ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Cerebrospinal Fluid p-Tau181/Amyloid Beta 42 Ratio Identifies Lymphatic-Venous Anastomosis Patients Who Respond to and Benefit from the Surgery for Relief of Cognitive Impairment with a Diagnostic Accuracy of 0.744

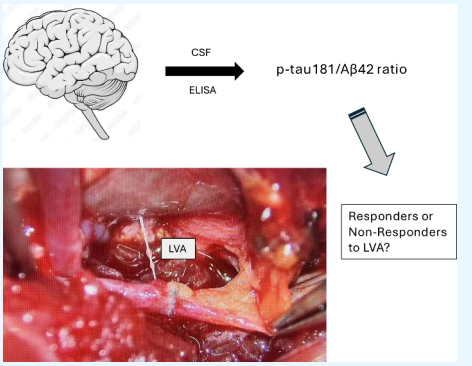
Guangqi Xu,[#] Qinghua Zhang,[#] Yinan Li,[#] Anqi Du,[#] Xiaona Xu, Ruyu Bai, Chao Bi, Yanxia Li, Tailun Zhang, Zhongfa Zhang, Ivan K. Chu, K. W. Michael Siu,* and Zhaoyan Yu*

Cite This: *ACS Omega* 2025, 10, 32029−32037

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Responders (R) and Non-Responders (NR) to lymphatic-venous anastomosis (LVA) surgery for relief of cognitive impairment show significant difference in their p-tau181/Aβ42 ratios via enzyme-linked immunosorbent assay (ELISA) of their cerebrospinal fluid (CSF). LVA involves the conjugation of a lymph vessel to a small vein in the neck to facilitate lymph flow. The R and NR groups were classified using five standard cognitive assessment tools: Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Alzheimer's Disease Assessment Scale − Cognitive subscale (ADAS-Cog), Activities of Daily Living (ADL) and Clinical Dementia Rating (CDR), and augmented by clinical assessments. ELISA results for 43 patients were available. Welch's *t* test and Mann−Whitney U-test revealed significant difference between R and NR's p-tau181 value and p-tau181/Aβ42 ratio. Using a p-tau181/Aβ42 cutpoint of 0.0923 allows patient selection for LVA with a specificity of 0.800, sensitivity of 0.667, positive predictive value (PPV) of 0.706, and negative predictive value (NPV) of 0.769. The diagnostic accuracy is 0.744. The receiver operating characteristic (ROC) curve gives an area under the curve (AUC) of 0.793, which compares well with those of biomarker tests currently in use: prostate specific antigen (PSA), Straticyte and Oncotype DX. The p-tau181/Aβ42 ratio could serve to predict LVA's effectiveness in alleviating AD patients' symptoms and to rule-in patients for LVA, once validation from a larger patient cohort is available.



INTRODUCTION

Lymphatic-venous anastomosis (LVA) is an experimental surgical therapeutic modality for alleviating symptoms of Alzheimer's disease (AD).[1,2] The Shandong Public Health Clinical Center Affiliated to Shandong University, a large provincial hospital in Shandong, China, began offering LVA on a provisional basis in November, 2024 to treat patients exhibiting clinical symptoms that are consistent with Stages 4−6 in the AD continuum as defined by the U.S. National Institute on Aging and the Alzheimer's Association (US NIA-AA).[3] Of the first cohort of 43 LVA patients documented with cerebrospinal fluid (CSF) AD biomarkers—amyloid beta 42 (Aβ42), Aβ42/40, p-tau181 and t-tau—via enzyme-linked immunosorbent assay (ELISA), 18 patients (41.9%) exhibited post-LVA cognitive improvement as judged by test scores in Chinese versions of five internationally recognized cognitive tests[4−8] and augmented by observations of three clinicians based on pre- and post-LVA testing and interviews. The US NIA-AA designates p-tau181 as a Core 1 biomarker for CSF analysis, and that the p-tau181/Aβ42 ratio as a diagnostic parameter in CSF assays.[3] A p-tau181/Aβ42 ratio of ∼0.1 and beyond is typically considered to be indicative of AD.[9,10]

In LVA, a lymph vessel is intimately conjugated (anastomosed) to a small vein to facilitate the lymph flow. It is most often practiced to relieve lymphedema of the extremities.[11] LVA has also been utilized for the management of head and neck lymphedema.[12] Alzheimer's disease is associated with plaques of amyloid and tau oligopeptdes; it has been postulated that LVA can offer the patient relief, as it provides additional pathways for clearance.[1,2] This hypothesis is contentious. However, there have been demonstrated cases of significant cognitive improvement postsurgery. Xie et al.[1] first reported the application of LVA on dementia patients. Recently, Li et al.[2] employed the "cervical shunting to unclog cerebral lymphatic systems"

Received: April 21, 2025
Revised: June 28, 2025
Accepted: July 2, 2025
Published: July 16, 2025

Table 1. Patients' Demographics, Outcomes of Cognitive Assessment, and Biomarker Values[a]

Patient ID	Age	Sex	Marital St	R or NR	Aβ42 (pg/mL)	Aβ42/40	p-tau181 (pg/mL)	p-tau181/Aβ42	t-tau (pg/mL)
# 2	71	F	M	NR	701	0.137	15	0.021	1132.6
# 6	56	F	M	NR	NA	NA	NA	NA	NA
# 7	65	F	M	NR	583.8	0.043	38.8	0.066	1634.3
# 8	70	F	M	NR	646.4	0.053	41.1	0.064	1291.1
# 12	84	F	M	NR	249.8	0.039	54.1	0.217	1339.9
# 13	65	M	M	NR	455.8	0.066	18.8	0.041	857.9
# 16	75	M	M	NR	228.4	0.04	21	0.092	363.5
# 17	72	F	M	NR	165.2	0.047	15	0.091	259.3
# 18	59	M	M	NR	373.5	0.053	33.7	0.09	623.5
# 21	74	F	M	NR	825.4	0.093	15	0.018	522.8
# 23	73	M	M	NR	482.8	0.095	15	0.031	588.9
# 24	67	M	M	NR	626.2	0.071	15	0.024	562.4
# 25	77	F	M	NR	414.6	0.049	38.1	0.092	453.8
# 28	61	M	M	NR	254.6	0.04	17.3	0.068	914.5
# 29	65	F	M	NR	345.2	0.049	38	0.11	1046
# 34	70	F	M	NR	NA	NA	NA	NA	NA
# 36	61	F	M	NR	585.7	0.048	24.7	0.042	1443.7
# 37	73	F	M	NR	222.2	0.047	18.1	0.081	198.2
# 41	72	F	M	NR	237.1	0.037	23.1	0.097	1138.5
# 47	67	F	M	NR	556.7	0.042	86.7	0.156	830.3
# 1	73	F	M	NR	918.9	0.053	64.1	0.07	1426.3
# 5	69	F	M	NR	604.9	0.097	24.9	0.041	1002.2
# 15	67	M	M	NR	258.6	0.063	22.3	0.086	259.3
# 33	60	M	M	NR	723.6	0.081	15	0.021	567.2
# 43	65	F	M	NR	583.8	0.043	38.8	0.066	1634.3
# 44	76	F	M	NR	275.5	0.042	16.1	0.058	764.3
# 45	62	F	M	NR	272.8	0.049	63.9	0.234	564.7
# 3	78	F	M	R	875.9	0.077	64	0.073	1996
# 4	78	F	M	R	342	0.06	30.5	0.089	1400.6
# 9	59	M	M	R	434.4	0.044	89	0.205	628.4
# 10	74	F	M	R	410.8	0.057	30.8	0.075	1150.3
# 11	71	M	M	R	353.7	0.079	21.2	0.06	524.1
# 14	66	F	M	R	499	0.057	74.2	0.149	536.7
# 19	61	F	M	R	NA	NA	NA	NA	NA
# 20	75	M	M	R	282.1	0.048	28.2	0.1	593.6
# 22	80	F	M	R	382.5	0.046	35.3	0.092	940.4
# 26	75	M	M	R	399.3	0.039	51.4	0.129	1864.2
# 27	67	F	M	R	472.5	0.03	53.4	0.113	1523.6
# 30	76	F	M	R	427.1	0.044	35.1	0.082	311.1
# 31	81	F	M	R	354.3	0.037	97.5	0.275	953.5
# 32	62	F	M	R	478.1	0.046	61.7	0.129	888.8
# 35	72	F	M	R	352.5	0.061	57.9	0.164	1344.8
# 38	67	F	M	R	260.5	0.03	26.6	0.102	529.1
# 39	67	M	M	R	193.8	0.042	47.5	0.245	1102.9
# 40	67	F	M	R	297.5	0.071	56.1	0.189	1159.3
# 42	69	F	M	R	428.3	0.06	44	0.103	1000.6
# 46	79	M	M	R	NA	NA	NA	NA	NA

