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The Association of Alzheimer's Disease-related Blood-based Biomarkers with Cognitive Test Performance in the Congolese Population

By

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Master of Public Health

Epidemiology

_____

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Bachelor of Science in Education
University of Georgia
2021

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ABSTRACT

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By Megan C. Schwinne

Alzheimer's Disease (AD), the leading cause of dementia and a major global burden, is commonly diagnosed via neuroimaging or cerebrospinal fluid (CSF) biomarker testing of phosphorylated tau (p-tau) 181 and amyloid (Aβ) 42/40. However, these diagnostic methods are invasive and expensive. This study aims to examine if AD-related blood-based biomarkers are associated with cognitive test performance, specifically in the Congolese population, where limited research has been conducted. In this cross-sectional study comprised of 81 individuals from the Democratic Republic of Congo (DRC), cognitive tests, including the Alzheimer's Questionnaire (AQ) and the Community Screening Interview for Dementia (CSID) were performed to distinguish dementia cases from healthy controls. Blood draws were then conducted to analyze p-tau 181 and Aβ-42/40 biomarkers. Multiple linear regression models were performed to analyze relationships between the biomarkers and cognitive test performance. Aβ-42/40 was significantly associated with a lower CSID score and a higher AQ score, which is typical of AD ($p<0.001$). These relationships were seen in healthy controls (CSID $p=0.01$, AQ $p=0.03$), but were not significant amongst dementia cases. However, p-tau 181 did not show significant associations with either cognitive test. Common risk factors and potential confounders did not alter these relationships. Understanding relationships between common AD-related cognitive tests and blood-biomarkers is a step towards utilization of blood-based biomarker tests as a screening tool for AD. With that said, further research needs to be conducted to evaluate blood biomarker test efficacy in larger samples and other populations.

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BACKGROUND

Alzheimer's Disease

Dementia, one of the top five causes of death globally, is an umbrella term encompassing a group of characteristic symptoms, which include difficulties with memory, language, problem-solving, and other thinking skills.[1,2] Alzheimer's Disease (AD), the leading cause of dementia in individuals aged 65 or older, is a large global burden, with 40-50 million people currently living with dementia[1] and predicted to be over 150 million by 2050.[3,4] Dementia is a clinical syndrome that does not require a diagnosis via biomarker measurements or PET-scans. However, AD is diagnosed based off of clinical symptoms of dementia along with a distinct biomarker profile or PET-scan.[5] AD is a slowly progressive neurodegenerative disease characterized by amyloid plaques and neurofibrillary tangles.[4] The changes in the brain from AD, such as the degeneration of nerve cells as well as the accumulation of the abnormal proteins, beta-amyloid and phosphorylated tau, are contributors to dementia.[2] While the underlying cause of the pathological changes in AD is still unknown, this multifactorial disease is associated with several risk factors.[4] The predominant risk factor is aging, in which the incidence is higher in age and most cases have a late onset of 65 years or older. Increased risk is also associated with environmental risk factors including air pollution, diet, infections, and metals, as they are proposed to induce oxidative stress and inflammation.[4] Additionally, cardiovascular disease, obesity, diabetes, and other medical conditions are associated with increased risk for AD development.[4] Nonetheless, genetic factors also play a major role, with 70% of AD cases relating to genetic factors.[4] Many cases are inherited in an autosomal dominant pattern and mutations in certain dominant genes, such as Amyloid precursor protein (APP) and apolipoprotein E (ApoE), are associated with AD.

[4] In fact, the e4 allele of the APOE gene is the strongest genetic risk factor for AD and the APOE e2 allele is the strongest genetic protective factor.[6]

Alzheimer's Disease in Congo

Given that AD risk increases with age, it is of specific importance to focus on the African population, since they are aging at an unprecedented rate.[2,7] This demographic transition is occurring faster in low and middle-income countries (LMIC) than it was in the previous century for high-income countries (HIC).[8] Thus, the largest proportion of the predicted increase in AD will take place in LMIC, especially East Asia and Sub-Saharan Africa, where over 70% of individuals with dementia are expected to live in 2040.[7,8] In a population-based prospective cohort study conducted in Congo, mortality risk was more than 2.5 times higher in the dementia group compared to those with normal cognition (HR 2.53; CI 95%, 1.42-4.49; p = 0.001).[7] Also, greater dementia severity was strongly associated with increased mortality risk (HR 1.91; CI 95%, 1.23-2.96; p = 0.004).[7] Given the predicted increase in prevalence of AD in LMIC, such as Congo, and the minimal literature on the effect of AD on the Congolese population, further research in Sub-Saharan Africa is needed in order to provide better screening techniques, resulting in better health outcomes.

