 8%-15%) of the results were in the intermediate zone (ie, between the 2 cutoff values). When the percentage of p-tau217 was used alone, the diagnostic accuracy was 93% (95% CI, 90%-96%), the positive predictive value was

96% (95% CI, 93%-100%), and the negative predictive value was 90% (95% CI, 85%-94%); however, 6% (95% CI, 3%-9%) of the results were in the intermediate zone (Figure 2A).

Diagnostic Performance of the APS2 vs P-Tau217 Alone in the Primary Care Cohort Using the Single-Batch Plasma Analysis

When the predefined single cutoff values were applied to the primary care cohort (single-batch analysis; $n = 307$), there was a diagnostic accuracy of 92% (95% CI, 88%-95%) with the APS2 compared with a diagnostic accuracy of 88% (95% CI, 85%-92%) with the percentage of p-tau217 alone. When the APS2 was used, the positive predictive value was 91% (95% CI, 87%-96%), the negative predictive value was 92% (95% CI, 87%-96%), and the AUC was 0.97 (95% CI, 0.95-0.99). When the percentage of p-tau217 alone was used, the positive predictive value was 86% (95% CI, 80%-91%), the negative predictive value was 92% (95% CI, 87%-96%), and the AUC was 0.96 (95% CI, 0.94-0.98) (Figure 1B).

With the 2 cutoff-value approach, use of the APS2 resulted in a diagnostic accuracy of 95% (95% CI, 92%-98%), a positive predictive value of 98% (95% CI, 95%-100%), and a negative predictive value of 93% (95% CI, 88%-97%); however, 15% (95% CI, 11%-19%) of the results were in the intermediate zone (ie, between the 2 cutoff values). When the percentage of p-tau217 was used alone, the diagnostic accuracy was 91% (95% CI, 87%-94%), the positive predictive value was 97% (95% CI, 94%-100%), and the negative predictive value was 86% (95% CI, 80%-91%); however, 8% (95% CI, 5%-11%) of the results were in the intermediate zone (Figure 2B).

Diagnostic Performance of the APS2 vs P-Tau217 Alone in the Primary and Secondary Care Cohorts Using the Prospective Plasma Analyses

When the preestablished single cutoff values were applied to the secondary care cohort (plasma samples were prospectively and continuously analyzed throughout the study period; $n = 398$), there was a diagnostic accuracy of 91% (95% CI, 88%-94%) with the APS2 compared with 90% (95% CI, 87%-93%) when the percentage of p-tau217 was used alone. When the APS2 was used, the positive predictive value was 91% (95% CI, 87%-95%), the negative predictive value was 91% (95% CI, 87%-95%), and the AUC was 0.97 (95% CI, 0.95-0.98). When the percentage of p-tau217 was used alone, the positive predictive value was 86% (95% CI, 81%-90%), the negative predictive value was 96% (95% CI, 93%-99%), and the AUC was 0.97 (95% CI, 0.95-0.98).

When the preestablished single cutoff values were applied to the primary care cohort ($n = 208$), the diagnostic accuracy was 89% (95% CI, 85%-93%) when the APS2 was used, the positive predictive value was 88% (95% CI, 81%-94%), the negative predictive value was 90% (95% CI, 84%-96%), and the AUC was 0.96 (95% CI, 0.94-0.98). When the percentage of p-tau217 alone was used, the diagnostic accuracy was 90% (95% CI, 86%-94%), the positive predictive value was 86% (95% CI, 79%-92%), the negative predictive value was 94% (95% CI, 89%-99%), and the AUC was 0.96 (95% CI, 0.93-0.98) (Figure 1C and D).

Table. Characteristics of the Primary and Secondary Care Cohorts

	Care cohort ^a		Standardized between-group difference ^b	
	Primary (n = 515)	Secondary (n = 698)	Median (95% CI)	% (95% CI)
Plasma analysis cohorts, No. of patients				
Single-batch analysis ^c	307	300		
Prospective analyses ^d	208	398		
Age, median (IQR), y	77.3 (72.6 to 81.4)	74.1 (67.3 to 78.6)	−0.395 (−0.486 to −0.265)	
Sex, No. (%)				
Female	257 (49.9)	324 (46.4)		−7.0 (−18.4 to 4.7)
Male	258 (50.1)	374 (53.6)		7.0 (−4.7 to 18.0)
Length of education				
No. of patients	515	658		
Median (IQR), y	11 (9 to 13)	12 (9 to 15)	0.29 (0 to 0.59)	
Mini-Mental State Examination				
No. of patients	511	658		
Score, median (IQR) ^e	27 (24 to 29)	26 (22 to 29)	−0.21 (−0.43 to 0)	
Cognitive stage, No. (%) ^f				
Subjective cognitive decline	140 (27.2)	139 (19.9)		−17.1 (−28.7 to −5.5)
Mild cognitive impairment	231 (44.9)	304 (43.6)		−2.6 (−14.2 to 8.6)
Dementia	144 (28.0)	255 (36.5)		18.4 (6.2 to 29.4)
Medical history, No./total (%)				
Cardiovascular disease	355/511 (69.5)	337/692 (48.7)		−43.2 (−55.1 to −31.4)
Hyperlipidemia	269/512 (52.5)	230/692 (33.2)		−39.7 (−51.9 to −27.6)
Chronic kidney disease	134/511 (26.2)	117/691 (16.9)		−22.7 (−34.1 to −11.1)
Diabetes	113/512 (22.1)	103/691 (14.9)		−18.4 (−30.2 to −7.2)
Carrier of apolipoprotein E ε4, No./total (%)	223/511 (43.6)	334/693 (48.2)		9.1 (−2.3 to 20.6)
Plasma results, median (IQR)				
Aβ42:Aβ40 ratio	0.09 (0.08 to 0.1)	0.10 (0.09 to 0.11)	0.35 (0.23 to 0.47)	
Percentage of p-tau217 ^g	3.7 (1.8 to 8.4)	3.9 (1.5 to 9.7)	0.039 (−0.12 to 0.20)	
Amyloid probability score 2 ^h	36 (12 to 86.5)	32 (8 to 90)	−0.11 (−0.38 to 0.27)	
Cerebrospinal fluid results, median (IQR)				
Aβ42:Aβ40 ratio	0.06 (0.05 to 0.1) ⁱ	0.06 (0.05 to 0.1)	0.056 (−0.27 to 0.28)	
P-tau217, pg/mL	13.3 (7.0 to 31.7) ⁱ	13.5 (5.3 to 35.1)	0.006 (−0.13 to 0.13)	
Positive pathology classification, No. (%)				
Amyloid-β ^j	276 (53.6)	405 (58.0)		−0.4 (−11.8 to 10.5)
Alzheimer disease ^k	257 (49.9)	347 (49.7)		−0.4 (−11.5 to 10.8)

^a Further cohort stratification, including for the entire study population, by Alzheimer disease pathology status appears in eTables 1-2 in [Supplement 1](#). The clinical diagnoses appear in eTable 3 in [Supplement 1](#).