[a]Legend. Sex: F = female; M = male. Marital status: M = married. R = Responders; NR = Non-Responders. Biomarker concentration in pg/mL; ratio is dimensionless NA = not available.

(CUCLS) procedure to treat AD patients specifically. Li stated that CUCLS differed from Xie's procedure both operationally as well as locationally, and these CUCLS-treated patients met the biological diagnostic criteria for AD.[2] It should be noted that in LVA the specific number of anastomosis junctions is operationally dependent on the specific patient undergoing surgery. The glymphatic and meningeal lymphatic systems are credited to play a major role in removal of potentially harmful proteins and peptides from the brain to the bloodstream for detoxification by the liver.[13−17] Blockage of this egress results in the accumulation of Aβ and tau protein in the brain and the formation of plaques and tangles.[13−18] Sleep is believed to play a significant role in enhanced clearance of undesirable proteins and metabolites from the brain; the lack of sleep, as a consequence of neurological disorders, exacerbates the deleterious outcomes.[18,19] This accelerated rate of removal during sleep, however, has recently been challenged.[20]

Herein we report our findings of LVA as a surgical treatment to relieve cognitive symptoms that are associated with AD. LVA is considered a minimally invasive procedure performed under

https://doi.org/10.1021/acsomega.5c03650
ACS Omega 2025, 10, 32029−32037

Table 2. Statistical Analyses of the Biomarker Values for Responders and Nonesponders[a]

Biomarker	NR mean ± SD n = 18	R mean ± SD n = 25	Welch's t (p)	MW U (p)	S/NS (α = 0.05) Welch's, MW
Aβ42 (pg/mL)	463.7000 ± 211.7935	402.4611 ± 142.8509	1.1317 (0.2643)	252.0 (0.5141)	(NS, NS)
Aβ42/40	0.0591 ± 0.0243	0.0516 ± 0.0146	1.2617 (0.2144)	256.0 (0.4523)	(NS, NS)
p-tau181 (pg/mL)	30.9440 ± 19.0617	50.2444 ± 21.5425	3.0397 (0.0045)	100.0 (0.0021)	(S, S)
p-tau181/Aβ42	0.0791 ± 0.0548	0.1319 ± 0.0614	2.9078 (0.0064)	93.0 (0.0012)	(S, S)
t-tau (pg/mL)	856.7800 ± 436.4574	1024.8889 ± 472.7001	1.1877 (0.2430)	179.0 (0.2626)	(NS, NS)

[a]NR = Non-Responders. R = Responders. SD = Standard Deviation. p = Probability value. MW = Mann−Whitney. S = Significant difference. NS = No significant difference.

microscopy.[1,2,21] We deem that after 47 operations over a period of approximately four months, this is now the appropriate time to share and discuss our initial results to support and inform research by others, given the extent of interests shown in some countries in the developing world (a news article in China reported >180 hospitals in China are currently engaging in LVA surgery) and that diversity in patient populations beyond Caucasians is desirable for tackling global challenges. We found that the p-tau181/Aβ42 ratio, as determined in the pre-LVA CSF biomarker assays, correlates with the clinical assignment of patient outcomes as Responders (R, those who showed post-LVA cognitive improvements) and Non-Responders (NR, those who did not). Once validated with a larger patient cohort, we deem adoption of this test to rule-in patients for LVA will optimize the success rate and ensure that precious surgical and therapeutic resources are allocated to patients who are most likely to benefit.

RESULTS

Patients, Enrollment, Surgery and Care. Patients elected or were enrolled by their guardians (spouses or adult children) to go through the LVA procedure. The participation and surgery were overseen by the hospital's ethics board, which had approved LVA as an experimental procedure provided on a provisional basis with continual assessment by the board (Approval number: GWLCZXEC2024-160). Patients admitted had to meet clinical staging within Stages 4−6, as defined by the US NIA-AA,[3] and had to fall within the age group of 50−89. They would have the experimental nature of LVA explained and would have been informed that success was far from guaranteed. They also had to meet anesthesia assessment for the hours long surgery, which is classified as minimally invasive. CSF was collected typically 1 day prior to surgery; the concentrations of the following antigens—Aβ42, Aβ40, tau and p-tau181—were assayed using standard sandwich enzyme-linked immunosorbent assay.[22] Standard cognitive tests were also conducted (see later).

LVA involves anastomosis of the internal jugular vein with deep cervical lymphatic vessels. An approximately 5 cm incision was made in the middle-to-lower third of the sternocleidomastoid muscle on each side of the neck. Several branches of the deep cervical lymphatic vessels were identified around the sternocleidomastoid muscle and were connected end-to-end with branches of the internal jugular vein. Postsurgery, the patients were housed in a specific unit of the hospital, where they were monitored and cared for on a continual basis by a dedicated medical team. Standard cognitive tests were repeated to gauge significant changes in cognitive functions. Most patients recovered sufficiently within 1 week to allow for suture removal and then discharge. These patients were followed up by phone or by in-person visits. No significant adverse effects of LVA were observed or reported. CSF ELISA records for 43 of the 47 LVA patients are documented; patients' demographic, molecular and clinical characteristics are also available (Table 1). Of the 47 LVA patients, 14 were males (68.1 ± 6.7 years old) and 33 females (70.3 ± 6.5 years old). The male/female ratio is a reflection of the rates at which patients sought treatment at the LVA clinic.