Alzheimer's Disease Cognitive Screening Tests

Common neurocognitive tests to screen for AD and related dementias include the Alzheimer's Questionnaire (AQ) and the Community Screening Interview for Dementia (CSID). Developed in the 1990s, The CSID is widely accepted as an appropriate dementia screening tool to use cross-culturally.[7,9] It serves to detect dementia in various populations with diverse

educational, cultural, and linguistic identities.[9] While this instrument is not the gold standard for diagnosis, it has been used in developing countries when higher quality screening instruments are not available. It evaluates the cognitive domains of language and expression, memory, learning, attention and calculation, praxis, orientation in space and time, and language comprehension. The test is comprised of cognitive tests administered to the patient as well as an informant interview about performance in everyday living.[9] Given that cognitive tests are susceptible to educational bias, the combination of the two types of tests yields better sensitivity and specificity for dementia diagnosis.[9]

The AQ, an informant-only assessment developed in 2010, quickly and accurately detects cognitive impairment.[10] It is advantageous in providing questions that require yes or no answers in a weighted format so that an absolute score is calculated, requiring no interpretation for individual components of the test.[10] The questions are also adapted from other commonly utilized informant-based assessments with adjustments to improve simplicity and speed of administering the test.[10] In past trials, the AQ has shown great accuracy with high sensitivities and specificities of 98.6% and 96% respectively.[10] This assessment evaluates the cognitive domains of memory, orientation, functional ability, visuospatial, and language of the participant.

The 42-question CSID scores can range from 0 to 55 with a lower score indicating worse cognition, while the 21-question AQ scores can range from 0 to 26 with a higher score indicating worse cognition. While the two screening tools have some different cognitive domains being tested, they both encompass semantic, executive, and memory knowledge. Given that both assessments have different attributes that may be advantageous to different populations, it is important to utilize multiple screening tools and supplementary tactics to identify cases of AD.

Alzheimer's Disease-related CSF and Blood-based Biomarkers

Given that neurodegenerative diseases, such as AD, are difficult to diagnose clinically, characteristic biomarkers of AD, such as total tau (T-tau), phosphorylated tau (p-tau), amyloid-β 42 (Aβ42), and amyloid-β 40 (Aβ40), are important for research and early diagnosis.[11,12] Increased levels of T-tau and p-tau with decreased Aβ42 in cerebrospinal fluid (CSF) is the biomarker pattern known as the "Alzheimer's CSF Profile", as they reflect key elements of AD pathophysiology.[13] Individuals presenting with AD have the A4 protein, also known as β-amyloid, in their brain tissue. Tau protein is primarily located in the neuronal axons and the tangles are composed of atypically hyperphosphorylated tau with approximately three times more phosphorylated sites than normal tau.[13] Tau normally binds to and stabilizes the neuronal microtubules but the hyperphosphorylation disrupts the microtubules, impairs the plasma and axon flow, and leads to loss of neuronal connectivity.[13] Studies show that patients presenting with early-onset AD, which are those with onset before age 65, have more severe plaque and tangle pathology, while those with late-onset AD have varying AD pathology. A lower Aβ42 reflects aggregation and deposition of protein in the brain. Aβ40 is the most abundant variant of Aβ in CSF so the Aβ42/40 ratio is utilized to compensate for individual differences of the Aβ isoforms to be a better predictor of the presence of brain amyloid than just the plasma concentrations alone.[13,14] A lower plasma Aβ42/40 ratio has been found in AD cases compared to healthy controls when using CSF and blood measures along with increased tau levels in plasma for AD cases.[13] Although obtaining the biomarkers via CSF has been customary practice, obtaining the biomarkers from blood is more accessible than CSF and is preferable for both screening and sampling purposes.[13] However, there are several caveats making blood more challenging than CSF for brain biomarkers. The minimal levels of brain proteins entering the

blood must be measured with high levels of other plasma proteins, which may present interference in analytic methods and dilution.[13] Additionally, the brain proteins released into blood may be degraded by proteases, which can cause a variance that may not be related to changes in the brain.[13] However, novel developments in ultrasensitive immunoassays and mass spectrometry bring promising results for the use of blood biomarkers over CSF biomarkers.