^b Additional information appears in the eMethods (statistical analysis subsection) in [Supplement 1](#).

^c Plasma samples were collected during the evaluation and analyzed at a single time point.

^d Plasma samples were shipped within 14 days from the evaluation and analyzed prospectively throughout the study period.

^e Scores range from 0 to 30 points. A higher score indicates better global cognition.

^f Classified based on cognitive test results and clinical assessments that were performed independently from the underlying etiology and Alzheimer disease biomarker results (additional information appears in the eMethods in [Supplement 1](#)).

^g The ratio of plasma phosphorylated tau 217 (p-tau217) relative to non-p-tau217 multiplied by 100.

^h The percentage of p-tau217 combined with the amyloid-β 42 and amyloid-β 40 (Aβ42:Aβ40) plasma ratio in a predefined logistic regression model.

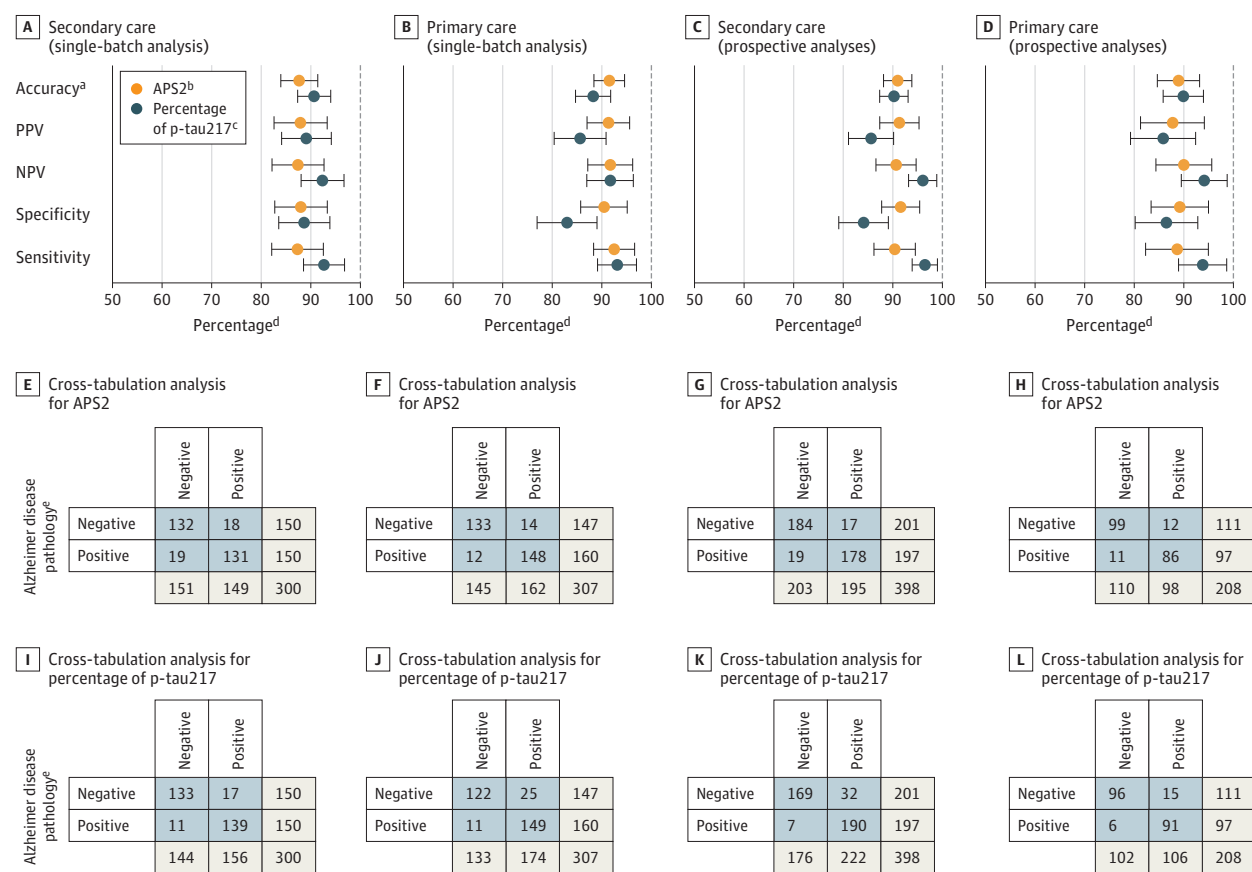
ⁱ There were 433 patients available for the analysis in the primary care cohort. The missing data are due to the patients who were unable to undergo lumbar puncture. These patients instead underwent assessment for Aβ positron emission tomography (PET) per the study design.

^j Based on cerebrospinal fluid–positive results for Aβ42:Aβ40 ratio (≤0.072) or positive for Aβ PET (did not undergo lumbar puncture).

^k Based on cerebrospinal fluid–positive results for p-tau217 and Aβ42:Aβ40 ratio. Other patients were positive for Aβ PET (did not undergo lumbar puncture).

When the 2 cutoff-value approach was used in the secondary care cohort, the APS2 had a diagnostic accuracy of 94% (95% CI, 91%-96%), a positive predictive value of 96% (95% CI, 93%-99%), and a negative predictive value of 91%

(95% CI, 87%-95%); however, 11% (95% CI, 8%-14%) of the results were in the intermediate zone (ie, between the 2 cutoff values). When the percentage of p-tau217 alone was used, the diagnostic accuracy was 93% (95% CI, 90%-95%), the positive

Figure 1. Performance Comparison of the Blood Tests Using the 1 Cutoff-Value Approach Along With Presence of Alzheimer Disease Pathology as an Outcome

APS2 indicates amyloid probability score 2; NPV negative predictive value; PPV, positive predictive value. The error bars indicate the 95% CIs. The detailed results for A-D appear in eTable 16 in Supplement 1. Cross-tabulation analyses are shown for secondary care (single-batch analysis) in E and I, for primary care (single-batch analysis) in F and J, for secondary care (prospective analyses) in G and K, and for primary care (prospective analyses) in H and L.

^aCorrectly classified participants.

^bThe percentage of plasma phosphorylated tau 217 (p-tau217) combined with the β -amyloid 42 and β -amyloid 40 (A β 42:A β 40) plasma ratio in a predefined logistic regression model. The cutoff value was 36.