Cognitive Assessment of LVA Patients. The cognitive functioning of each patient pre- and post-LVA was evaluated by means of the Chinese versions of Mini-Mental State Examination (MMSE),[4] Montreal Cognitive Assessment (MoCA),[5] Alzheimer's Disease Assessment Scale − Cognitive subscale (ADAS-Cog),[6] Activities of Daily Living (ADL)[7] and Clinical Dementia Rating (CDR);[8] each of these tests measured different aspects of cognition. These standard tests were augmented by independent assessments from three physicians (Supplementary Table 1). AD research protocols typically use multiple cognitive tests to capture different outcome domains and with augmentation by clinical judgment.[23,24] In our study, if two or more of the five cognitive tests of a given patient showed post-LVA improvement, that patient was considered a Responder (having shown improvement after LVA). For patients showing improvement in only one cognitive test and scoring 7 or above in the total clinicians' assessment, they were also considered Responders. All other patients were considered to be Non-Responders. A total of 35 patients were classified as Responders or Non-Responders based solely on their cognitive assessments (having two or more of the tests showing post-LVA improvement), which were judged to be less subjective than clinicians' assessments. Of the 12 patients whose cognitive scores were 1 (showing improvement in only one test), clinicians' assessments classified five as R and seven as NR (see Supplementary Table 1 for details). The total number of Responders thus identified is 20 and that of Non-Responders is 27.

Statistical Tests for Significant Differences between Non-Responders and Responders. We have documented ELISA results of 43 out of the first cohort of 47 LVA patients. The five biomarker values and ratios between the two groups—NR and R—were compared in an examination of significance differences between the two. The results are shown in Table 2. We employed two statistical tests: (1) Welch's t test[25] is a test used to determine whether the means of a given NR and R biomarker set are significantly different from each other; Welch's t test differs from the better-known Student t test in that the former does not assume the NR and R groups have equal variances. (2) Mann−Whitney U test[26] compares the medians of an NR and R biomarker set; there is no assumption of normal distribution of the biomarker values. It is readily apparent from Table 2 that of the five biomarker values or ratios examined, only the p-tau181 concentration itself and the p-tau181/Aβ42 ratio

https://doi.org/10.1021/acsomega.5c03650
ACS Omega 2025, 10, 32029−32037

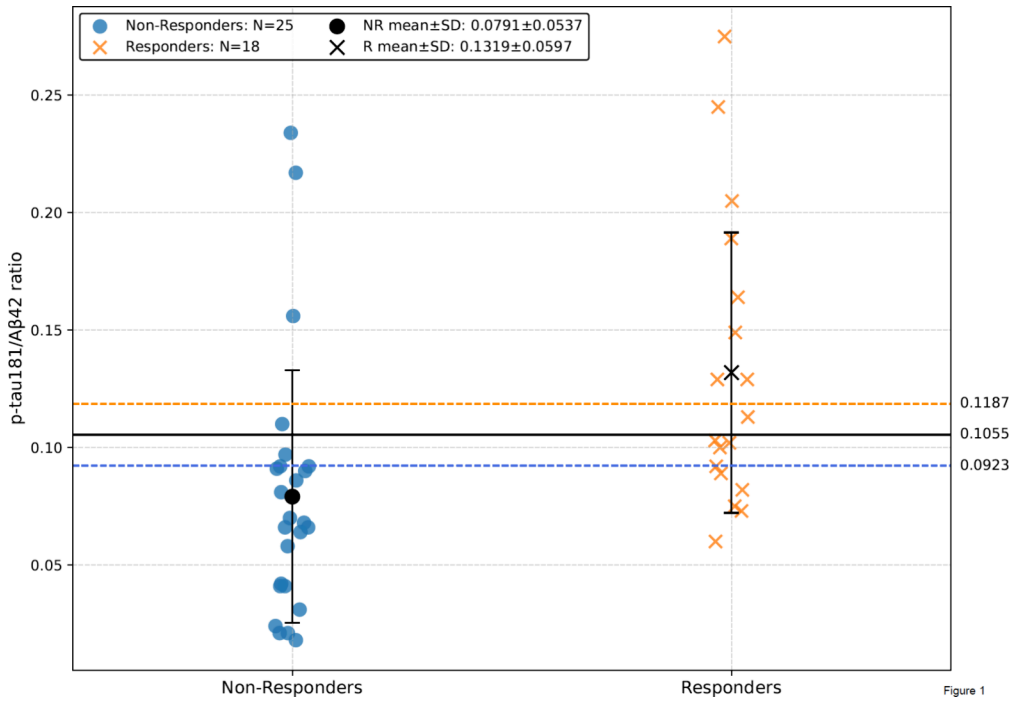


Figure 1. p-tau181/Aβ42 biomarker ratios between Responders and Non-Responders and cutpoints in biomarker testing.

of the NR and R groups show any significant difference and that the same findings are evident in both statistical tests. Neither the Aβ42 concentrations alone, nor the Aβ42/40 ratios, differ significantly between the two groups.

p-tau181/Aβ42 Ratio as a Parameter to Select Patients for LVA. Table 2 shows that the Non-Responders have a mean p-tau181/Aβ42 ratio of 0.0791, whereas the Responders have a mean ratio of 0.1319. We envision that a reasonable starting point in evaluating the discriminating ability of the p-tau181/Aβ42 ratio to delineate Responders from Non-Responders would be the midpoint between the two means, or (0.0791 + 0.1319)/2 = 0.1055. Employing this cutpoint of 0.1055 leads to the following biomarker test figures of merit: TN = 21 (correctly identified as NR), FP = 4 (incorrectly identified as R), TP = 9 (correctly identified as R), and FN = 9 (incorrectly identified as NR) (see Figure 1 and Table 1). These mean that we have the following performance metrics:

1. Sensitivity (True Positive Ratio) = TP/(TP + FN) = 9/(9 + 9) = 0.500 or 50.0%
2. Specificity (True Negative Ratio) = TN/(TN + FP) = 21/(21 + 4) = 0.840 or 84.0%
3. PPV (Positive Predictive Value) = TP/(TP + FP) = 9/(9 + 4) = 0.692 or 69.2%
4. NPV (Negative Predictive Value) = TN/(TN + FN) = 21/(21 + 9) = 0.700 or 70.0%

We envision that a biomarker test, such as the current test employing the p-tau181/Aβ42 ratio, which has a high specificity and high PPV, would be useful in cases where a test will help to rule-in patients for surgery or treatment that is resource intensive.