Rationale for Thesis

Substantial research has been implemented to prove that the CSID and AQ cognitive tests as well as CSF biomarkers, such as Aβ42/40 and p-tau 181, can be used to screen for AD; however, current research on the association between AD diagnosis and these biomarkers in blood has been predominantly limited to studies conducted in high-income countries. Very few studies have occurred in Sub-Saharan Africa.

Given the potential educational bias that the CSID and AQ tests have on participants of varying backgrounds and the invasiveness of retrieving biomarkers via CSF, it is critical to determine other plausible screening methods to evaluate individuals for AD. Furthermore, with the increasing prevalence of AD in LMIC, in specific Africa, it is important to focus research on these populations, especially since the majority of AD-related research is conducted in populations of European ancestry and in high-income countries.

Through past research on the associations between AD-related CSF biomarkers with cognitive test results observed in high-income countries and populations of European ancestry, we hypothesized that blood-based AD-related biomarkers would be associated with CSID and AQ results in a population in Sub-Saharan Africa. Decreased Aβ-42/40 and increased p-tau

levels would be associated with a decreased score on the CSID and an increased score on the AQ.

METHODS

Study Design and Participants

From 2019 to 2022, a cross-sectional study using community-based recruitment was carried out in Kinshasa, the capital of the Democratic Republic of Congo (DRC). 1432 individuals were recruited from churches, clinics, hospitals, door-to-door, and older adult associations to then be screened. Eligibility criteria required that participants be 50 years or older, have a close contact to serve as a collateral informant, have no current or past history of neurodevelopmental, mental, psychiatric, or neurogenerative diagnosis other than dementia, able to give informed consent, fluent in French or Lingala, and have adequate sensory perceptual skills to be able to see and draw for cognitive tests. Cognitive test data was collected between 2019-2021 and blood specimens for biomarker analysis were collected between 2021-2022. Only some participants, less than those who had cognitive tests, were given the option to proceed with donating blood specimens. The study was approved by the Ethics Committee and Institutional Review Boards of the University of Kinshasa and written informed consent was obtained from participants as well as financial compensation.

Cognitive Measurements

Participants and their informants were administered the CSID as well as the AQ test to screen for dementia and to be further assigned into the dementia group or the control group, which comprised of individuals with normal cognitive aging. Participants were first classified

using CSID scores as cognitively impaired (CSID score of < 25.5) or as cognitively unimpaired (CSID score of ≥ 25.5). Next, participants were classified within each category of cognition via AQ scores as cognitively impaired (AQ score of > 13) or as cognitively unimpaired (AQ score of ≤ 13). Given the two cognitive tests and classifications, 4 separate groups were created, which were major neurocognitive disorder (CSID < 25.5 and AQ > 13), mild neurocognitive disorder (CSID < 25.5 and AQ ≤ 13), subjective cognitive impairment (CSID ≥ 25.5 and AQ > 13), and normal cognition (CSID ≥ 25.5 and AQ ≤ 13). Only the individuals with major neurocognitive disorder, which were considered to have dementia, and the individuals with normal cognition, which were considered healthy controls, were included for this analysis. Out of the 1432 initial participants, 271 met the above criteria for major neurocognitive disorder or normal cognition, in which 88 individuals were classified as having major neurocognitive disorder and 183 individuals were classified as healthy subjects with normal cognition. Following this classification, an expert panel of neuropsychologists, neurologists, and psychiatrists assessed and confirmed 55 individuals to have major neurocognitive disorder and then matched 59 healthy controls on age, education, and sex. Due to the participants not having PET-scans or CSF biomarker tests in this study, participants with major neurocognitive disorder will be characterized as having dementia and will not be identified as individuals with possible AD.[5]

Descriptive Measurements

Participants were given self-report questionnaires and interviews to obtain demographic, socioeconomic, and medical history information. Individuals were categorized into age groups of 50-64, 65-74, 75-84, and 85+. Education levels were also categorized into levels of primary school (1-6 years), secondary school (7-12 years), some or completion of university (13-17

years), and beyond university (18+ years). Medical residents measured hypertension by using a manual sphygmomanometer and three measurements of systolic and diastolic blood pressure were collected. Having an average systolic blood pressure over 140 mmHg or a diastolic blood pressure over 90 mmHg was defined as hypertension.

Biomarker Measurements

For the individuals that consented to blood donation for blood biomarker measurements, a phlebotomist drew the blood at the Medical Center of Kinshasa (CMK) blood laboratory by venipuncture into ethylenediaminetetraacetic acid (EDTA). Blood samples were centrifuged within 15 minutes and 5 ml were aliquoted into 0.5 ml tubes. The samples were temporarily stored at -20 ^{o} Celsius for less than a week and then at -80 ^{o} C for longer-term storage at the CMK laboratory freezer. The samples were then shipped on dry ice to Emory University laboratory and analyzed by C_2N Diagnostics (amyloid-β 40 and amyloid-β 42 proteins) and by Dr. Blaine Roberts's lab at Emory University (p-tau 181). For the amyloid-β 42/40 ratios, plasma samples were spiked with stable isotope labeled recombinant proteins. Plasma proteins were extracted using proprietary antibodies conjugated to magnetic beads, eluted from the beads, and then digested with a site-specific protease to form C-terminal peptides specific to amyloid-β 40 and amyloid-β 42 proteins. The peptides were separated using micro-flow liquid chromatography and electro-sprayed into the source of a high resolution orbitrap mass spectrometer. This procedure identifies the peptides of interest based on known amino acid sequence and mass to charge ratio. It then quantifies the ion signal intensity from the endogenous peptides by comparison to a calibration curve created with a stable isotope labeled internal standard peptide. The amyloid-β 40 and amyloid-β 42 concentrations were quantified by comparing the signal intensities for the

endogenous peptides to those obtained from the stable isotope labeled proteins spiked into the sample. Amyloid-β 42/40 ratios were measured via the SISAQ^{TM} Platform. To analyze p-tau 181 concentrations, EDTA plasma samples were prepared according to manufacturer's instructions from the p-tau 181 kit v2. Samples were thawed at room temperature for 45 minutes and then centrifuged at 5000xg for 10 minutes. The plasma samples were then diluted four times on bench and measured on the Simoa HDX platform.

Statistical Analysis

Of the study population, 81 of these individuals had both biomarker and cognitive data (43 dementia cases and 38 healthy controls, Figure 1). Preliminary analysis involved obtaining frequencies and means of sex, age, education level, and basic medical history, for the overall sample population and the two groups of differing neurological status. Multiple linear regression models were utilized to analyze associations between cognitive test scores (dependent variable) and blood biomarkers (main independent variable). Neurological status was also a primary indicator variable in a model analyzing associations between cognitive tests or blood biomarkers with status. Analyses considered the overall CSID and AQ scores, as well as domain-specific scores (executive, semantic, and memory) separately. The biomarkers evaluated were p-tau 181 and Aβ-42/40 values. These models analyzed associations overall as well as stratified by neurological status (dementia or healthy control). Aβ-42/40 was modeled in 0.01 increments, its standard deviation, to represent more meaningful findings in relation to associations with cognitive test scores. All models controlled for age, sex, and education, as these covariates may be possible confounders and bias the measures of association. The results were expressed as β coefficients with corresponding 95% confidence intervals. Tests for potential interactions

between biomarkers and covariates, including sex, age, education, and APOE status were conducted to understand if these variables significantly affect the relationship between biomarkers and cognitive tests. Tests for interaction involving the variables age and education were assessed on a continuous scale. The presence of the e4 allele in APOE genotypes, a known risk factor for AD, was assessed as a categorical variable, in which individuals were dichotomized as either having the e4 allele or not.[6] All statistical tests were two-sided, and p-values < 0.05 were considered to be statistically significant. All analyses were conducted using SAS version 9.4 statistical software.

RESULTS

Descriptive and Clinical Characteristics of the Sample Population

Baseline characteristics of the 81 individuals, including demographics and medical history, were reported in Table 1. The sample population consisted of 43 dementia cases and 38 healthy controls with a mean age of 73 years (ranging from 50-88 years old). Sex, body mass index, age groups, and education levels were similar between the dementia and control groups, confirming matching was performed appropriately. Regarding medical history, a large proportion of the participants (53%) had hypertension, with more dementia cases having prevalent hypertension compared to the controls (60% and 45% respectively). Additionally, more of the dementia cases (28%) reported alcohol abuse compared to the control group (11%). The remaining relevant medical history and mental conditions, such as high cholesterol, poor nutrition, anxiety, and depression were minimally reported among the sample.

Descriptive Characteristics of Cognitive Tests

Upon comparison of CSID and AQ cognitive test scores between dementia cases and healthy controls (Table 2), all overall scores as well as the semantic, executive, and memory domain scores were significantly different between the two groups ($p < 0.01$). Given that the higher the CSID score, the better the cognition, on average, the healthy controls scored higher in all CSID domains compared to the dementia cases. The CSID memory domain had the largest difference between groups and the CSID semantic domain scores were the least impacted. Furthermore, the healthy control group scored lower on the AQ cognitive test compared to the dementia cases. Again, the AQ memory domain yielded the largest difference and the AQ semantic domain scores yielded the smallest difference between groups.

Descriptive Characteristics of Blood Biomarkers

Average, Aβ-42, Aβ-40, Aβ-42/40 ratio, and p-tau 181 measures are represented in Table 2. Aβ-42/40 was significantly larger in the control group compared to the dementia group ($p=0.002$). Aβ-42 and Aβ-40 were not significantly different between groups, but only the ratio is clinically relevant for being a biomarker of AD. While Aβ-42/40 was statistically significant, analysis of p-tau 181 yielded essentially identical concentrations between the two groups of differing neurological statuses ($p=0.94$).

Association between Cognitive Tests and Biomarkers

Upon exploration of potential associations between blood biomarkers and cognitive test scores among the whole study population, Aβ-42/40 was strongly associated with both CSID and AQ overall scores ($p<0.001$), while p-tau 181 was not. CSID overall scores and Aβ-42/40 demonstrated a positive association while AQ overall scores and Aβ-42/40 demonstrated a

negative association. For every 0.01 increase in Aβ-42/40, on average the CSID overall score was 3.77 points higher, after adjusting for age, sex, and education. Furthermore, for every 0.01 increase in Aβ-42/40, on average the AQ overall score was 4.58 points lower after adjustment. Aβ-42/40 was only significantly associated with the CSID test (p=0.01) and the AQ test (p=0.03) among the healthy controls and not among the individuals with dementia (Table 3). Potential interaction between biomarkers with covariates, including sex, age, education, and APOE status was assessed. The test for interactions between these variables all resulted in p-values > 0.05, meaning interaction was not present and these variables did not modify the relationship between cognitive tests and biomarkers.

Cognitive tests were also stratified into their 3 domains, semantic, executive, and memory knowledge (Table 4). Only the CSID semantic domain was associated with p-tau 181 (p=0.03), while all other domains were not significantly associated with this biomarker. However, all cognitive test domains were associated with Aβ-42/40 ($p \leq 0.001$) except for the CSID semantic domain. For both the CSID and AQ tests, the memory domain had the strongest difference by 0.01 Aβ-42/40 increments (3.47 and -2.4 respectively), while the semantic domain had the smallest rate of change (0.38 and -0.78 respectively).

DISCUSSION

Major Findings

In a community-based sample from the DRC, we found associations of blood Aβ-42/40 with CSID and AQ scores, with lower Aβ-42/40 correlating with a lower CSID score and higher AQ score, which is characteristic of AD and other dementias. These relationships were present for healthy controls but not participants with dementia. However, circulating p-tau 181 was not

associated with either cognitive test. Age, sex, education, and presence of the APOE e4 allele, which is a major known risk factor for AD, did not significantly modify these associations.

Previous Literature

Previous research suggests that the use of biomarkers alongside neurocognitive tests is the future of clinical practice, as they aid in early identification and potential for prevention of AD manifestation or progression.[3] The literature has repeatedly shown that reduced Aβ-42/40 and high p-tau is characteristic of AD, and these relationships have commonly been seen in CSF and more recently studied in blood.[13] Plasma Aβ-42/40 had similar results with CSF tests, however, p-tau did not align as clearly and showed a weaker relationship.[15] This supports these present findings since Aβ-42/40 was associated with tests displaying cognitive impairment, but p-tau was not. With that said, a recent study investigating associations between these plasma biomarkers and their relationship with AD-associated neuroimaging results show that both biomarkers are significant.[16] They also found that the most significant relationship was revealed when p-tau 181 and Aβ-42 were utilized together in a ratio, which was not considered in this study.

Biological Plausibility

Memory significantly differs for those with dementia most likely due to pathophysiological changes, such as the accumulation of amyloid-beta and the development of hyperphosphorylated tau protein tangles.[3] This then leads to secretion of neurotoxins and inflammatory factors, resulting in neuronal death in specific brain areas and causes memory impairment.

Aβ-42/40's apparent association compared to p-tau 181's insignificant association with cognitive status may be due to the differences in pathophysiological processes between the biomarkers. Aβ-42/40 is an index for increased neurotoxicity, amyloidogenicity, as well as disease severity, whereas p-tau 181 is an index for tau hyperphosphorylation, neurofibrillary tangles formation, and degenerative axonal loss in the brain.[16] These processes may manifest differently in blood samples compared to CSF samples or may differ between individuals. It is also possible that the lack of association between p-tau 181 and cognitive status in this study is due to the small sample size and may not represent a true finding.

While blood is cheaper, less invasive, and more accessible to sample from individuals compared to CSF, there are inevitable caveats to blood biomarker analysis.[17] It is more difficult to reliably measure blood biomarkers that are related to cognitive disorders because the biomarkers are present at lower concentrations in the blood compared to CSF, which is closer to the brain and allows for a free exchange of molecules.[13,15,18] Only a fraction of brain proteins enters the blood stream and dilution may occur. Brain proteins released in the blood may be degraded by proteases, metabolized in the liver, or cleared by kidneys, leading to potential for varying measurements that may not necessarily be representative of cognitive impairment. Lastly, the low levels of brain proteins entering the blood are mixed in a matrix containing high levels of unrelated plasma proteins that may skew results.[13]

Strengths and Limitations

This study had numerous strengths through the study design, setting, and statistical analyses methods. First, Sub-Saharan Africa, specifically the DRC, is an understudied area for dementia and AD, therefore this research strengthens and adds to knowledge of AD and its

associated biomarkers for this population. The research staff were also familiar with the area and the population of interest so there was enhanced partnership and no language barrier. Third, the sample was relatively healthy and varied in age, sex, and education levels. Furthermore, multiple cognitive tests (CSID and AQ) as well as an expert panel were utilized to establish and confirm the participants cognitive ability to prevent misclassification. Additionally, dementia cases and healthy controls were matched on age, sex, and education as part of the study design but these covariates were also controlled for in analysis, decreasing the possibility of confounding. Lastly, statistical tests prevented further potential bias by confirming there were no outliers or key variables causing interaction.

As with all research studies, there are inevitably limitations. While Sub-Saharan Africa, specifically DRC is an understudied area, the population within the region lacks variability in race and ethnicity, so there is lack of generalizability to other populations. Additionally, given the location, it is a complicated setting with less access to advanced technology. Variables that may have been risk factors or confounders, such as physical activity and family history were not considered and the sample size of 81 is rather low, decreasing the study power. Lastly, there was a discrepancy in the number of questions in this study's CSID test compared to the most common CSID test (36 vs. 42 questions), which decreases comparability.

CONCLUSION

Understanding the AD-specific blood biomarkers Aβ-42/40 and p-tau 181 relationships with neurocognitive tests related to AD is a promising next step in the implementation of blood-based biomarkers in order to overcome access and cost barriers. Instruments for quantifying blood biomarkers are becoming more sensitive and implementation is increasing. 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 comparison and further validation. