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Plasma biomarkers of Alzheimer's disease and related dementia for the prediction of hippocampal atrophy amongst adults in Kinshasa, Democratic Republic of Congo

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Abstract

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ABSTRACT

Objective: Hippocampus is one of the first brain structures affected by Alzheimer's disease (AD) and its atrophy is a strong indicator of the disease. This study aims to investigate the ability of plasma biomarkers of AD and AD-related dementias amyloid-β (Aβ42/40), phosphorylated tau-181 and 217 (p-tau181, p-tau217), neurofilament light (NfL), and glial fibrillary acidic protein (GFAP) to predict hippocampal atrophy in adult individuals in Kinshasa, Democratic Republic of Congo (DRC).

Methods: 85 adult individuals (40 healthy and 45 suspected AD) over 65 years old were evaluated using the Community Screening Instrument for Dementia and Alzheimer's Questionnaire (AQ). Core AD biomarkers (Aβ42/40, p-tau181, p-tau217), and non-specific AD biomarkers (NfL, GFAP) were measured in blood samples collected at study visit. Hippocampal volumes were measured from study magnetic resonance imaging (MRI). Multiple linear regression was used to evaluate differences in biomarker concentrations by neurological status. Logistic regression models were used to create receiver operating characteristic curves and calculate areas under curve (AUCs) with and without clinical covariates to determine the ability of biomarker concentrations to predict hippocampal atrophy. Plasma biomarkers were used either as single or in combination in the models.

Results: Reduced Aβ42/40 and elevated p-tau 181 were associated with decreased left hippocampal (LH) volume. Elevated p-tau 181 was similarly associated with total hippocampal (TH) atrophy, with stronger associations for LH than TH volumes. AUC of plasma biomarkers without the clinical covariates individually to discriminate LH, RH, and TH atrophy ranged between 85.1% to 94.3%; 78.0% to 81.8%; and 82.3% to 85.6%, respectively. The AUC of models including clinical covariates and AD biomarkers used in combination to discriminate LH, RH, TH ranged between 83.9%-96.0%; 77.4%-94.9%; and 81.3%-89.0% respectively. Only higher p-tau 181 concentrations were significantly associated with 1.6 to 3.0-fold increased odds of hippocampal atrophy per standard deviation.

Conclusion: These results indicate that, consistent with studies in other settings, core AD plasma biomarkers can predict hippocampal atrophy in a population in Sub-Saharan Africa.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is associated with hippocampal atrophy.[1] AD is notable for cognitive decline predominantly impacting memory functioning alongside deficits in other neurocognitive domains.[1] Ongoing research of AD pathology has expanded the number of fluid (e.g., cerebral spinal fluid [CSF], blood) biomarkers utilized in the screening, diagnosis, and monitoring of AD,[2] including the core biomarkers of AD [ratio of amyloid-β (Aβ42/40), phosphorylated tau-181 and 217 (p-tau181, p-tau217)], and non-specific biomarkers to AD [neurofilament light (NfL), and glial fibrillary acidic protein (GFAP)]. These studies have shown a positive and stronger correlation between CSF and plasma mostly with Aβ42 and Aβ40 levels.[2–7]

Amyloid and tau deposition, axonal damage and astrocyte death are integral to neurodegenerative cerebral atrophy.[8] Aβ accumulation pathway begins with the cortices, allocortical regions, midbrain, cerebellum, and brain stem.[9] In contrast, tau accumulation starts in cortical lesions and continues to the allocortical and neocortical regions of the temporal lobe, subsequently extending to the parietal lobe, occipital, prefrontal areas, premotor areas, and finally into the neocortical primary fields.[10]

Researchers have shown that the hippocampus is one of the structures of the brain affected by AD.[1,11] Hippocampus is known for its key roles in forming, storing, consolidating and retrieving memory, which is a key deficit in AD patients.[12] Therefore, hippocampal atrophy is considered as an important clinical feature and diagnostic criteria of AD. The atrophy of hippocampus is a strong indicator of AD, and the rate of its shrinkage is used to predict the progression of AD.[13] Currently, the literature is mixed

regarding the association between plasma AD biomarkers and hippocampal volume. In participants from the Baltimore Longitudinal Study of Aging (BLSA), baseline plasma Aβ42/40 ratio, GFAP, NfL, and p-tau181 were not associated with baseline hippocampal volume.[2] However, for those with lower amyloid burden, greater baseline ptau-181 was associated with accelerated decline in hippocampal volume, possibly reflecting greater impact during the earlier course of AD pathology. In another study examining four older adult cohorts across the United States, plasma GFAP was not associated with hippocampal volume.[3] Similarly, in a German cohort, plasma biomarkers of inflammation (TNFα, interleukin 6 [IL-6]) were not related to whole hippocampus volumes.[6] In contrast, one study found that plasma AD biomarkers (p-tau 217, p-tau 181, Aβ42/40, NfL) predicted hippocampal atrophy in a sample of non-demented older adults, with p-tau 217 being the best predictor of hippocampal atrophy.[4] Additionally, those predictions were specific to AD, as there were no associations found in a sample of non-AD individuals.[4]

Overall, most AD biomarker studies have primarily been conducted with Western populations samples made up of predominantly White individuals of European ancestry. Thus, understanding the role of plasma AD biomarkers within non-White populations is crucial. There are few studies on the association of core AD biomarkers (Aβ and p-tau) and hippocampal atrophy in culturally diverse populations. For example, in an enthnoracially diverse sample of community dwelling older adults, hippocampal volume was found to be a significant mediator between Aβ42/40 and NfL on baseline episodic memory and executive function measures.[14] In a Singaporean cohort with varying cognitive status, p-tau181, p-tau 181/t-tau, Aβ42/40, and p-tau 181/Aβ42 ratios were all

significantly associated with hippocampal volume.[15] Similar associations were seen in a primarily Hispanic sample (>60% Hispanic participants), where hippocampal volume was significantly related to plasma NfL in individuals with AD, but not for those who were considered cognitively normal.[16]

The current study aims to investigate ability of AD core plasma biomarkers (Aβ 42/40, p-tau 181, p-tau 217) and non-specific AD biomarkers (NfL and GFAP) to discriminate the severity of hippocampal atrophy in adult individuals in Kinshasa, Democratic Republic of the Congo (DRC), in Sub-Saharan Africa (SSA). We hypothesized that core (Aβ42/40 and p-tau 181/217) and non-specific (NfL and GFAP) plasma biomarkers of AD will be associated with hippocampal atrophy in this sample. Given the pathophysiological impact and the usefulness of both p-tau 181 and p-tau 217 in the diagnosis of AD pathology, alongside the promising superiority of p-tau 217 to p-tau 181,[17] we hypothesize that p-tau 181 and 217 will have greater discriminatory ability of hippocampal atrophy than other plasma biomarkers. Finally, we predict that Aβ42/40, NfL, and GFAP will demonstrate adequate to good sensitivity to discriminate between individuals with and without hippocampal atrophy.