Consequence of Adopting Higher or Lower Cutpoints. Picking cutpoints above or below the midpoint of 0.1055 for the p-tau181/Aβ42 ratio test leads to predictable consequences in the change of sensitivity, specificity, PPV and NPV. Figure 1 illustrates the changes in TN, FP, TP and FN for a low cutpoint of 0.0923 (one-fourth between the means of R and NR), as well as for a high cutpoint of 0.1187 (three-fourths between the two means). For the low cutpoint of 0.0923, here are the performance metrics:

1. Sensitivity (True Positive Ratio) = TP/(TP + FN) = 12/(12 + 6) = 0.667 or 66.7%
2. Specificity (True Negative Ratio) = TN/(TN + FP) = 20/(20 + 5) = 0.800 or 80.0%
3. PPV (Positive Predictive Value) = TP/(TP + FP) = 12/(12 + 5) = 0.706 or 70.6%
4. NPV (Negative Predictive Value) = TN/(TN + FN) = 20/(20 + 6) = 0.769 or 76.9%

And for the high cutpoint of 0.1187:

1. Sensitivity (True Positive Ratio) = TP/(TP + FN) = 8/(8 + 10) = 0.444 or 44.4%
2. Specificity (True Negative Ratio) = TN/(TN + FP) = 22/(22 + 3) = 0.880 or 88.0%
3. PPV (Positive Predictive Value) = TP/(TP + FP) = 8/(8 + 3) = 0.727 or 72.7%
4. NPV (Negative Predictive Value) = TN/(TN + FN) = 22/(22 + 10) = 0.688 or 68.8%

These results are in accordance with the general expectation that adopting a lower cutpoint increases the sensitivity but decreases the specificity of the test, while picking a higher cutpoint decreases the sensitivity but increases the specificity. Selection of the optimal cutpoint for a given test will need to fit the purpose or design of the test (see Discussion).

It is noteworthy that a p-tau181/Aβ42 ratio of ~0.1 is indicative of AD.[9,10] This means that there is a correlation between the effectiveness of LVA and the presence of AD in LVA patients.

p-Tau's Performance as a Biomarker. A similar evaluation can also be performed on p-tau181 alone as a biomarker in discriminating Responders from Non-Responders. Employing the midpoint between the two means, or (30.944 + 50.244)/2 pg/mL = 40.594 pg/mL as the cut point, leads to the

https://doi.org/10.1021/acsomega.5c03650
ACS Omega 2025, 10, 32029−32037

following biomarker test figures of merit: TN = 12, FP = 6, TP = 14, and FN = 11. These figures then give the following performance metrics:

1. Sensitivity (True Positive Ratio) = TP/(TP + FN) = 11/(11 + 7) = 0.611 or 61.1%
2. Specificity (True Negative Ratio) = TN/(TN + FP) = 20/(20 + 5) = 0.800 or 80.0%
3. PPV (Positive Predictive Value) = TP/(TP + FP) = 11/(11 + 5) = 0.688 or 68.8%
4. NPV (Negative Predictive Value) = TN/(TN + FN) = 20/(20 + 7) = 0.741 or 74.1%

These metrics are comparable to those of p-tau181/Aβ42. However, as US NIA-AA designates the assay of the latter as diagnostic of AD (and not p-tau181alone), we will move our investigation forward with the p-tau181/Aβ42 ratio.

Performance Evaluation: Splitting the LVA Patients into a 2/3rd Training Set and a 1/3rd Testing Set. Table 3

Table 3. 2/3rd to 1/3rd Random Splitting of LVA Patients into a Training and a Testing Group, Respectively

	10 random splits		100 random splits		1000 random splits	
	Mean	SD	Mean	SD	Mean	SD
Training Sensitivity	0.542	0.056	0.554	0.079	0.545	0.084
Training Specificity	0.844	0.050	0.831	0.061	0.843	0.053
Training PPV	0.725	0.074	0.715	0.080	0.724	0.083
Training NPV	0.711	0.033	0.714	0.042	0.713	0.044
Testing Sensitivity	0.617	0.224	0.558	0.223	0.542	0.215
Testing Specificity	0.811	0.122	0.846	0.128	0.842	0.116
Testing PPV	0.705	0.138	0.738	0.175	0.712	0.185
Testing NPV	0.780	0.119	0.757	0.103	0.746	0.097

shows the outcome of randomly splitting the LVA patient p-tau181/Aβ42 ratios 10, 100 and 1,000 times, and using the 2/3rd training set to select the cutpoint (the mean between the R and the NR groups) to calculate the performance metrics of the 1/3rd testing set in a simulation of a prospective study. It is apparent that the average sensitivity, specificity, PPV and NPV of the larger training set are comparable to their equivalents in the complete set and that the % SDs are comparable across the 10, 100 and 1,000 splits at ~9.1%. The mean performance metrics of the testing set are comparable to those of the larger training set although the % SDs of the former at ~22.6% are larger. We would expect that % SD of the testing set to decrease on increasing the absolute number of LVA patients. These results support adoption of the p-tau181/Aβ42 ratio to predict LVA outcomes on a prospective basis.

Receiver Operating Characteristics (ROC). Figure 2a shows a plot of sensitivity (true positive ratio) versus (1 − specificity) (false positive ratio) for the p-tau181/Aβ42 biomarker ratio test at different cutpoints in the form of a receiver operating characteristic curve. The area under the curve (AUC) is ~0.793, which signifies that this biomarker test has acceptable performance on the cusp of being excellent. In practice, this means that a randomly selected Responder will have a higher p-tau181/Aβ42 ratio than that of a randomly selected Non-Responder ~79% of the time. Bootstrap resampling[27−29] of the data 1,000 times to simulate performance in a larger patient cohort leads to an average AUC of 0.792 with a 95% confidence interval between 0.646 and 0.914 (Figure 2b).

DISCUSSION

It is evident from Figure 1 and from the figures of merit presented in the Results section that although a significant fraction of patients whose p-tau181/Aβ42 ratios are above the cutpoints respond to the LVA surgery and have their clinical symptoms improved, there are also a few who do not and that some patients whose p-tau181/Aβ42 ratios are below the cutpoints exhibit post-LVA improvement. A key factor for consideration is how a given biomarker test is used and what clinical questions are being addressed.

The AUC of a biomarker test is a useful measure that signifies its overall performance.[30] The AUC of 0.793 of the p-tau181/Aβ42 (as it stands in the current limited data set) compares well against a number of biomarker tests that have currently found clinical utility. The prostate-specific antigen (PSA) test has an AUC of 0.678 (in discriminating prostate cancer versus no cancer).[31−33] The Straticyte test of Proteocyte AI[34,35] has an AUC of 0.605.[35] The Oncotype DX Breast Recurrence Score Test has an AUC of 0.59 − 0.75.[36,37] These tests serve different purposes clinically.

The PSA test is one of the most widely used biomarker tests.[32] PSA is prostate-specific but not prostate cancer-specific, and there lies challenges to the use of PSA for screening. The commonly used cutpoint of 4.1 ng/mL has a specificity of 0.938 or 93.8%, but only a sensitivity of 0.205 or 20.5%; lowering the cutpoint to 1.1 ng/mL will substantially increase sensitivity to 0.834 (83.4%) but decrease specificity to 0.389 (38.9%).[31] Adopting this low cutpoint would mean subjecting 61.1% of patients to prostate biopsy unnecessarily. As it is, the PSA test is not recommended to screen the general population for prostate cancer but is primarily used to monitor prostate cancer recurrence.[33]

The Straticyte test[34] uses a combination of immunohistochemical staining of the S100A7 biomarker and image analysis of oral premalignant lesion (OPL) biopsies to evaluate the probability of malignant transformation within five years.[34,35] Straticyte is a relatively new prognostic test for a given patient's probability in being diagnosed of oral cancer in the future; the test has received Clinical Laboratory Improvement Amendments accreditation for use in the U.S. and in Canada. As Straticyte is intended to identify patients' precancerous lesions that are likely to transform into oral cancers (despite surgical removal of the OPL), the cutpoint is set so as to catch as many such patients as possible: sensitivity = 0.962 (96.2%) and specificity = 0.205 (20.5%).[35] These patients are then recommended for active surveillance over the next five years. The cost of this increase in surveillance is small relative to the cost of surgery and care for late-stage oral cancer. For patients with low risks of transformation, they are considered healthy after a single follow-up at six months postbiopsy.

Oncotype DX Breast Recurrence Score Test is an assay based on the expression of 21 genes and is designed for evaluating the probability of recurrence in a distant organ, as well as the value of chemotherapy as an adjuvant therapy for estrogen receptor-positive and human epidermal growth factor receptor 2-negative early stage breast cancer patients.[36,37]

The p-tau181/Aβ42 biomarker ratio could serve as a test to predict the effectiveness of LVA as treatment to alleviate AD symptoms once we have confirmatory data from a larger set of patients. In this intended clinical use, the p-tau181/Aβ42

https://doi.org/10.1021/acsomega.5c03650
ACS Omega 2025, 10, 32029−32037

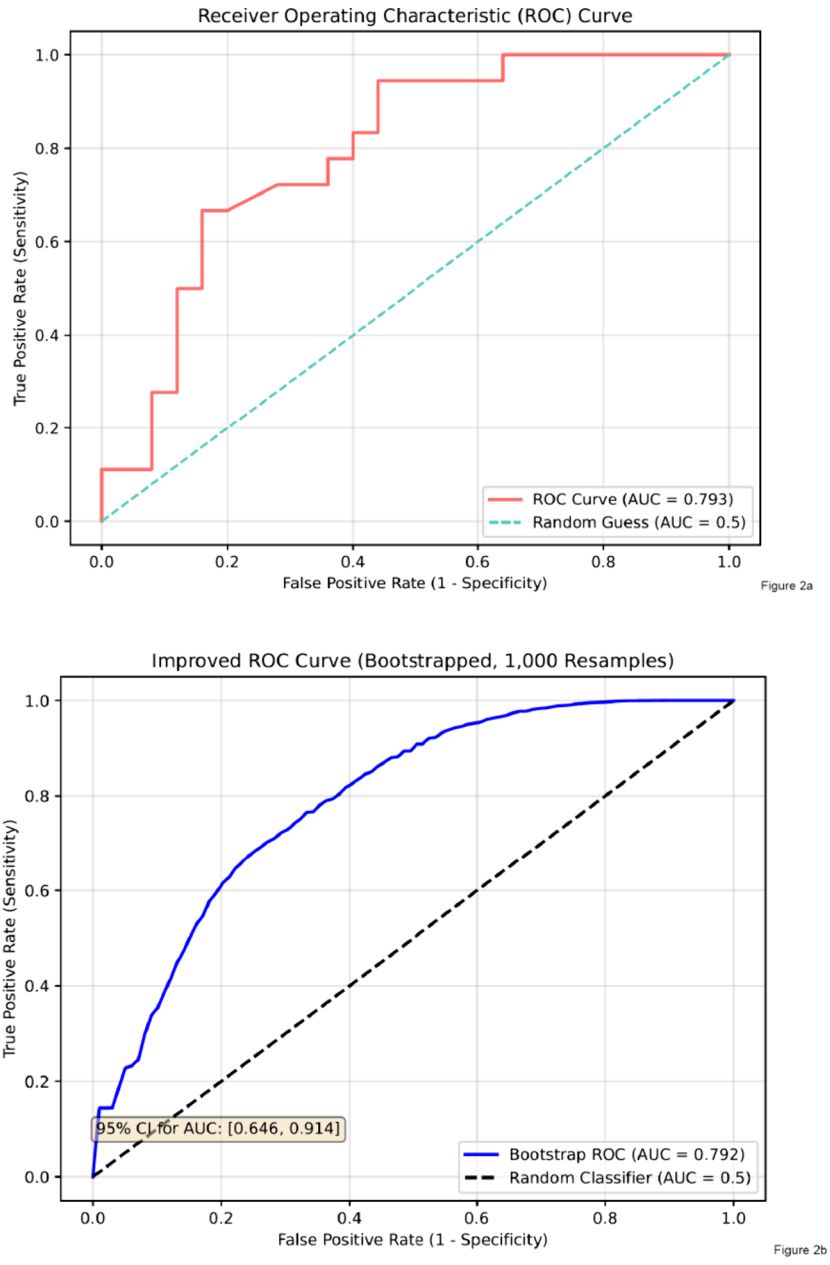


Figure 2. (a) ROC of the p-tau181/Aβ42 biomarker ratio test on 43 LVA patients. (b) ROC as a result of 1,000 bootstrap resampling with replacement.

biomarker ratio test is most similar to the Oncotype DX Breast Recurrence Score test of the three tests thus discussed. The use of biomarkers in predicting therapeutic response is one application that has experienced the most clinical success in the biomarker field.[38,39] Considering the balance between specificity and sensitivity, an optimal cutpoint for the p-tau181/Aβ42 biomarker ratio test could be set at ~0.0923 (the low cutpoint discussed above) for a sensitivity of 0.667 and a specificity of 0.800; trading a small decrease in specificity results in a relatively large gain in sensitivity. At this cutpoint, the diagnostic accuracy = (TP + TN)/(TP + TN + FP + FN) = (12 + 20)/(12 + 20 + 5 + 6) = 32/43 = 0.744 or 74.4%; this means the test will identify LVA patients who respond to the treatment and who do not three times out of four. Triaging patients whose p-tau181/Aβ42 ratios are $\geq$0.0923 to undergo LVA ensures that those patients who are most likely to benefit from the procedure are given priority. For patients whose p-tau181/Aβ42 ratios are <0.0923, they would only have a chance of FN/(FN + TN) = 6/(6 + 20) = 0.231 or 23.1% in responding to treatment, if they elected to go through with it. They (or their guardians) would need to decide with their healthcare providers whether the LVA surgery is advisible even if availability is not an issue.

Limitation of Current Study. The performance metrics of the p-tau181/Aβ42 ratio biomarker test are supportive of the test's use as a means to select patients for LVA surgery. In the small first cohort of 43 patients, the test exhibits a diagnostic accuracy of 74.4% and an AUC of 0.793. This performance, albeit encouraging, will need to be verified on a much larger cohort. The optimal cutpoint of 0.0923 is commonly accepted as indicative of AD, meaning that those patients who benefit from LVA have AD. Given the intention of LVA is to provide extra clearance for toxic brain biomolecules and the fact that LVA is

https://doi.org/10.1021/acsomega.5c03650
ACS Omega 2025, 10, 32029−32037

effective for relieving symptoms for patients having high p-tau181/Aβ42 ratios or high p-tau181 load, it would appear that there is a correlation between clearance and p-tau181 load. A correlation is only the beginning, and it is hoped that this study will stimulate work toward providing a molecular or mechanistic model. Our current ethics approval precludes us from obtaining post-LVA CSF samples, which could provide biological evidence of the molecular changes manifested by the CSF composition. Evidence of post-LVA molecular changes, and especially the differences between Responders and Non-Responders, would inform the underlining mechanisms in symptom relief resulting from LVA. The cognition assessments that we employed provided only a primitive and blunt measure of recovery; we are of the opinion that a more graded assessment or evaluation is desirable to better reflect the different extents of recoveries that are apparent. The most striking result could be interpreted as an almost complete regain of cognitive functions that are required for social functioning. As the majority of AD patients have mixed dementia,[40] the p-tau181/Aβ42 test may or may not be indicative of the full cognitive impairment of any given patient. This may be a reason some patients who tested positive do not respond to LVA surgery, while others who tested negative do respond. We are still in the beginning of LVA surgery and research, and it will take time to work out all of the details. Be that as it may, the p-tau181/Aβ42 biomarker test appears to be effective in ruling-in patients for the LVA treatment. The development and application of additional biomarker tests in combination with the p-tau181/Aβ42 test may offer better discrimination in the future.

AD is an incapacitating affliction that has few effective therapeutic means, despite decades of research and heavy investment. The limited results of LVA are thus far promising, and it is our hope that they would encourage the scientific community to be receptive and engaged to advance the field.

METHODS

ELISA of CSF AD Biomarkers. A pre-LVA CSF sample (2−3 mL) was collected via lumbar puncture from each patient one day prior to the surgery. The CSF samples were processed at room temperature within eight hours postcollection. Sandwich ELISA tests[22] of CSF Aβ42, Aβ40, t-tau and p-tau181 with commercial ELISA kits (IBL International GmbH, Germany) were carried out in Jiangsu Simcere Diagnostic Laboratory (Jiangsu Simcere Diagnostics Co., Ltd., Nanjing 210023, China) according to the manufacturer's instructions. Briefly, 100 μL of standards, controls (with known concentrations), and prediluted patient samples were measured in wells of a microtiter plate coated with monoclonal antibody against t-tau (amino acids 155−165 of human tau protein), p-tau181 (amino acids 117−124 of the human tau protein), Aβ42 (C-terminus of the amyloid-beta (1−42) peptide), or Aβ40 (C-terminus of the amyloid-beta (1−40) peptide). The microtiter plate was covered with black adhesive foil and incubated in the dark for 120 min at room temperature (18−25 °C) on an orbital shaker (500 rpm). After incubation, the adhesive foil was removed and the plate washed five times with 300 μL of diluted wash buffer. 100 μL of diluted Enzyme Conjugate was added to each well. The plate was then covered and incubated for another 60 min at room temperature on an orbital shaker (500 rpm). After washing, 100 μL of Tetramethylbenzidine (TMB) Substrate Solution was added into each well and the mixture incubated for 30 min. Finally, to stop the substrate reaction, 100 μL of TMB Stop Solution was added to each well. To determine optical densities (OD) of the samples, the plates were read using a microplate photometer (Sunrise, Tecan, Switzerland) at 450 nm (with reference wavelength: 600−650 nm). The protein concentrations (pg/mL) were deduced by fitting the OD on standard curves.

As the ratios of Aβ42/40 and p-tau181/Aβ42 are now the recommended parameters for the diagnosis of AD,[3] these ratios were calculated from the measured biomarker concentrations (Table 1).

Statistical Analyses. Statistical analyses were performed using Python 3.10.12 (http://www.python.org) on PyCharm Community Edition 2024.3.4 (https://www.jetbrains.com). Welch's t tests[23] and Mann−Whitney U tests (Wilcoxon Rank Sum tests)[24] were employed to determine whether the biomarker concentrations or their ratios exhibited significant difference between the Responders and Non-Responders (see Supplementary Table 2). For a given biomarker or ratio that showed significant difference, values (e.g., the midpoint) between the mean of the R and that of the NR are then used as cutpoints to compute the sensitivity, the specificity, and other performance metrics of LVA for alleviating symptoms of cognitive impairment. Sensitivity is defined as TP/(TP + FN), specificity is defined as TN/(TN + FP), PPV = TP/(TP + FP), and NPV = (TN)/(TN + FN). Diagnostic accuracy equals (TP + TN)/(TP + TN + FP + FN) (see the Results section for marker-specific presentation).

The ruggedness in performance of the biomarker concentrations or ratios was evaluated by randomly splitting the LVA patients into a 2/3rd training set and a 1/3rd testing set (using a custom script written in Python; see Supplementary Table 3) in a simulation of a prospective study. The training and testing data sets were split 10, 100 and 1,000 times, using the 2/3rd training set to select the cutpoint (the mean between the R and the NR groups) to calculate the performance metrics of the 1/3rd testing set. The numbers of Responders and Non-Responders within the training and testing sets were identical across the splits. This works out to be 16 NR and 12 R in the training set and nine NR and six R in the testing set. The initial cutpoint of such an evaluation was taken as the midpoint of the mean values of NR and R in the training set. Sensitivity, specificity, PPV and NPV of the training and testing sets (mean ± standard deviation (SD)) were calculated.

Bootstrapping Resampling. The bootstrapping resampling technique[27−29] was employed to estimate the variability and confidence intervals for the AUC in the ROC curve. One thousand bootstrap sample sets were generated by sampling the original data with replacement,[28,29] calculating the AUC for each sample set and then determining the 2.5th and 97.5th percentile of the distribution thus produced to estimate the 95% confidence interval (see Python script in Supplementary Table 4).

Consent Statement. The surgical participation and procedures were approved by the Shandong Public Health Clinical Center Affiliated to Shandong University's ethics board (Approval number: GWLCZXEC2024-160). Testing and assessment were conducted as a part of the medical and surgical care provided by the hospital; all patients provided informed consent.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.5c03650.

https://doi.org/10.1021/acsomega.5c03650
ACS Omega 2025, 10, 32029−32037

Details of cognitive assessments and clinicians' assessment (Table S1) (XLSX)

Python scripts are available in Supplementary Tables S2−S4 (PDF)

AUTHOR INFORMATION

Corresponding Authors

K. W. Michael Siu − *Center for Mass Spectrometry Research and Clinical Application, and Center for Integrative and Translational Medicine, Shandong Public Health Clinical Center Affiliated to Shandong University, Jinan, Shandong 250013, China; Department of Chemistry and Biochemistry, University of Windsor, Windsor, Ontario N9B 3P4, Canada;* orcid.org/0000-0002-9551-6506; Email: kwmsiu@hotmail.com

Zhaoyan Yu − *Center for Mass Spectrometry Research and Clinical Application, and Center for Integrative and Translational Medicine, Shandong Public Health Clinical Center Affiliated to Shandong University, Jinan, Shandong 250013, China*; Email: janezhen1213@gmail.com

Authors

Guangqi Xu − *Department of Reconstructive Surgery and Wound Repair, Shandong Public Health Clinical Center Affiliated to Shandong University, Jinan, Shandong 655442, China*

Qinghua Zhang − *Department of Neurology, Shandong Public Health Clinical Center Affiliated to Shandong University, Jinan, Shandong 655442, China*

Yinan Li − *Center for Mass Spectrometry Research and Clinical Application, and Center for Integrative and Translational Medicine, Shandong Public Health Clinical Center Affiliated to Shandong University, Jinan, Shandong 250013, China*

Anqi Du − *Center for Mass Spectrometry Research and Clinical Application, and Center for Integrative and Translational Medicine, Shandong Public Health Clinical Center Affiliated to Shandong University, Jinan, Shandong 250013, China*

Xiaona Xu − *Center for Mass Spectrometry Research and Clinical Application, and Center for Integrative and Translational Medicine, Shandong Public Health Clinical Center Affiliated to Shandong University, Jinan, Shandong 250013, China*

Ruyu Bai − *Center for Mass Spectrometry Research and Clinical Application, and Center for Integrative and Translational Medicine, Shandong Public Health Clinical Center Affiliated to Shandong University, Jinan, Shandong 250013, China; Center for Experimental Education, Faculty of Medicine, Huaqiao University, Quanzhou, Fujian 362021, China*

Chao Bi − *Department of Reconstructive Surgery and Wound Repair, Shandong Public Health Clinical Center Affiliated to Shandong University, Jinan, Shandong 655442, China*

Yanxia Li − *Department of Neurology, Shandong Public Health Clinical Center Affiliated to Shandong University, Jinan, Shandong 655442, China*

Tailun Zhang − *School of Foreign Languages and Literature, Shandong University, Jinan, Shandong 250100, China*

Zhongfa Zhang − *Department of Reconstructive Surgery and Wound Repair and Department of Neurology, Shandong Public Health Clinical Center Affiliated to Shandong University, Jinan, Shandong 655442, China; Center for Mass Spectrometry Research and Clinical Application, and Center for Integrative and Translational Medicine, Shandong Public Health Clinical Center Affiliated to Shandong University, Jinan, Shandong 250013, China*

Ivan K. Chu − *Center for Mass Spectrometry Research and Clinical Application, and Center for Integrative and Translational Medicine, Shandong Public Health Clinical Center Affiliated to Shandong University, Jinan, Shandong 250013, China; Department of Chemistry, University of Hong Kong, Hong Kong 999077, China;* orcid.org/0000-0003-0652-975X

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.5c03650

Author Contributions

G.X., Q.Z., I.K.C., K.W.M.S. and Z.Y. conceptualized and contributed to planning of this study. G.X., Q.Z. and C.B. performed the surgery. Y.L. and A.D. performed the analyses. K.W.M.S. and Z.Y. wrote the manuscript with contributions from coauthors. All authors contributed to the study and approved the manuscript for submission.

Author Contributions

[#]G.X., Q.Z., Y.L., and A.D. contributed equally to this work

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was funded by the Shandong Public Health Clinical Center Affiliated to Shandong University as well as the Shandong Science and Technology Department. Q.Z. thanks the State Administration of Traditional Chinese Medicine, Shandong Provincial Health and Wellness for support (GZY-KIS-SD-2003055). I.K.C. acknowledges support by Hong Kong's Research Grants Council. Informative discussion with Drs. Adrienne G. Siu and Kenneth H. Pritzker on biomarker science is gratefully acknowledged.

REFERENCES

(1) Xie, Q.; Louveau, A.; Pandey, S.; Zeng, W.; Chen, W. F. Rewiring the brain: the next frontier in supermicrosurgery. *Plast. Reconstr. Surg.* **2024**, *153*, 494e−495e.

(2) Li, X.; Zhang, C.; Fang, Y.; Xin, M.; Shi, J.; Zhang, Z.; et al. Promising outcomes 5 weeks after a surgical cervical shunting procedure to unclog cerebral lymphatic systems in a patient with Alzheimer's disease. *General Psychiatry.* **2024**, *37*, No. e101641.

(3) Jack, C. R., Jr.; Andrews, J. S.; Beach, T. G.; Buracchio, T.; Dunn, B.; Graf, A.; et al. Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's Association Workgroup. *Alzheimer's Dementia* **2024**, *20*, 5143−5169.

(4) Arevalo-Rodriguez, I.; Smailagic, N.; Roque i Figuls, M.; Ciapponi, A.; Sanchez-Perez, E.; Giannakou, A.; et al. *Mini-Mental Examination (MMSE) for the detection of Alzheimer's disease and other dementias in people with mild cognitive impairment (MCI)*; The Cochrane Collaboration; John Wiley & Sons, Ltd., 2015.

(5) Nasreddine, Z. S.; Phillips, N. A.; Bedirian, V.; Charbonneau, S.; Whitehead, V.; Collin, I.; et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J. Am. Geriat. Soc.* **2005**, *53*, 695−699.

(6) Kueper, J. K.; Speechley, M.; Montero-Odasso, M. The Alzheimer's Disease Assessment Scale − Cognitive Subscale (ADAS-Cog): modifications and responsiveness in pre-dementia populations. A narrative review. *J. Alzheimer's Disease* **2018**, *63*, 423−444.

(7) Edmekong, P. F.; Bomgaars, D. L.; Sukumaran, S, School, C. Activities of Daily Living. StatPearls (Internet) Treasure Island (FL); StatPearls Publishing, 2005; https://www.ncbi.nlm.nih.gov/books/NBK470404/.

(8) Morris, J. C. Clinical dementia rating: a reliable and valid diagnostic and staging measure for dementia of the Alzheimer type. *Int. Psychogeriatrics.* **1997**, *9*, 173−176.
(9) Shaw, L. M.; Vanderstichele, H.; Knapik-Czajka, M.; Clark, C. M.; Aisen, P. S.; Petersen, R. C.; et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann. Neurol.* **2009**, *65*, 403−413.
(10) Chong, J. R.; Ashton, N. J.; Karikari, T. K.; Tanaka, T.; Saridin, F. N.; Reilhac, A.; et al. Plasma p-tau181 to Aβ42 ratio is associated with brain amyloid burden and hippocampal atrophy in an Asian cohort of Alzheimer's disease patients with concomitant cerebrovascular disease. *Alzheimer's Dementia* **2021**, *17*, 1649−1662.
(11) Yamamoto, T.; Rodriguez, J. R. Step-by-step instruction: lymphaticovenular anastomosis (LVA) techniques. In *Multimodal management of upper and lower extremity lymphedema*; Schaverien, M. V., Dayan, J. H., Eds.; Springer, 2022; pp 59−69.
(12) Lin, Y.-S.; Liu, C.-J.; Chou, C.-H. Lymphovenous anastomosis for the external and internal types of head and neck lymphedema: a case series and preliminary clinical results. *PRS Global Open* **2024**, *12*, e5872.
(13) Iliff, J. J.; Wang, M.; Liao, Y.; Plogg, B. A.; Peng, W.; Gundersen, G. A.; et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Sci. Transl. Med.* **2012**, *4*, 147.
(14) Hladky, S. B.; Barrand, M. A. Mechanisms of fluid movement into, through and out of the brain: evaluation of the evidence. *Fluids Barriers CNS.* **2014**, *11*, 26.
(15) Louveau, A.; Smirnov, I.; Keyes, T. J.; Eccles, J. D.; Rouhani, S. J.; Peske, J. D.; et al. Structural and functional features of central nervous system lymphatic vessels. *Nature.* **2015**, *523*, 337−341.
(16) Zhou, L.; Nguyen, T. D.; Chiang, G. C.; Wang, X. H.; Xi, K.; Hu, T.-W.; et al. Parenchymal CSF fraction is a measure of brain glymphatic clearance and positively associated with amyloid beta deposition on PET. *Alzheimer's Dementia* **2024**, *20*, 2047−2057.
(17) Yamamoto, E. A.; Bagley, J. H.; Geltzeiler, M.; Sanusi, O. R.; Dogan, A.; Liu, J. J.; Piantino, J.; et al. The perivascular space is a conduit for cerebrospinal fluid flow in humans: A proof-of-principle report. *Proc. Nat. Acad. Sci. U. S. A.* **2024**, *121*, No. e2407246121.
(18) Selkoe, D. J.; Hardy, J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Molecular Medicine.* **2016**, *8*, 595−608.
(19) Xie, L.; Kang, H.; Xu, Q.; Chen, M. J.; Liao, Y.; Thiyagarajan, M.; et al. Sleep drives metabolite clearance from the adult brain. *Science.* **2013**, *342*, 373−377.
(20) Miao, A.; Luo, T.; Hsieh, B.; Edge, C. J.; Gridley, M.; Wong, R. T. C.; et al. Brain clearance is reduced during sleep and anesthesia. *Nat. Neurosci.* **2024**, *27*, 1046−1950.
(21) Fang, R.; Jin, L.; Lu, H.; Xie, Q.; Yang, X.; Kueckelhaus, M. A Novel Microsurgical Rat Model of Cervical Lymph Node-to-Vein Anastomosis (LNVA) for Studying Brain Lymphatic Outflow. *bioRxiv* **2025**, DOI: 10.1101/2025.03.15.643475.
(22) Golde, T. E.; Eckman, C. B.; Younkin, S. G. Biochemical detection of Aβ isoforms: implications for pathogenesis, diagnosis, and treatment of Alzheimer's disease. *Biochim. Biophys. Acta* **2000**, *1502*, 172−187.
(23) Sheehan, B. Assessment scales in dementia. *Ther. Adv. Neurol. Disord.* **2012**, *5*, 349−358.
(24) Atri, A.; Dickerson, B. C.; Clevenger, C.; Karlawish, J.; Knopman, D.; Lin, P.-J.; et al. The Alzheimer's Association clinical practice guideline for the diagnostic evaluation, testing, counseling, and disclosure of suspected Alzheimer's disease and related disorders (DETeCD-ADRD): Validated clinical assessment instruments. *Alzheimer's Dementia* **2025**, *21*, No. e14335.
(25) West, R. M. Best practice in statistics: use the Welch t-test when testing the difference between two groups. *Ann. Clin. Biochem.* **2021**, *58*, 267−269.
(26) Mann, H. B.; Whitney, D. R. On a test of whether one of two random variables is stochastically larger than the other. *Ann. Math. Statist.* **1947**, *18*, 50−60.
(27) Efron, B. Bootstrap methods: another look at the jackknife. *Ann. Stat.* **1979**, *7*, 1−26.
(28) Mooney, C. Z.; Duval, R. D. *Bootstrapping*; Quantitative Applications in the Social Sciences; SAGE Publicaitons Inc., 2011; DOI: 10.4135/9781412983532
(29) Chong, S. F.; Choo, R. Introduction to bootstrap. *Proc. Singap. Healthc.* **2011**, *20*, 236−240.
(30) Bewick, V.; Cheek, L.; Ball, J. Statistics review 13: receiver operating characteristic curves. *Critical Care.* **2004**, *8*, 508−512.
(31) Catalona, W. J.; Smith, D. S.; Ratliff, T. L.; Dodds, K. M.; Coplen, D. E.; Yuan, J. J. J.; et al. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. *New Engl. J. Med.* **1991**, *324*, 1156−1161.
(32) Thompson, I. M.; Ankerst, D. P.; Chi, C.; Lucia, M. S.; Goodman, P. J.; Crowley, J. J. Operating characteristics of prostate-specific antigen in men with an initial PSA level of 3.0 ng/mL or lower. *JAMA* **2005**, *294*, 66−70.
(33) National Cancer Institute, National Institutes of Health. Prostate-specific antigen (PSA) test. https://www.cancer.gov/types/prostate/psa-fact-sheet.
(34) Proteocyte AI. https://proteocyte.com/straticyte.
(35) Hwang, J. T. K.; Dammling, C.; McCord, C.; McGuire, T.; Park, E. P.; Filkowski, J.; et al. External validation of Straticyte, a quantitative biomarker-based risk assay in predicting oral cancer. *J. Maxillofac. Oral Surg.* **2024**, DOI: 10.1007/s12663-024-02362-7.
(36) Baltres, A.; Al Masry, Z.; Zemouri, R.; Valmary-Degano, S.; Arnould, L.; Zerhouni, N.; et al. Prediction of Oncotype DX recurrence score using deep multi-layer perceptrons in estrogen receptor-positive, HER2-negative breast cancer. *Breast Cancer.* **2020**, *27*, 1007−1016.
(37) Marazzi, F.; Barone, R.; Masiello, V.; Magri, V.; Mule, A.; Santoro, A.; et al. Oncotype DX predictive nomogram for recurrence score output: the novel system ADAPTED01 based on quantitative immunochemistry analysis. *Clin. Breast Cancer* **2020**, *20*, e600−e611.
(38) Kulasingam, V.; Diamandis, E. P. Strategies for discovering novel cancer biomarkers through utilization of emerging technologies. *Nat. Clin. Pract.* **2008**, *5*, 588−599.
(39) Diamandis, E. P., Fritche, H. A., Lilja, H, Chan, D. W., Schwartz, M. K., Eds. *Tumor Markers: Physiology, Pathobiology, Technology, and Clinical Applications*; AACC Press: Washington, DC, 2002.
(40) *2025 Alzheimer's disease facts and figures. Special report: American perspectives on early detection of Alzheimer's disease in the era of treatment*; US Alzheimer's Association, 2025; Vol. *21*, Issue 5.