^cThe ratio of plasma p-tau217 relative to non-p-tau217 multiplied by 100. The cutoff value was 3.26.

^dPercentage of accuracy, PPV, NPV, specificity, or sensitivity.

^eDefined as having cerebrospinal fluid-positive results for A β 42:A β 40 ratio (≤ 0.072)²⁰ and p-tau217 (>11.42 pg/mL).²³ For participants who could not undergo lumbar puncture, Alzheimer disease pathology was based on positron emission tomographic-positive results for A β .

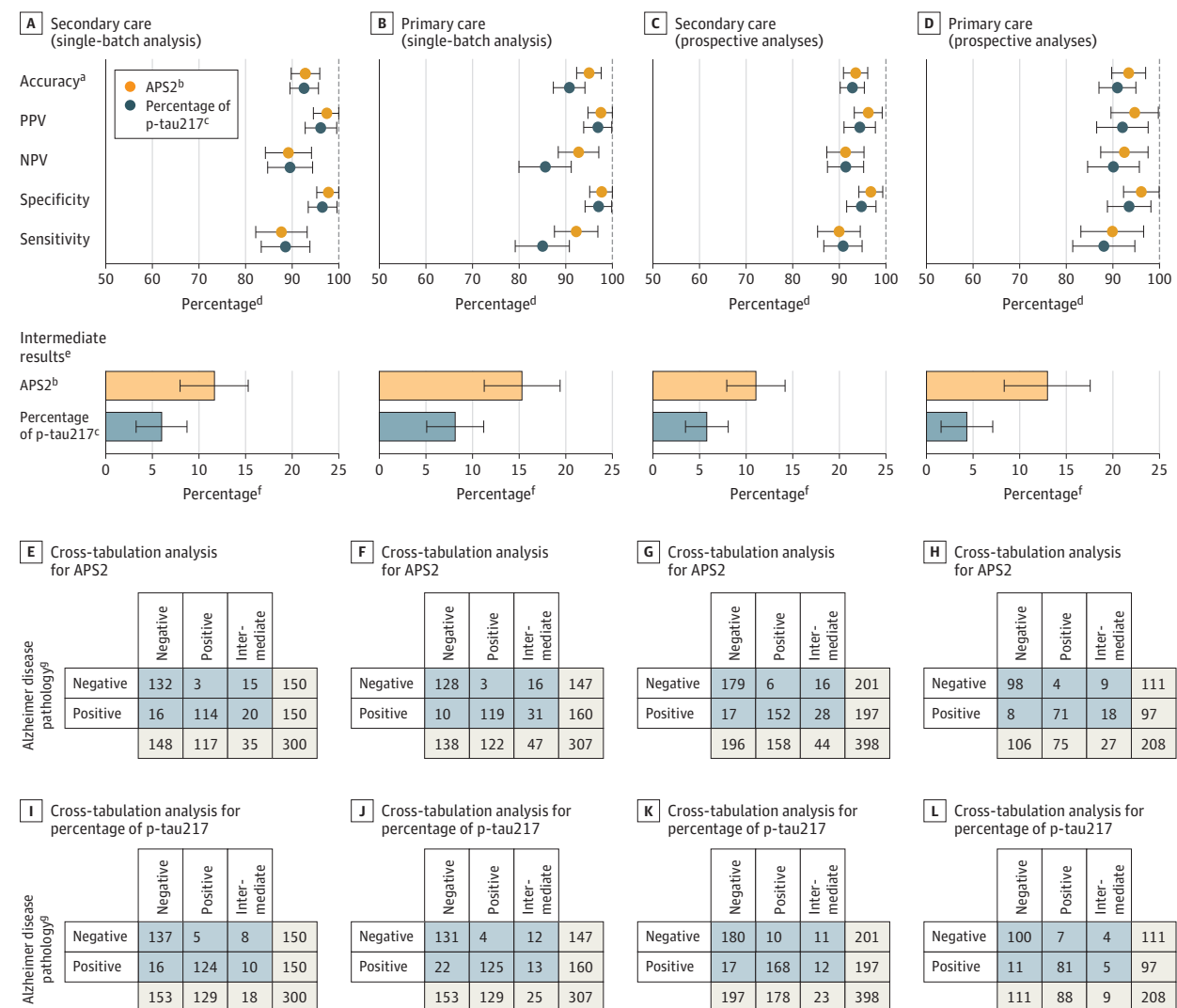
predictive value was 94% (95% CI, 91%-98%), and the negative predictive value was 91% (95% CI, 87%-95%); however, 6% (95% CI, 3%-8%) of the results were in the intermediate zone.

Similar performance was observed in the primary care cohort when the APS2 was used; the diagnostic accuracy was 93% (95% CI, 90%-97%), the positive predictive value was 95% (95% CI, 90%-100%), and the negative predictive value was 92% (95% CI, 87%-98%); however, 13% (95% CI, 8%-18%) of the results were in the intermediate zone (ie, between the 2 cutoff values). When the percentage of p-tau217 was used alone, the diagnostic accuracy was 91% (95% CI, 87%-95%), the positive predictive value was 92% (95% CI, 87%-98%), and the negative predictive value was 90% (95% CI, 85%-96%); however, 4% (95% CI, 2%-7%) of the results were in the intermediate zone (Figure 2C and D).

Blood Biomarker Performance vs Standard Clinical Evaluation

After a standard clinical evaluation (no biomarker data were used) in the secondary care cohort (the prospectively analyzed part), dementia specialists had an overall diagnostic accuracy of 71% (95% CI, 67%-76%) when Alzheimer disease pathology was used as an outcome, which was significantly lower than the prospectively measured diagnostic accuracy of 92% (95% CI, 89%-95%) when the APS2 was used and 91% (95% CI, 88%-94%) when the percentage of p-tau217 alone was used (Figure 3A). The mean certainty of the assessment by dementia specialists was a score of 6 (95% CI, 5.8-6.2) on a scale from 0 (very low) to 10 (very high) for the level of diagnostic confidence.

After the standard clinical evaluation (no biomarker data were used) in the primary care cohort (prospectively analyzed part), the primary care physicians had an overall diagnostic

Figure 2. Performance Comparison of the Blood Tests Using the 2 Cutoff-Value Approach Along With Presence of Alzheimer Disease Pathology as an Outcome

APS2 indicates amyloid probability score 2; NPV, negative predictive value for 2 cutoff values; PPV, positive predictive value for 2 cutoff values. Error bars indicate the 95% CIs. The detailed results for A-D appear in eTable 17 in Supplement 1. Cross-tabulation analyses are shown for secondary care (single-batch analysis) in E and I, for primary care (single-batch analysis) in F and J, for secondary care (prospective analyses) in G and K, and for primary care (prospective analyses) in H and L.

^aCorrectly classified participants.

^bThe percentage of plasma phosphorylated tau 217 (p-tau217) combined with the β -amyloid 42 and β -amyloid 40 (A β 42:A β 40) plasma ratio in a predefined logistic regression model. The cutoff values were 31 (lower) and 62 (upper).

^cThe ratio of plasma p-tau217 relative to non-p-tau217 multiplied by 100. The cutoff values were 3.93 (lower) and 5.18 (upper).

^dPercentage of accuracy, PPV, NPV, specificity, or sensitivity.

^eRefers to participants between the upper and lower cutoff values (who were not included when calculating diagnostic accuracy).

^fPercentage of intermediate results using the APS2 or percentage of p-tau217 alone.

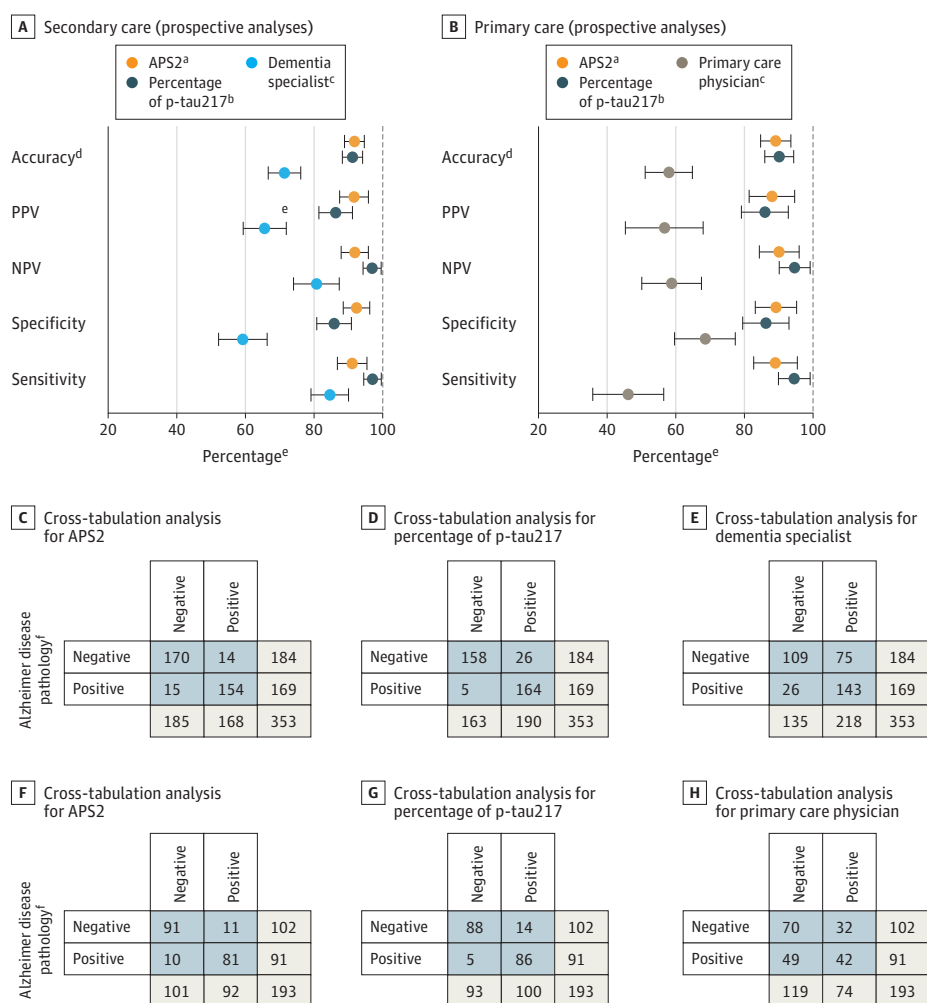
^gDefined as having cerebrospinal fluid-positive results for A β 42:A β 40 ratio (≤ 0.072)²⁰ and p-tau217 (>11.42 pg/mL).²³ For participants who could not undergo lumbar puncture, Alzheimer disease pathology was based on positron emission tomographic-positive results for A β .

accuracy of 58% (95% CI, 51%-65%) when Alzheimer disease pathology was used as an outcome, which was significantly lower than the diagnostic accuracy of the prospectively measured APS2 (89% [95% CI, 85%-94%]) and the percentage of p-tau217 alone (90% [95% CI, 86%-94%]) (Figure 3B). The mean certainty of the assessment by primary care physicians was a score of 5.8 (95% CI, 5.5-6.1) on a scale from 0 (very low)

to 10 (very high) for the level of diagnostic confidence. The results from the 2 cutoff-value approach appear in eFigure 3 in Supplement 1.

When clinical Alzheimer disease (based on a consensus diagnosis including cerebrospinal fluid analysis or PET) was used as an outcome, and when the estimation of clinical Alzheimer disease was made by dementia specialists (no biomarker data

Figure 3. Comparison Between the Diagnostic Performance of the Physicians and the Blood Tests Using the 1 Cutoff-Value Approach Along With Presence of Alzheimer Disease Pathology as an Outcome



Data are from a subpopulation of patients with available physician questionnaires. APS2 indicates amyloid probability score 2; NPV, negative predictive value; PPV, positive predictive value. Error bars indicate the 95% CIs. The detailed results for A-D appear in eTable 18 in Supplement 1.

Cross-tabulation analyses are shown for secondary care (prospective analyses) in C, D, and E and for primary care (prospective analyses) in F, G, and H. The comparisons between the diagnostic accuracy of physicians and the use of blood biomarkers with 2 cutoff values and with clinical Alzheimer disease as an outcome appear in eFigures 3-4 in Supplement 1.

^aThe percentage of plasma phosphorylated tau 217 (p-tau217) combined with the β -amyloid 42 and β -amyloid 40 (A β 42:A β 40) plasma ratio in a predefined logistic regression model. The cutoff value was 36.

^bThe ratio of plasma p-tau217 relative to non-p-tau217 multiplied by 100. The cutoff value was 3.26.

^cPhysicians were asked if they thought their patient has Alzheimer disease pathology in the brain after the standard investigation, but prior to seeing any Alzheimer disease biomarker result (plasma sample, cerebrospinal fluid, or positron emission tomographic [PET] scan).

^dCorrectly classified participants.

^ePercentage of accuracy, PPV, NPV, specificity, or sensitivity.

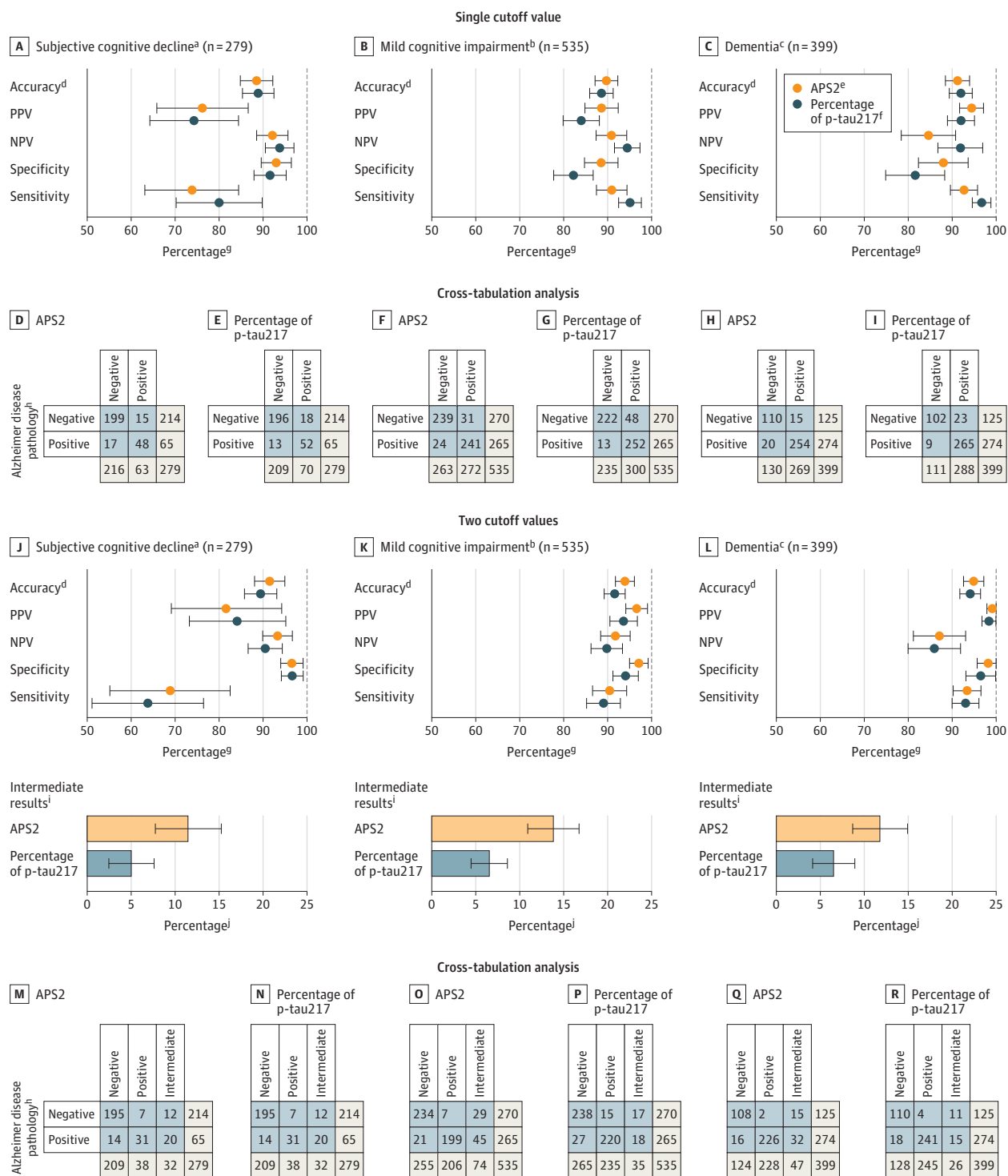
^fDefined as having cerebrospinal fluid-positive results for A β 42:A β 40 ratio (≤ 0.072)²⁰ and p-tau217 (>11.42 pg/mL).²³ For participants who could not undergo lumbar puncture, Alzheimer disease pathology was based on positive results for A β using PET.

were used), the diagnostic accuracy was 73% (95% CI, 68%-79%) in patients with mild cognitive impairment and dementia compared with 91% (95% CI, 88%-95%) for the APS2 and 91% (95% CI, 87%-94%) for the percentage of p-tau217 alone. For primary care physicians, the diagnostic accuracy was 61% (95% CI, 53%-69%) vs 91% (95% CI, 86%-96%) for the APS2 and was 91% (95% CI, 86%-95%) for the percentage of p-tau217 alone (eFigure 4 and eTable 4 in Supplement 1).

Additional Secondary Analyses

To evaluate the diagnostic accuracy of the tests across levels of cognitive severity (subjective cognitive decline, mild cognitive impairment, and dementia), the data were pooled from the secondary care cohort and the primary care cohort (Figure 4 and eFigure 5, eTables 5-6, and the eResults in Supplement 1 for comparisons between cognitive stages). When applying the 2 cutoff values, the diagnostic accuracy was significantly

Figure 4. Performance of the Blood Tests in Different Cognitive Stages in Pooled Data Along With Alzheimer Disease Pathology as an Outcome



Error bars indicate 95% CIs. The detailed results for A-C and J-L appear in eTables 19 and 20, respectively, in [Supplement 1](#).

^aDid not fulfill criteria for mild cognitive impairment or dementia.

^bCognitive symptoms and abnormal performance on cognitive testing.

^cClassified according to the *DSM-5*.²⁵

^dCorrectly classified participants.

^eThe percentage of plasma phosphorylated tau 217 (p-tau217) combined with

the β -amyloid 42 and 40 (A β 42:A β 40) plasma ratio (cutoff value: 36).

^fPlasma p-tau217 to non-p-tau217 $\times$ 100 (cutoff value: 3.26).

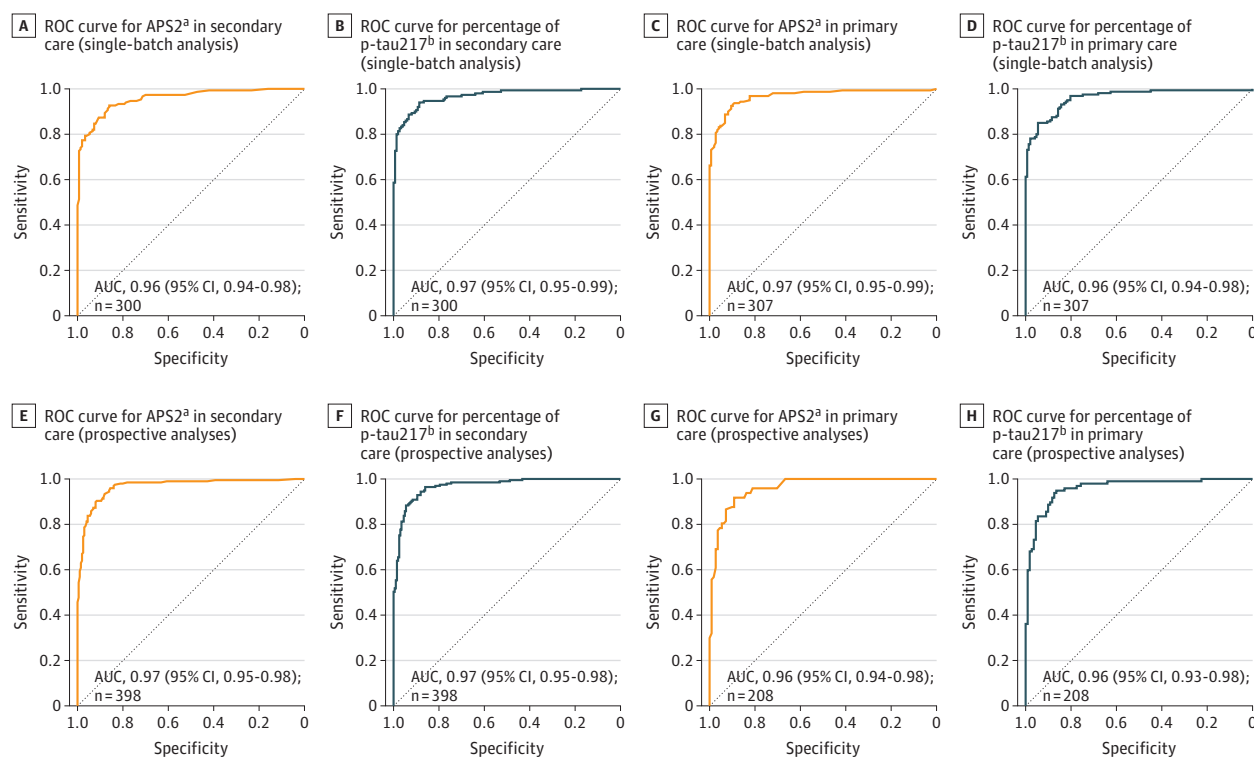
^gPercentage of accuracy, PPV, NPV, specificity, or sensitivity.

^hCerebrospinal fluid-positive results for A β 42:A β 40 ratio (≤ 0.072)²⁰ and p-tau217 (>11.42 pg/mL)²³ or based on positron emission tomographic results.

ⁱBetween the cutoff values (not included in diagnostic accuracy calculation).

^jPercentage of intermediate results using APS2 or percentage of p-tau217.

Figure 5. Receiver Operating Characteristic (ROC) Curve Analysis of the Blood Tests Along With Alzheimer Disease Pathology as an Outcome



Presence of Alzheimer disease pathology was defined as having cerebrospinal fluid–positive results for β -amyloid 42 and β -amyloid 40 ($A\beta_{42}:A\beta_{40}$) ratio (≤ 0.072)²⁰ and plasma phosphorylated tau 217 (p-tau217; >11.42 pg/mL).²³ For participants who could not undergo lumbar puncture, Alzheimer disease pathology was based on positron emission tomographic–positive results for $A\beta$. APS2 indicates amyloid probability score 2; AUC, area under the curve.

^aThe percentage of p-tau217 combined with the $A\beta_{42}:A\beta_{40}$ plasma ratio in a predefined logistic regression model.

^bThe ratio of plasma p-tau217 relative to non-p-tau217 multiplied by 100.

increased (as expected), and this was driven by the increased number of positive predictive values (eFigure 6 and eTable 7 in Supplement 1). Performance of the APS2 and the percentage of p-tau217 alone using clinically available cutoff values provided by C2N Diagnostics appear in eTable 8 in Supplement 1.

The results for the use of $A\beta_{42}:A\beta_{40}$ ratio compared with the APS2 and the percentage of p-tau217 alone are described in the eResults and appear in eTable 9 in Supplement 1. The AUC values for the APS2 and the percentage of p-tau217 alone (from the single-batch analysis and the prospective analyses) that were used in classifying Alzheimer disease pathology appear in Figure 5. The cerebrospinal fluid concentrations for the p-tau217 groups (negative, intermediate, or positive) appear in eFigure 7 in Supplement 1. The positive results for $A\beta$ PET appear in eFigure 8 in Supplement 1. Additional secondary outcomes appear in eTables 10-15 in Supplement 1 (clinical Alzheimer disease, $A\beta_{42}:A\beta_{40}$ ratio in cerebrospinal fluid, and $A\beta_{42}$ to p-tau181 ratio in cerebrospinal fluid). Details of the results visualized in Figures 1, 2, 3, and 4 appear in eTables 16-20 in Supplement 1. The correlations between the Alzheimer disease biomarkers analyzed in plasma and in cerebrospinal fluid appear in eFigure 9 in Supplement 1.

Discussion

In this study, we demonstrated that applying predefined blood biomarker cutoff values for the percentage of p-tau217 combined with $A\beta_{42}:A\beta_{40}$ ratio (the APS2) resulted in high diagnostic accuracy, positive predictive values, and negative predictive values for plasma samples collected from patients treated at primary and secondary care clinics and when using Alzheimer disease pathology as an outcome. Notably, the APS2 performed consistently in prospectively collected plasma samples analyzed biweekly, indicating the robustness of the assay performance.

Despite clear differences in patient demographics and clinical characteristics between the primary care and secondary care cohorts (Table), the blood biomarkers exhibited comparable performance in both contexts (Figures 1 and 2). Moreover, the diagnostic accuracy of the blood test surpassed that of dementia specialists, and especially primary care physicians, after a standard clinical evaluation that did not include collection of biomarker data, highlighting the potential of these blood biomarkers for improving the diagnostic accuracy when assessing patients with possible

Alzheimer disease (Figure 3 and eFigure 4 in [Supplement 1](#)). Importantly, the blood test performed accurately despite a relatively high rate of medical comorbidities, including kidney disease (26% in the primary care cohort).

The key novel methods used in this study include (1) the application of predefined cutoff values derived from an independent cohort, (2) use of prospectively analyzed plasma samples, and (3) validation of the diagnostic performance of blood biomarker data collected from a diverse cohort of patients treated in primary care. It is challenging to accurately identify Alzheimer disease in primary care (especially in patients with mild cognitive symptoms). The use of the APS2 and the percentage of p-tau217 alone demonstrated superior diagnostic accuracy (89%-90% for the APS2 and the percentage of p-tau217 alone with Alzheimer disease pathology as an outcome) compared with the diagnostic accuracy among primary care physicians (58%) using current diagnostic tools (Figure 3).

The improved diagnostic accuracy of the APS2 and percentage of p-tau217 alone was also evident when using clinical Alzheimer disease as an outcome (91% for both the APS2 and the percentage of p-tau217 alone) in patients with mild cognitive impairment or dementia compared with a diagnostic accuracy of 61% for primary care physicians (eFigure 4 in [Supplement 1](#)). The higher diagnostic accuracy of the blood test indicates that it could be suitable for implementation in primary care, but future studies need to examine its effect on clinical care. In addition to improving diagnostic accuracy, a positive test result could further support the initiation of widely available treatments (such as cholinesterase inhibitors). Even more importantly, it could aid in identifying potential candidates for timely antiamyloid treatment and who should be referred to secondary care.

In the secondary care cohort, dementia specialists correctly identified Alzheimer disease pathology in 71% of patients before reviewing cerebrospinal fluid test results (Figure 3), which is consistent with reports of a misdiagnosis rate between 25% and 30%.¹⁶ Use of blood biomarkers also had a higher rate of accuracy (91% for the blood test) than dementia specialists (73%) when using clinical Alzheimer disease as an outcome (eFigure 4 in [Supplement 1](#)), indicating the value of using the blood test at clinics in which Alzheimer disease biomarkers analyzed from cerebrospinal fluid (lumbar puncture) and PET are not readily available. The diagnostic accuracy of the blood tests is on par with FDA-cleared cerebrospinal fluid biomarkers,¹⁵ and because blood tests are more time-effective, cost-effective, and convenient for the patient, they could also potentially replace cerebrospinal fluid tests and PET.

For a blood biomarker to be used as a confirmatory test to detect Alzheimer disease pathology, a very high positive predictive value is crucial, especially before the initiation of antiamyloid treatment. The 2 cutoff-value approach (also used for 1 of the 2 FDA-cleared cerebrospinal fluid tests²⁰), achieved positive predictive values of 97% to 99% in patients with cognitive impairment (Figure 4), which is the target population of currently available antiamyloid treatments.^{5-7,16} Although negative predictive values (87%-92% using the APS2) were

slightly lower in patients with cognitive impairment (Figure 4 and eFigure 5 and eTables 5-6 in [Supplement 1](#)), we argue that a very high positive predictive value is probably more important in diagnosing patients as having Alzheimer disease, especially before initiating costly and burdensome antiamyloid treatment.^{7,22,24,33}

Importantly, even in places with limited access to these new therapies, an accurate, biomarker-verified Alzheimer disease diagnosis can have a positive effect on clinical care⁴ and prognostication.³⁴ The positive predictive values were suboptimal for accurate identification of Alzheimer disease pathology in patients at the subjective cognitive decline stage—regardless of the cutoff value approach used (Figure 4), which could be a disadvantage for clinical trials including patients with presymptomatic Alzheimer disease, but not in clinical practice because there are no clinical criteria for diagnosing Alzheimer disease at the subjective cognitive decline stage.²⁴ On the other hand, the negative predictive values were higher in patients with subjective cognitive decline (91%-94% for the APS2 or the percentage of p-tau217 alone, regardless of cutoff value approach used). This indicates that the blood test would be more useful for ruling out underlying Alzheimer disease when only subtle symptoms are present.

Even though the APS2 and the percentage of p-tau217 alone showed similarly high diagnostic accuracy for identifying clinical Alzheimer disease (eTables 10-11 and eFigure 4 in [Supplement 1](#)) as Alzheimer disease pathology (eTable 9 in [Supplement 1](#)), it is crucial to emphasize that a biomarker for Alzheimer disease pathology, however accurate, should not serve as a standalone diagnostic test for Alzheimer disease but must be interpreted in a clinical context. This is important because Alzheimer disease pathology can be asymptomatic for many years,³⁵ and cognitive symptoms in some patients with Alzheimer disease pathology can be primarily caused by other conditions. Incorrect interpretation of a positive Alzheimer disease biomarker could thus lead to underdiagnosis of relatively common non-Alzheimer disease conditions (such as limbic-predominant age-related TDP-43 encephalopathy³⁶).

Both the APS2 and the percentage of p-tau217 alone showed robust test performance in the current analysis. The results were very similar between these biomarkers using Alzheimer disease pathology as an outcome in both the secondary care and primary care cohorts (eTable 9 in [Supplement 1](#)). In participants with subjective cognitive decline or mild cognitive impairment, the APS2 demonstrated significantly higher diagnostic accuracy with the use of 2 cutoff values rather than only 1, although this approach resulted in a higher number of intermediate results compared with the percentage of p-tau217 alone (Figure 4 and eFigure 6 in [Supplement 1](#)). Participants with intermediate results are not as straightforward to manage in clinical practice. In secondary care, these patients may be candidates for further biomarker examination using cerebrospinal fluid tests or PET. Future research should explore the optimal workflow in primary care. Depending on the level of suspicion for Alzheimer disease and local or regional guidelines, these patients might be appropriate for referral to secondary care or for a repeat blood test in primary care.

Limitations

There are some limitations to this study. First, the results proved robust across settings and plasma analysis designs, but validation in cohorts from other countries is essential, especially in cohorts that may have a lower prevalence of amyloid positivity and in primary care (where the performance of Alzheimer disease blood biomarkers is less known).¹⁶

Second, future studies should also evaluate fully automated immunoassays that may be more practical for implementation at local clinical chemistry laboratories. Currently, mass spectrometry assays might have drawbacks (such as higher costs and requirement of high technical expertise).

Third, we used the ratio of p-tau217 to non-p-tau217 (percentage of p-tau217) because the results from a prior study¹² showed the percentage of p-tau217 can mitigate the effect of non-Alzheimer disease-related comorbid conditions (such as chronic kidney disease) on p-tau217. However, the

current study did not further examine the potential advantage of percentage of p-tau217 vs p-tau217 or if there are specific settings or subgroups for which percentage of p-tau217 is especially useful. It would be of value to address the comparison of percentage of p-tau217 vs p-tau217 in future studies, especially because other assays only measure p-tau217 alone.

Conclusions

The APS2 and percentage of p-tau217 alone had high diagnostic accuracy for identifying Alzheimer disease among individuals with cognitive symptoms in primary and secondary care using predefined cutoff values. Future studies should evaluate how the use of blood tests for these biomarkers influences clinical care.

ARTICLE INFORMATION

Accepted for Publication: June 27, 2024.

Published Online: July 28, 2024.

doi:10.1001/jama.2024.13855

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Author Contributions: Dr Palmqvist had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Acquisition, analysis, or interpretation of data: All authors.

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Statistical analysis: Palmqvist, Mattsson-Carlsson, West, Salvadó.

Obtained funding: Palmqvist, Mattsson-Carlsson, Ossenkoppele, Blennow, Salvadó, Hansson.

Administrative, technical, or material support: Palmqvist, Schindler, Smith, Calling, Verghese, Braunstein, Hansson.

Supervision: Palmqvist, West, Braunstein, Blennow, Hansson.

Conflict of Interest Disclosures: Dr Palmqvist reported receiving institutional research support from ki Elements, Alzheimer's Drug Discovery Foundation, and Avid Radiopharmaceuticals and receiving consultancy or speaker fees from Biogen, Biogen, Eisai, Eli Lilly, and Roche. Dr Schindler reported receiving personal fees from Eisai for serving on advisory boards and that her institution (Washington University) has a partial ownership interest in C2N Diagnostics; however, she has no personal financial involvement with C2N Diagnostics and will not receive any compensation. Dr Smith reported receiving personal fees from Hoffmann-La Roche Ltd. Dr Ossenkoppele reported receiving institutional research support from the European Research Council, ZonMw, Nederlandse Organisatie voor Wetenschappelijk Onderzoek, the National Institutes of Health, the Alzheimer Association, Alzheimer Nederland, Stichting Dioraphte, the Cure Alzheimer's Fund, Health Holland, ERA PerMed, Alzheimerfond, Hjärnfonden, Avid Radiopharmaceuticals, Janssen, Roche, Quantex, and Optina Diagnostics; receiving speaking fees from GE Healthcare; and serving as an advisory board member for Asceneuron and as a steering committee member for Bristol Myers Squibb. Drs West, Monane, Verghese, and Braunstein reported being salaried employees or consultants for C2N Diagnostics and receiving compensation from the company in the form of a salary or equity. Dr Blennow reported serving as a consultant or on advisory boards for AbbVie, AC Immune, Acumen, ALZPath, AriBio, BioArctic, Biogen, Eisai, Lilly, Moleac Pte Ltd, Neurimmune, Novartis, Ono Pharma, Prothena, Roche Diagnostics, and Siemens Healthineers; serving on data monitoring committees for Julius Clinical and Novartis; receiving speaking fees for lectures, educational materials, or educational programs from AC Immune, Biogen, Celdara Medical, Eisai, and Roche Diagnostics; and being a

cofounder of Brain Biomarker Solutions, which is a part of the GU Ventures Incubator Program. Dr Hansson reported receiving personal fees from AC Immune, Alzpath, BioArctic, Biogen, Cerveau, Eisai, Eli Lilly, Fujirebio, Roche, Bristol Myers Squibb, Merck, Novartis, Novo Nordisk, Roche, Sanofi, and Siemens and receiving institutional research support from ADx, AVID Radiopharmaceuticals, Biogen, Eli Lilly, Eisai, Fujirebio, GE Healthcare, Pfizer, and Roche. No other disclosures were reported.

Funding/Support: The study was supported by grant R01AG083740 from the National Institute on Aging; grants SG-23-1061717 and ZEN24-1069572 from the Alzheimer's Association; funding from the GHR Foundation; grant ADG-101096455 from the European Research Council; grants 2022-00775, 2021-02219, and 2018-02052 from the Swedish Research Council; grant 2021-184 from ERA PerMed; grant 2022-0231 from the Knut and Alice Wallenberg Foundation; funding from the Strategic Research Area MultiPark at Lund University (a multidisciplinary research collaborative for Parkinson disease); grants AF-994229, AF-980942, AF-980907, and AF-981132 from the Swedish Alzheimer Foundation; grants FO2023-0163, FO2022-0204, and FO2021-0293 from the Swedish Brain Foundation; grants FRS-0003 and FRS-0004 from the Family Rönström's Foundation; grant 1412/22 from the Parkinson Foundation of Sweden; funding from the Cure Alzheimer's Fund; funding from the Konung Gustaf V:s och Drottning Victorias Frimurarestiftelse; grant 2020-0000028 from the Skåne University Hospital Foundation; grant 2019-03401 from the EU Joint Programme Neurodegenerative Diseases; grant 22-066 from the Wallenberg AI, Autonomous Systems, and Software Program and the SciLifeLab and Wallenberg national program for Data-Driven Life Science joint call for research projects; grant 2022-1259 from the Regionalt Forskningsstöd; agreements 2022-Projekt0107 and 2022-Projekt0080 from the Swedish federal government; Marie Skłodowska-Curie action grant 101061836 from the European Union's Horizon 2020 research and innovation program; grant AARF-22-972612 from the Alzheimer's Association research fellowship; and funding from GE Healthcare for the precursor [¹⁸F]flutemetamol. The

study was carried out as an academic collaboration between Lund University and C2N Diagnostics.

Role of the Funder/Sponsor: The funders/sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. Lund University and its affiliated researchers did not receive funding or compensation from C2N Diagnostics. C2N Diagnostics performed the plasma analyses while blinded to any biomarker or clinical data and had no role in the statistical analysis or results.

Meeting Presentation: Presented in part at the Alzheimer's Association International Conference; July 28, 2024; Philadelphia, Pennsylvania.

Data Sharing Statement: See [Supplement 2](#).

Additional Contributions: We acknowledge the following employees of C2N Diagnostics: Venky Venkatesh, PhD (for samples logistics and C2N data sharing), Matt Meyer, PhD (for plasma test development), Kris Kirmess, PhD (for samples analysis and data acquisition), Justine Coppinger, MS (for critical review of the manuscript), and Stephanie Eastwood, PhD and Mary Holubasch, MT (for plasma analysis). We also acknowledge the following individuals at Lund University: Ayesha Fawad, MD, and Tove Freiburghaus, MD (for assessing and extracting comorbidities from medical records), Divya Bali, MSc, and Anna Orduña Dolado, MSc (for cerebrospinal fluid analysis), Antoine Leuzy, PhD, and Olof Strandberg, PhD (for processing ¹⁸F)flutemetamol scans), Erik Nilsson, MD, and Lisa Bjartmar, MD (for clinical assessments), Lyduine Collij, PhD (for reading ¹⁸F)flutemetamol scans), Danielle van Westen, MD (for assessing computed tomographic and magnetic resonance imaging scans), and Patrik Midlöv, MD (for study support). We also acknowledge the following individual at Sahlgrenska University Hospital: Hayder Shweliyya, BS (for cerebrospinal fluid analysis). None of these individuals received special compensation beyond their standard salaries.

Additional Information: During the preparation of this work, the authors used ChatGPT (OpenAI) to improve the readability of certain parts of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content included in this publication.

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