METHODS

Study population

Participants of this study are community-dwellers from Kinshasa, DRC, selected from our previous study.[18] Participants were included if they were at least 65 years or older, had a family member or close friend to serve as an informant, and were fluent in French or Lingala. We excluded participants who had history of schizophrenia, neurological disease other than dementia, or other medical conditions potentially

affecting the central nervous system (CNS). To establish neurological status in the absence of established diagnostic criteria for AD in SSA, we screened participants using the Alzheimer's Questionnaire (AQ)[19] and the Community Screening Instrument for Dementia (CSID).[20] The AQ was used to assess activities of daily living and symptoms of AD in participants.[19] The CSID Questionnaire, which is extensively used in many SSA dementia studies,[20] was used to screen cognitive abilities. Based on cognitive and functional deficits per the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision (DSM-5-TR) diagnostic criteria,[21] we used Congo-Brazzaville cut-offs of CSID, the closest city from Kinshasa, to classify participants.[22] Similar to our prior study,[23] participants were classified using CSID and AQ scores (see Figure 1), which yielded 4 groups:dementia, mild neurocognitive disorder (MND), subjective cognitive impairment, and healthy control (HC), i.e., normal cognition. A panel consisting of a neurologist, psychiatrist and neuropsychologist reviewed screening tests, clinical interview, and neurological examination of subjects, of whom 56 were confirmed with a diagnosis of dementia and 58 were considered HC. Of these 114 participants, 29 refused to provide blood samples, leaving 85 participants (75%) in whom plasma biomarkers were obtained (44 dementia and 41 HC). Written informed consent was obtained prior to participants' undergoing any study procedures. Participants were financially compensated for their time. The procedures were approved by the Ethics Committee/Institutional Review Boards of the University of Kinshasa and Emory University.

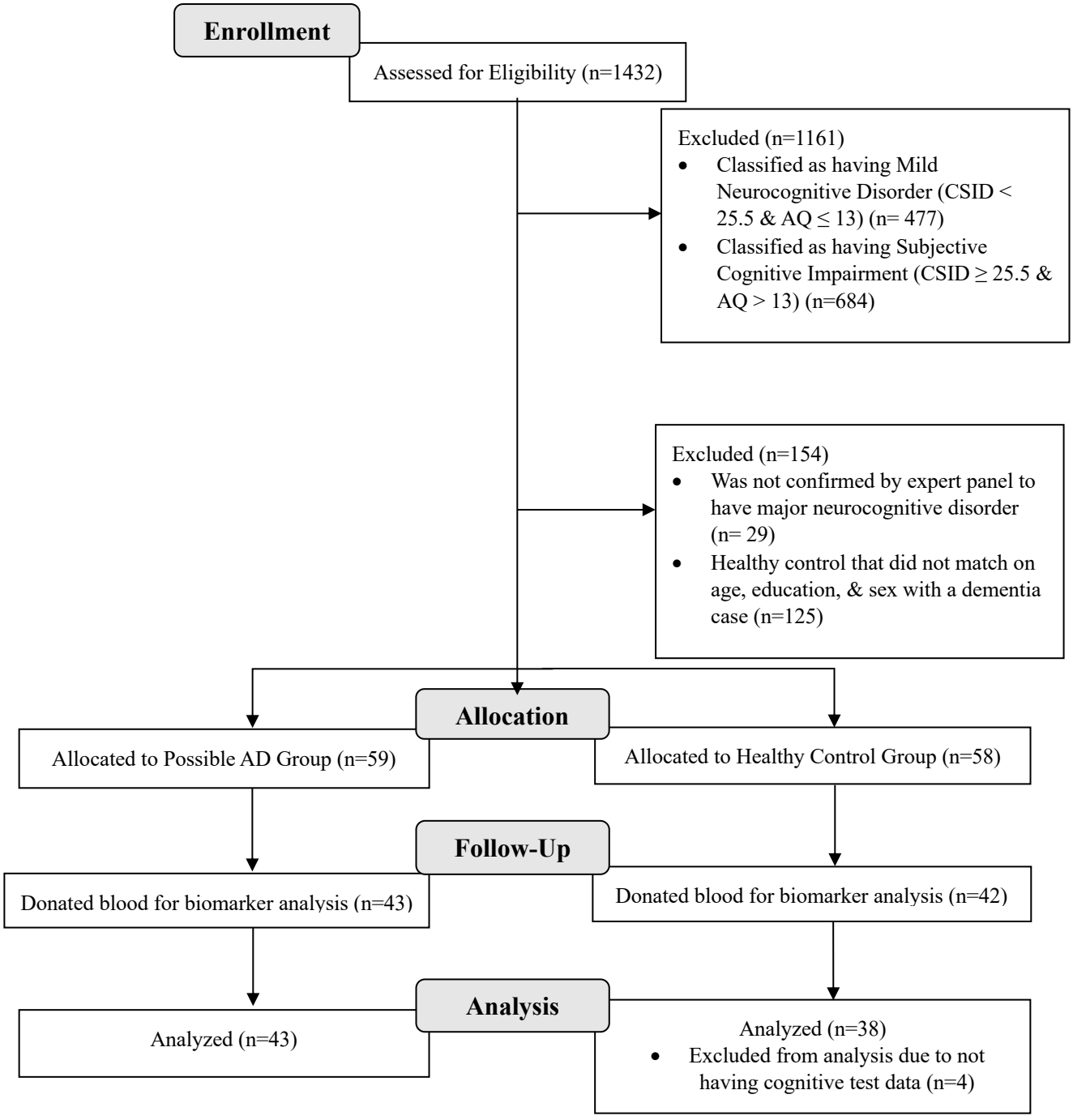


Figure 1. Flow Chart of Recruitment Status from those assessed for eligibility at enrollment (n=1432) to the individuals that were allocated to the dementia or control group and analyzed (n=85)

Procedure

Participants underwent a comprehensive clinical evaluation, including cognitive testing, self-report questionnaires, and standard psychiatric and neurological evaluations to be diagnosed with dementia or to be considered as HC by an expert panel (neurologist, psychiatrist, and neuropsychologist). Subjects were interviewed to obtain demographic, socioeconomic, and medical history. Afterwards, blood samples were obtained at Medical Center of Kinshasa (CMK) by a phlebotomist. Sample collection protocol and quantification of fluid biomarkers are presented below.

Measures

Plasma biomarkers

Blood samples were drawn in the CMK blood laboratory by venipuncture into dipotassium ethylene diamine tetra acetic acid (K_2 EDTA) tubes. Samples were centrifuged within 15 minutes at 1800 g house temperature, and 5 mL of plasma was aliquoted into 0.5 mL polypropylene tubes and stored initially at -20^{o} C for less than a week and then moved to a -80 ^{o}C freezer for longer term storage at a CMK laboratory.[24] These aliquots were shipped frozen on dry ice to Emory University.

Plasma biomarker concentrations were measured using commercially available Neurology 4-PLEX E (Aβ40, Aβ42), P-tau181 (P-tau181 v2; l) Quanterix kits for the Simoa HD-X platform (Billerica, MA) at the University of San Francisco. P-tau217 was measured using the proprietary ALZpath P-tau-217 CARe Advantage kit (lot #MAB231122, ALZpath, Inc.) for the Simoa HD-X platform. The instrument operator was blinded to clinical variables. All analytes were measured in duplicate. For Aβ40 and Aβ42, all samples were measured above the lower limit of quantification (LLOQ) of 1.02

pg/mL and 0.378 pg/mL. The average coefficient of variation (CV) for Aβ40 and Aβ42 were 6.0% and 6.5%. For P-tau181, all samples were measured above the kit lower limit of quantification (LLOQ) of 0.085 pg/mL, with an average CV of 11.6%. For P-tau217 the LLOQ was 0.024 pg/mL and the average CV was 19.8%.

Neuroimaging

All subjects were imaged on a 1.5 Tesla MRI unit (Siemens, Magneton Sonata) scanner at HJ Hospitals in Kinshasa using the same standardized imaging acquisition protocol based on the Alzheimer's Disease Research Center protocol of Emory University. This consisted of sagittal volumetric T1-weighted (MPRAGE), coronal T2-weighted, and axial diffusion-weighted, T2-weighted, and T2-FLAIR sequences. Typical acquisition parameters for the MPRAGE sequence were TR = 2200 ms, minimum full TE, TI = 1000 ms, flip angle = 8°, FOV = 25 cm, with a 192 × 184 acquisition matrix, yielding a voxel size of approximately 1.25 × 1.25 × 1.2 mm.[25] Images were reviewed by an experienced neuroradiologist. White matter hyperintensity was graded according to the age-related white matter changes (ARWMC) scale.[26] Number of chronic brain parenchymal microhemorrhages were recorded. Lobar volume loss pattern of the brain was assessed. MPRAGE images were reoriented into the oblique coronal plane orthogonal to the principal axis of the hippocampal formation, and medial temporal lobe atrophy (MTLA) and entorhinal cortex atrophy (EriCa) scores were assessed.[27] Finally, the presence or absence of any additional abnormalities was noted, and patients were excluded if neuroimaging evidence indicated an etiology other than probable AD (e.g., presence of a brain tumor).

Quantitative volumetric analysis using Freesurfer

The 3D T1w images were segmented using Freesurfer (v.6, MGH, MA), which includes a full processing stream for MR imaging data that involves skull-stripping, bias field correction, registration, and anatomical segmentation as well as cortical surface reconstruction, registration, and parcellation. Regional brain volume for both cortical and subcortical brain regions were calculated. The left and right hippocampal (LH, RH) volume were averaged. Interindividual variation in head size were accounted for in further statistical analysis by controlling for total intracranial volume.

Statistical Analyses

Statistical analyses were performed using SAS statistical software. Descriptive statistics for continuous, normally distributed variables are presented as mean ± standard deviation (SD) and categorical variables are expressed using counts and proportions. Winsorization of plasma biomarkers to the 95th percentile was used to limit the effect of extreme outliers. We standardized the hippocampus volume by subtracting the mean hippocampal volume of the sample and dividing by the sample hippocampal volume standard deviation. In addition, we also standardized the biomarkers by subtracting the mean biomarker of the sample and dividing by the sample plasma biomarker standard deviation. Standardized hippocampal volumes and biomarkers were obtained for LH, RH, and total hippocampal (TH) volume. We calculated the difference between hippocampal volumes by cognitive status. We defined hippocampal atrophy based on the established cutoffs (Mondragón, et al. 2016):[28]

- ≥ 3000 mm^3 or < 3000 mm^3 to define normal or atrophy for LH and RH, respectively.
- ≥ 6000 mm^3 or < 6000 mm^3 to define normal or atrophy for TH, respectively.

Based on these cutoffs, we calculated the prevalence of LH, RH and TH atrophy.

Multiple linear regression adjusting for age, sex, and education was used to evaluate differences in biomarkers by neurological status. We also used multiple linear regressions to assess associations between biomarkers and hippocampal volumes adjusting for age, sex, education, intracranial volume and depression. Logistic regression was conducted to create receiver operating characteristic curves (ROC) and to calculate areas under curve (AUCs) to evaluate the ability of plasma biomarkers (Aβ42/40, p-tau 181, NfL, GFAP) to predict hippocampal atrophy controlling for age, education, gender, depression score, and intracranial volume. Cutoff scores for each plasma biomarkers were determined based on optimal sensitivity and specificity for detecting the presence of hippocampal atrophy determined by the value maximizing the Youden's index (sensitivity + specificity – 100). We used Hosmer and colleagues ROC-AUC categories (Hosmer et al., 2013), which considered the value of <0.600 as "failure", values between 0.600 and 0.699 as "poor", values between 0.700 and 0.799 as "fair", values between 0.800 and 0.899 as "good", and values 0.900 or greater as "excellent".

RESULTS

Demographic data, cognitive screening scores, hippocampal volumes, and plasma biomarker characteristics stratified by neurological status are presented in Table 1. There were no significant differences in sex between groups. Education level was lower in participants with suspected dementia. There were significant differences in cognitive screening scores used in distinguishing neurological status, with HC having higher scores than those with suspected AD. There were significant differences in LH,

RH, and TH volumes between HC and suspected AD, with suspected AD showing lower hippocampal volumes. NfL and GFAP also differed significantly by neurological status after controlling for age, gender, and education (Table 1).

Table 1. Characteristics of the Study Sample Stratified by Cognitive Status‡

	HC mean (SD), (n=40)	**Suspected AD** mean (SD), (n=45)	**All Patients** mean (SD), (n=85)	**p-value***
Age (years)	72.6 (8.1)	73.8 (7.1)	73.2 (7.6)	0.86
Sex Male (n,%)†	18 (45.0%)	20 (44.4%)	38 (44.2%)	0.39
Education (years)	9.2 (5.2)	7.39 (5.4)	8.30 (5.4)	0.08
CSID	31.8 (2.9)	19.6 (5.6)	24.9 (7.6)	<.0001
AQ	3.5 (2.9)	19.0 (3.9)	14.1 (8.5)	<.0001
GDS	4.0 (2.4)	7.7 (3.7)	6.0 (3.7)	<.0001
Estimated Intracranial Volume (mm^3)	1433766 (159920)	1415062 (261524)	1422350 (226362)	0.51
Left hippocampal vol (mm^3)	3413 (451)	3046 (560)	3189 (548)	0.022
Right hippocampal Vol (mm^3)	3383 (441)	3017 (560)	3159 (544)	0.034
Total hippocampal vol (mm^3)	6795 (858)	6063 (1036)	6348 (1030)	0.037
$A\beta_{42}$ (pg/ml)	3.83 (2.30)	3.81 (2.02)	3.82 (2.14)	0.73
$A\beta_{40}$ (pg/ml)	68.1 (51.6)	78.4 (50.1)	73.5 (50.7)	0.65
$A\beta_{42/40}$	0.078 (0.040)	0.062 (0.030)	0.069 (0.036)	0.11

p-tau 181 (pg/ml)	2.21 (1.49)	3.03 (2.26)	2.67 (1.97)	0.15
p-tau217 (pg/ml)	0.44 (0.47)	0.34 (0.34)	0.38 (0.40)	0.34
NfL (pg/ml)	37.8 (31.2)	62.7 (41.5)	50.6 (39.0)	0.006
GFAP (pg/ml)	165.5 (97.8)	241.0 (143.6)	205.9 (128.8)	0.007

CSID = Community Screening Instrument for Dementia; AQ = Alzheimer's Questionnaire; GDS= Geriatric Depression Scale; LH= Left hyppocampal volume, RH= right hippocampal volume; NfL= neurofilament light; GFAP= glial fibriliary acidic protein;
‡ n=77
† relative and absolute frequency of male in this sample.
*p-values were calculated using multiple linear regressions adjusted for age, education, and gender.

The difference between the TH volume of HC and suspected AD was 367 mm^3. The difference between the LH and RH volumes of HC was 30 mm^3 , while the difference between LH and RH volumes of suspected AD was 29 mm^3. Prevalence of LH, RH and TH atrophy was 32.5 %, 34.2%, and 33.3%, respectively.

Table 2 presents the association between hippocampal volume and plasma biomarkers. LH volume was significantly associated with Aβ42/40, and p-tau 181 concentration. TH volumes showed significant associations only with Aβ42/40. RH was not associated with any of the plasma biomarkers. Hippocampal volume was not associated with p-tau 217, NfL, and GFAP.

Table 2: Association between standardized plasma biomarkers and hippocampal volume

Biomarkers	β_1 Parameter estimate	95% CI	p-value
Left hippocampal volume			
$A\beta_{42/40}$	-0.13	-0.246, -0.004	0.042
p-tau 181	0.118	0.361, 0.201	0.005
p-tau 217	-0.015	-0.152, 0.120	0.816
NfL	0.056	-0.044, 0.156	0.266

GFAP	0.089	-0.007, 0.185	0.09
Right hippocampal volume			
$A\beta_{42/40}$	-0.125	-0.265, 0.014	0.077
p-tau 181	0.094	-0.004, 0.192	0.059
p-tau 217	-0.039	-0.204, 0.125	0.629
NfL	0.041	-0.075, 0.157	0.480
GFAP	0.049	-0.063, 0.163	0.382
Total hippocampal volume			
$A\beta_{42/40}$	-0.149	-0.281, -0.017	0.026
p-tau 181	0.083	-0.011, 0.178	0.082
p-tau 217	-0.014	-0.174, 0.146	0.858
NfL	0.052	-0.058, 0.163	0.349
GFAP	0.074	-0.33, 0.181	0.173

*β_1 represents the average magnitude in which plasma biomarkers are associated with low hippocampal volume, adjusting for age, gender, education, depression and intracranial volume (unless testing that covariate).
LH= Left hyppocampal volume, RH= right hippocampal volume; NfL= neurofilament light; GFAP= glial fibriliary acidic protein;
‡ n=77

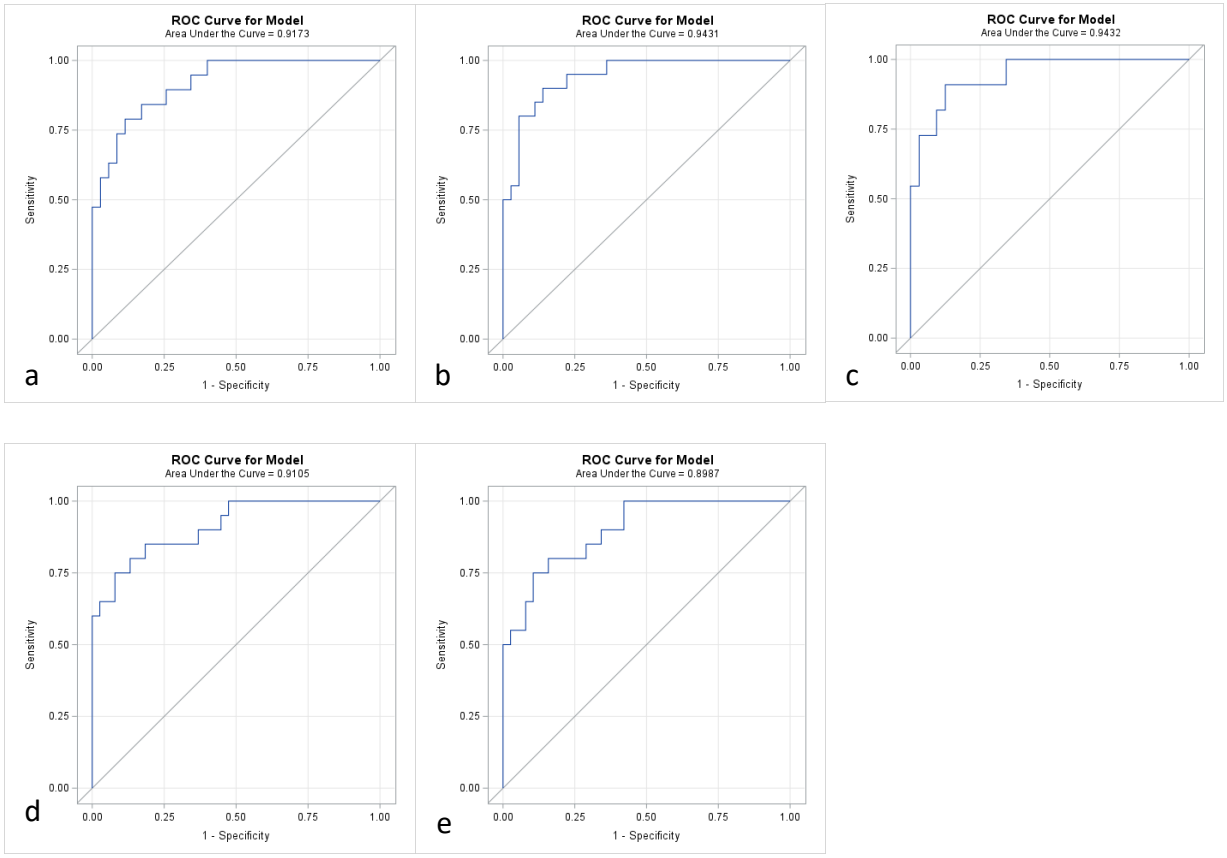
Table 3 presents biomarkers' cutoffs, sensitivity, specificity, and accuracy of the plasma biomarkers to discriminate LH atrophy. Sensitivity varied from poor (63.2%) to fair (75.0%) with NfL and GFAP being the highest, while specificity was good (ranging from 81.6% to 88.6%). In addition, the AUC of plasma biomarkers to discriminate LH atrophy varied between good (85.1%) to excellent (94.3%) with GFAP having lowest AUC and p-tau 181 and 217 having the highest AUC. We show the various AUC of plasma biomarkers discriminating LH in figure 3. We also developed models using a combination of plasma biomarkers to find their ability to discriminate LH atropy. The AUC of the combined plasma biomarkers to discriminate LH atrophy varies between 83.9% to 96.0%. The AUC of a model including covariates only (without any biomarkers) to discriminate LH atrophy was 85.1% (see table 3).

Table 3: *Sensitivity, specificity, and accuracy of plasma biomarkers in discriminating LH atrophy*

Biomarker	**Cutoff**	**Se / Spe**	**Crude AUC** (95% CI)**	**AUC** (95% CI)***
Covariates only*				85.1 (76.7 - 93.6)
$A\beta_{42/40}$	0.048	63.2/88.6	66.4 (50.9 - 81.7)	91.7 (84.1 - 99.3)
p-Tau 181	0.629	70.0/86.1	73.9 (57.7 - 90.2)	94.3 (88.7 - 99.8)
p-Tau 217	0.145	72.7/81.3	48.7 (29.1 - 68.3)	94.3 (87.1 - 100)
NfL	23.8	75.0/81.6	64.4 (49.3 - 79.6)	91.1 (83.3 - 98.8)
GFAP	202	75.0/81.6	77.5 (63.8 - 91.2)	89.9 (81.9 - 97.8)
Models				
$A\beta_{42/40,}$ p-tau 181	----	----	77.5 (62.5 - 92.5)	94.9 (89.5 - 100.0)
$A\beta_{42/40,}$ p-tau 217	----	-----	69.5 (52.2 - 86.8)	94.4 (86.3 - 100.0)
$A\beta_{42/40,}$ p-tau 181, NfL	----	-----	77.4 (62.2 - 92.6)	95.6 (90.0 - 100.0)
$A\beta_{42/40,}$ p-tau 217, NfL	----	-----	73.3 (56.2 - 90.5)	95.9 (89.3 - 100.0)
$A\beta_{42/40,}$ p-tau 181, GFAP	---	----	86.3 (75.8 - 96.9)	94.7 (89.2 - 100.0)
$A\beta_{42/40,}$ p-tau 217, GFAP	---	----	80.1 (64.7 - 95.4)	95.9 (90.5 - 100.0)
$A\beta_{42/40,}$ NfL & GFAP	----	----	77.4 (62.2 - 92.6)	93.7 (87.4 - 100.0)
P-tau 181,NfL & GFAP	----	----	87.6 (77.7 - 97.6)	94.9 (89.0 - 100.0)
P-tau 217, NfL & GFAP	----	----	75.7 (55.8 - 95.5)	96.0 (90.3 - 100.0)
$A\beta_{42/40,}$ p-tau 181, NfL &GFAP	----	----	86.6 (76.3 - 96.9)	95.6 (90.0 -100.0)
$A\beta_{42/40,}$ p-tau 217, NfL &GFAP	----	----	80.1 (64.3 - 95.9)	83.9 (71.4 - 96.5)

* Covariates only model includes the following variables: included only age, gender, education, depression score, and intracranial volume without corresponding biomarkers
** Model including only the corresponding biomarkers.
*** Model including covariates and corresponding biomarkers.
LH= Left hyppocampal volume, RH= right hippocampal volume; NfL= neurofilament light; GFAP= glial fibriliary acidic protein;
‡ n=77

Figure 3: AUC discriminations of plasma biomarkers predicting left hippocampal atrophy



[a] AUC of $A\beta_{42/40}$ discriminating LH atrophy
[b] AUC of p-tau 181 discriminating RH atrophy
[c] AUC of p-tau 217 discriminating LH atrophy
[d] AUC of NfL discriminating LH atrophy
[e] AUC of GFAP discriminating LH atrophy

Table 4 presents biomarkers' cutoffs, sensitivity, specificity, and discrimination ability of the plasma biomarkers for RH atrophy. The sensitivity of plasma biomarkers to discrimante RH atrophy varies between 0% and 80.0% with p-tau 217 showing the lowest and p-tau 181 being the highest. The specificity ranges between 56.8% and 100%. The AUC of biomarkers were between fair (78.0%) to good (81.8%) with p-tau 181 showing the highest. The AUC of a covariates-only model for RH atrophy prediction was 78.0%. The AUC of biomarkers predicting RH atrophy varied between

77.4% to 94.9%. Like with LH, the presence of $A\beta_{42/40}$, p-tau 181, and NfL had more impact in the model (see table 4).

Table 4: *Sensitivity, specificity, and accuracy of plasma biomarkers in discriminating low right hippocampal volume*

Biomarker‡	**Cutoff**	**Se** / **Spe**	**Crude AUC**	**AUC** (95% CI)
Covariates only				77.96 (67.6 - 88.3)
$A\beta_{42/40}$	0.049	73.7/62.9	70.3 (55.6 - 85.1)	80.3 (68.8 - 91.8)
p-Tau 181	1.510	80.0/63.9	68.9 (53.5 - 84.2)	81.8 (70.6 - 93.1)
p-Tau 217	0.821	0/100	56.3 (37.6 - 74.9)	77.2 (62.8 - 91.6)
NfL	36.1	71.4/64.9	64.6 (49.9 - 79.2)	76.8 (64.7 - 88.9)
GFAP	133	76.2/56.8	61.5 (45.7 - 77.4)	76.9 (64.9 - 89.1)
Models				
$A\beta_{42/40}$, p-tau 181	----	-----	72.8(57.9 - 87.7)	94.9 (89.5 - 100.0)
$A\beta_{42/40}$, p-tau 217	----	-----	73.3 (57.1 - 89.6)	82.4 (69.2 - 95.6)
$A\beta_{42/40}$, p-tau 181, NfL	---	-----	75.7 (61.1 - 90.2)	83.8 (73.2 - 94.4)
$A\beta_{42/40}$, p-tau 217, NfL	---	----	73.3 (57.1 - 89.6)	83.9 (71.4 – 96.5)
$A\beta_{42/40}$, p-tau 181, GFAP	----	----	72.5 (56.8 - 88.3)	82.7 (71.7 - 93.7)
$A\beta_{42/40}$, p-tau 217, GFAP	----	----	70.0 (51.9 - 88.0)	83.3 (70.6 - 96.1)
p-tau 181, NfL, GFAP	----	----	71.9 (56.6 - 87.3)	81.4 (70.1 - 92.7)
p-tau 181, NfL, GFAP	----	----	65.4 (47.1 - 83.7)	77.4 (63.3 - 91.6)
$A\beta_{42/40}$, p-tau 181, NfL & GFAP	----	----	74.1 (59.0 - 89.2)	83.8 (73.2 - 94.4)
$A\beta_{42/40}$, p-tau 217, NfL & GFAP	----	----	72.5 (55.8 - 89.2)	83.9 (71.4 - 96.5)

LH= Left hyppocampal volume, RH= right hippocampal volume; NfL= neurofilament light; GFAP= glial fibriliary acidic protein;
‡ n=77

Table 5 represents biomarkers' cutoffs, sensitivity, specificity, and discriminative ability of the plasma biomarkers to predict TH atrophy. The sensitivity of plasma

biomarkers to discriminate TH atrophy ranged from 42.9 to 90.5 with p-tau 217 showing the lowest and p-tau 181 the highest. The specificity varied between 51.4 to 79.9. The AUC of plasma biomarkers to discriminate TH atrophy was between 82.3 to 85.6 with p-tau 217 having the lowest AUC and p-tau 181 having the highest AUC. In the model, the AUC of the covariates-only model was 79.3%. Like with LH and RH, the AUC in the crude and the full models increases with the number of biomarkers in the models. The AUC varied between 81.3% to 89.0% (see table 5).

Table 5 *Sensitivity, specificity, and accuracy of plasma biomarkers in discriminating low total hippocampal volume*

Biomarker	**Cutoff**	**Se/ Spe**	**Crude AUC** (95% CI)	**AUC** (95% CI)
Covariates only				79.3 (69.2 - 89.3)
$A\beta_{42/40}$	0.061	90.5/57.6	69.7 (55.2 - 84.3)	85.6 (75.9 - 95.3)
p-Tau 181	0.610	63.6/76.5	66.3 (49.8 - 82.7)	85.8 (76.1 - 95.7)
p-Tau 217	0.613	42.9/79.3	51.2 (31.7 - 70.7)	82.3 (70.1 - 94.9)
NfL	48.2	87.0/57.1	63.5 (48.9 - 78.1)	83.0 (72.6 - 93.4)
GFAP	133	78.3/60.0	70.5 (56.2 - 84.7)	83.5 (73.4 - 93.6)
Models				
$A\beta_{42/40,}$ p-tau 181	----	----	70.5 (54.8 - 86.2)	88.0 (79.1 - 96.9)
$A\beta_{42/40,}$ p-tau 217	----	----	71.6(55.4 - 87.8)	87.8 (76.9 - 98.5)
$A\beta_{42/40,}$ p-tau 181, NfL	----	----	71.3 (55.5 - 87.1)	89.0 (80.4 - 97.7)
$A\beta_{42/40,}$ p-tau 217, NfL	----	----	77.2 (61.9 - 92.4)	87.5 (76.5 - 98.5)
$A\beta_{42/40,}$ p-tau 181, GFAP	----	----	76. 2 (62.5 - 89.9)	87.7 (78.7- 96.7)
$A\beta_{42/40,}$ p-tau 217, GFAP	----	----	78.8 (64.1 - 93.5)	85.8 (74.3 - 97.2)
p-tau 181, NfL, GFAP	----	----	77.2 (63.3 - 90.9)	86.4 (76.8 - 95.8)
p-tau 217, NfL, GFAP	---	----	65.2 (46.1 - 84.3)	81.3 (68.5 - 94.1)
$A\beta_{42/40,}$ p-tau 181, NfL & GFAP	----	----	77.7 (64.9 - 90.5)	89.0 (80.4 - 97.7)

$A\beta_{42/40,}$ p-tau 217, NfL & GFAP	---	----	79.8 (65.3 - 94.4)	87.5 (76.5 - 98.5)

The covariates in the covariate model are age, gender, education, intracranial volume and depression score; Crude AUC refers to the biomarker as the only predictor of the model without any other predictors.
LH= Left hyppocampal volume, RH= right hippocampal volume; NfL= neurofilament light; GFAP= glial fibriliary acidic protein;
‡ n=77

Finally, Table 6 presents the odds ratio describing the strength of the association between plasma biomarkers and hippocampal atrophy (LH, RH, and TH). Higher Aβ42/40 was associated with 49%-57% reduction in the odds of hippocampal atrophy per standard deviation. Additionally, the association between lower p-tau 217 and hippocampal atrophy ranged between a 13.7% to 30.1% reduced odds of hippocampal atropy per standard deviation. Higher p-tau 181 concentrations were significantly associated with 1.6 to 3.0-fold increased odds of hippocampal atrophy per standard deviation. NfL and GFAP were associated with 1.3 to 1.7 and 1.1 to 1.3-fold increased odds of hippocampal atrophy per standard deviation, respectively (see Table 6).

Table 6 Odd Ratio of plasma biomarkers

Biomarker ‡	**LH (OR, 95% CI)**	**RH (OR, 95% CI)**	**TH (OR, 95% CI)**
$A\beta_{42/40}$	0.430 (0.145 - 1.271)	0.509 (0.222 - 1.167)	0.436 (0.183 - 1.042)
p-Tau 181	3.032 (1.203 - 7.639)	1.573 (0.935 - 2.647)	1.588 (0.882 - 2.858)
p-Tau 217	0.863 (0.162 - 4.593)	0.699 (0.260 - 1.884)	0.862 (0.321 - 2.313)
NfL	1.693 (0.676 - 4.244)	1.251 (0.668 - 2.342)	1.474 (0.690 - 3.146)
GFAP	1.144 (0.541 - 2.416)	1.195 (0.673 - 2.121)	1.266 (0.673 - 2.382)

OR= Odds Ratio per standard deviation, LH= Left hyppocampal volume, RH= right hippocampal volume; NfL= neurofilament light; GFAP= glial fibriliary acidic protein;
‡ n=77

Discussion

In the current study, we examined the discriminative ability of core AD plasma biomarkers (Ab42/40, p-tau 181, and p-tau 217) and non-specific AD biomakers (NfL, GFAP) in predicting hippocampal atrophy in adult Congolese with and without probable AD from Kinshasa, DRC. This is one of the first exploratory studies that used the AD plasma biomarkers based on Alzheimer's Association criteria[29] in a SSA population. Our results found that patients with AD have significantly smaller hippocampi than HC subjects, including both unilateral and total volumes which is consistent with broader research literature.[30] In our previous study, we did not find significant relationships between mood severity and hippocampal volume.[30] Due to lack of significant association with hippocampal volume, we used mood severiry as covariate to be conservative in our analyses. We found elevated rate of non-specific AD biomarkers (NfL and GFAP) in our patients with probable AD, which appears consistent with reports that NfL and GFAP are involved in other neurodegeneration pathology.[31]

Our first hypothesis which predicted that core (Aβ42/40 and p-tau 181/217) and non-specific (NfL and GFAP) plasma biomarkers of AD would be associated with hippocampal atrophy was partially supported. We found significant associations between Aβ42/40 and p-tau 181 plasma biomarkers with LH volumes. However, p-tau 217 and non-specific (NfL and GFAP) AD plasma biomarkers were not associated with hippocampal atrophy in this sample. These findings are similar with studies in other populations, which have found associations between AD core plasma biomarkers with hippocompal atrophy in elderly subjects with dementia.[32,33] These findings also highlight the importance of core AD plasma biomarkers in the evaluation of adults with and without AD making the Aβ42/40 and p-tau 181 better plasma AD biomarkers in the

diagnostic assessment of cognitive aging. Our results also support the utility of using these two plasma biomarkers to discriminate hippocampal atrophy in adults with and without AD in SSA populations. In addition, the core AD plasma biomarkers (Aβ42/40 and p-tau 181) had greater discriminative ability of hippocampal atrophy than p-tau 217 and non-specific AD biomarkers (NfL and GFAP). These results demonstrated good specificity of plasma biomarkers in discriminating hippocampal atrophy either as crude or adjusted models. However, the sensitivity of plasma biomarkers was still poor in discriminating hippocampal atrophy. Only plasma p-tau 181 concentrations were significantly associated with increased odds of hippocampal atrophy in this sample.

While lateralized hippocampal findings are frequently observed in the research literature, our study did find some evidence of laterality with regards to the ability of plasma biomarkers in discriminating hippocampal atrophy. The ability to discriminate atrophy was slightly better for LH than RH. The discriminative strength remained almost the same if intracranial volume is also added as a covariate and after adjustment for some covariates. These results can be explained by the fact that screening measures which were used for the selection of the participants had many items that are more verbally loaded. Other studies have reported similar findings in assessing the sensitivity and specificity of plasma biomarkers in discriminating hippocampal volumes in Western elderly subjects. The findings of this study provide evidence of the usefulness of Alzheimer's Association criteria of AD in this sample to discriminante hippocampal atrophy. Our analyses also showed the importance of MRI as a diagnostic tool of AD with specific emphasis on hippocampal atrophy. Our findings have also demonstrated the synergical effect of plasma biomarkers in discriminanting hippocampal atrophy.

This study is the first in the SSA to attempt to discriminante hippocampal volumes based on AD core and non-specific AD plasma protein biomarkers in a sample of adults in the DRC with and without dementia. Our findings should be interpreted considering several limitations, such as the cross-sectional nature of the study, low sensitivity, and lack of amyloid PET imaging confirming AD pathology. These analyses should be further validated in longitudinal data. Another limitation includes a small sample of participants, which limited the detection of differences that could have been clinically and significantly relevant to find adequate discriminative strength of the plasma biomarkers. Thus, future studies should replicate these findings with larger sample sizes. Third, the screening measures used (CSID and AQ) have not been validated in SSA in general and the DRC in particular. Fourth, this study included only subjects with suspected dementia and healthy controls. Those with cognitive difficulties seen in between these two categories (e.g., MCI, subjective memory complaints) were excluded, leaving only the extremes of the dementia spectrum. Future studies should conduct statistical analyses across all 4 groups (healthy controls, MCI, subjective memory complaint and dementia). Furthermore, future studies should also aim to replicate our findings using amyloid and tau brain PET, or mass spectrometry to measure biomarkers. A major caveat is that our AD biomarkers were determined by Simoa, which is not optimal. The gold standard of core AD biomarkers assessment is amyloid and tau brain PET. Thus, continued investigation into racial disparities in AD biomarkers and relation to AD-dementia using these gold standard techniques (e.g., brain amyloid PET, CSF).

Conclusions

Understanding the ability of AD core plasma biomarkers (Aβ-42/40, p-tau 181, and p-tau 217) and non-specific plasma biomarkers (NfL and GFAP) to discriminate hippocampal atrophy in adult individuals is a promising next step in clinical and research settings. While blood biomarkers are not equivalent to an AD diagnosis, they can be utilized as a screening tool before resorting to PET-scan neuroimaging or CSF biomarker analysis. Future studies are needed in which AD-related blood and CSF biomarkers are tested from the same individuals for better discrimination of the hippocampal atrophy . Additionally, larger studies with greater sample sizes and diversity in races and ethnicities should be employed to increase generalizability.

References

1. Rao YL, Ganaraja · B, Murlimanju · B V, Joy T, Krishnamurthy A, Agrawal A. Hippocampus and its involvement in Alzheimer's disease: a review. *SpringerYL Rao, B Ganaraja, BV Murlimanju, T Joy, A Krishnamurthy, A Agrawal3 Biotech, 2022•Springer*. 123AD;12(2):55. doi:10.1007/s13205-022-03123-4
2. Blennow K, medicine HZJ of internal, 2018 undefined. Biomarkers for Alzheimer's disease: current status and prospects for the future. *Wiley Online LibraryK Blennow, H ZetterbergJournal of internal medicine, 2018•Wiley Online Library*. 2018;284(6):643-663. doi:10.1111/joim.12816
3. Babić Leko M, Nikolac Perković M, Klepac N, et al. IL-1β, IL-6, IL-10, and TNFα Single Nucleotide Polymorphisms in Human Influence the Susceptibility to Alzheimer's Disease Pathology. *Journal of Alzheimer's Disease*. 2020;75(3):1029-1047. doi:10.3233/JAD-200056
4. Giacomucci G, Mazzeo S, Bagnoli S, et al. Plasma neurofilament light chain as a biomarker of Alzheimer's disease in Subjective Cognitive Decline and Mild Cognitive Impairment. *SpringerG Giacomucci, S Mazzeo, S Bagnoli, A Ingannato, D Leccese, V Berti, S Padiglioni, G GaldoJournal of neurology, 2022•Springer*. 1234;269(8):4270-4280. doi:10.1007/s00415-022-11055-5
5. Gulisano W, Maugeri D, Baltrons MA, et al. Role of Amyloid-β and Tau Proteins in Alzheimer's Disease: Confuting the Amyloid Cascade. *Journal of Alzheimer's Disease*. 2018;64(s1):S611-S631. doi:10.3233/JAD-179935
6. Kim K, Shin K, Cells KC, 2023 undefined. GFAP as a potential biomarker for Alzheimer's disease: a systematic review and meta-analysis. *mdpi.comKY Kim, KY Shin, KA ChangCells, 2023•mdpi.com*. 2023;12(9). doi:10.3390/cells12091309
7. Lyra Silva NM, Gonçalves RA, Pascoal TA, et al. Pro-inflammatory interleukin-6 signaling links cognitive impairments and peripheral metabolic alterations in Alzheimer's disease. *nature.comNM Lyra e Silva, RA Gonçalves, TA Pascoal, RAS Lima-Filho, EPF Resende, ELM VieiraTranslational psychiatry, 2021•nature.com*. 2021;11(1):251. doi:10.1038/s41398-021-01349-z
8. Jack CR, Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: An updated hypothetical model of dynamic biomarkers. *Lancet Neurol*. 2013;12(2):207-216. doi:10.1016/S1474-4422(12)70291-0
9. Hampel H, Hardy J, Blennow K, et al. The amyloid-β pathway in Alzheimer's disease. *nature.comH Hampel, J Hardy, K Blennow, C Chen, G Perry, SH Kim, VL Villemagne, P AisenMolecular psychiatry, 2021•nature.com*. 2021;26(10):5481-5503. doi:10.1038/s41380-021-01249-0
10. Braak H, Cortex KDTC, 2018 undefined. Spreading of tau pathology in sporadic Alzheimer's disease along cortico-cortical top-down connections. *academic.oup.comH Braak, K Del TrediciCerebral Cortex, 2018•academic.oup.com*. 2018;28(9):3372-3384. doi:10.1093/cercor/bhy152
11. Maruszak A, Silajdžic E, Lee H, et al. Predicting progression to Alzheimer's disease with human hippocampal progenitors exposed to serum. *academic.oup.comA Maruszak, E Silajdžić, H Lee, T Murphy, B Liu, L Shi, C De Lucia, A Douiri, E SaltaBrain, 2023•academic.oup.com*. 2023;146(5):2045-2058. doi:10.1093/brain/awac472

12. Fortin NJ, Agster KL, Eichenbaum HB. Critical role of the hippocampus in memory for sequences of events. *Nat Neurosci*. 2002;5(5):458-462. doi:10.1038/NN834
13. Xiao Y, Hu Y, Huang K. Atrophy of hippocampal subfields relates to memory decline during the pathological progression of Alzheimer's disease. *Front Aging Neurosci*. 2023;15. doi:10.3389/FNAGI.2023.1287122/FULL
14. Constantinides VC, Paraskevas GP, Boufidou F, et al. CSF Aβ42 and Aβ42/Aβ40 ratio in Alzheimer's disease and frontotemporal dementias. *mdpi.comVC Constantinides, GP Paraskevas, F Boufidou, M Bourbouli, ES Pyrgelis, L StefanisDiagnostics, 2023•mdpi.com*. 2023;13(4). doi:10.3390/diagnostics13040783
15. Lewczuk P, Matzen A, Blennow K, et al. Cerebrospinal fluid Aβ 42/40 corresponds better than Aβ 42 to amyloid PET in Alzheimer's disease. *content.iospress.comP Lewczuk, A Matzen, K Blennow, L Parnetti, JL Molinuevo, P Eusebi, J KornhuberJournal of Alzheimer's Disease, 2017•content.iospress.com*. 2017;55(2):813-822. doi:10.3233/JAD-160722
16. Janelidze S, Teunissen CE, Zetterberg H, et al. Head-to-head comparison of 8 plasma amyloid-β 42/40 assays in Alzheimer disease. *jamanetwork.com*. 2021;78(11):1375-1382. doi:10.1001/jamaneurol.2021.3180
17. Thijssen EH, La Joie R, Strom A, et al. Plasma phosphorylated tau 217 and phosphorylated tau 181 as biomarkers in Alzheimer's disease and frontotemporal lobar degeneration: a retrospective diagnostic performance study. *Lancet Neurol*. 2021;20(9):739-752. doi:10.1016/S1474-4422(21)00214-3
18. Ikanga J, Patrick SD, Schwinne M, et al. Sensitivity of the African neuropsychology battery memory subtests and learning slopes in discriminating APOE 4 and amyloid pathology in adult individuals in the Democratic Republic of Congo. *Frontiers in Neurology* . 2024;15. doi:10.3389/FNEUR.2024.1320727/FULL
19. Malek-Ahmadi M, Sabbagh MN, The Cleo Roberts M. Development and Validation of the Alzheimer's Questionnaire (AQ). *J Nat Sci*. 2015;1(5):e104. Accessed December 18, 2022. /pmc/articles/PMC4423544/
20. Hall KS, Gao S, Emsley CL, Ogunniyi AO, Morgan O, Hendrie HC. Community screening interview for dementia (CSI 'D'); performance in five disparate study sites. *Int J Geriatr Psychiatry*. 2000;15(6):521-531. doi:10.1002/1099-1166(200006)15:6<521::aid-gps182>3.0.co;2-f
21. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. American Psychiatric Association; 2013.
22. Guerchet M, M'Belesso P, Mouanga AM, et al. Prevalence of Dementia in Elderly Living in Two Cities of Central Africa: The EDAC Survey. *Dement Geriatr Cogn Disord*. 2010;30(3):261-268. doi:10.1159/000320247
23. Ikanga J, Reyes A, Kaba D, et al. Prevalence of suspected dementia in a sample of adults living in Kinshasa-Democratic Republic of the Congo. *Alzheimer's & Dementia*. Published online 2023. doi:10.1002/ALZ.13003
24. Verberk IMW, Misdorp EO, Koelewijn J, et al. Characterization of pre-analytical sample handling effects on a panel of Alzheimer's disease–related blood-based biomarkers: Results from the Standardization of Alzheimer's Blood Biomarkers

(SABB) working group. *Alzheimer's & Dementia*. 2022;18(8):1484-1497. doi:10.1002/ALZ.12510
25. Wahlund LO, Barkhof F, Fazekas F, et al. A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke*. 2001;32(6):1318-1322. doi:10.1161/01.STR.32.6.1318
26. Claus JJ, Staekenborg SS, Holl DC, et al. Practical use of visual medial temporal lobe atrophy cut-off scores in Alzheimer's disease: validation in a large memory clinic population. *SpringerJJ Claus, SS Staekenborg, DC Holl, JJ Roorda, J Schuur, P Koster, CEM TielkesEuropean radiology, 2017•Springer*. 2017;27(8):3147-3155. doi:10.1007/s00330-016-4726-3
27. Enkirch SJ, Traschütz A, Müller A, et al. The ERICA score: An MR imaging-based visual scoring system for the assessment of entorhinal cortex atrophy in Alzheimer disease. *Radiology*. 2018;288(1):226-233. doi:10.1148/RADIOL.2018171888
28. Mondragón JD, Celada-Borja C, Barinagarrementeria-Aldatz F, Burgos-Jaramillo M, Barragán-Campos HM. Hippocampal Volumetry as a Biomarker for Dementia in People with Low Education. *Dement Geriatr Cogn Dis Extra*. 2016;6(3):486-499. doi:10.1159/000449424)
29. Jack Jr CR, Scott Andrews J, Beach TG, et al. Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's Association Workgroup. *Alzheimer's & Dementia*. Published online June 27, 2024. doi:10.1002/ALZ.13859
30. Ikanga J, Hickle S, Schwinne M, et al. Association Between Hippocampal Volume and African Neuropsychology Memory Tests in Adult Individuals with Probable Alzheimer's Disease in Democratic Republic of Congo. *Journal of Alzheimer's Disease*. 2023;96(1):395-408. doi:10.3233/JAD-230206
31. Wang X, Shi Z, Qiu Y, Sun D, medicine HZB, 2024 undefined. Peripheral GFAP and NfL as early biomarkers for dementia: longitudinal insights from the UK Biobank. *SpringerX Wang, Z Shi, Y Qiu, D Sun, H ZhouBMC medicine, 2024•Springer*. 2024;22(1). doi:10.1186/s12916-024-03418-8
32. Li K, Qu H, Ma M, et al. Correlation Between Brain Structure Atrophy and Plasma Amyloid-β and Phosphorylated Tau in Patients With Alzheimer's Disease and Amnestic Mild Cognitive Impairment Explored by Surface-Based Morphometry. *Front Aging Neurosci*. 2022;14. doi:10.3389/FNAGI.2022.816043/FULL
33. Chong JR, Ashton NJ, Karikari TK, 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. *Wiley Online LibraryJR Chong, NJ Ashton, TK Karikari, T Tanaka, FN Saridin, A Reilhac, EG Robins, YH NaiAlzheimer's & Dementia, 2021•Wiley Online Library*. 2021;17(10):1649-1662. doi:10.1002/alz.12332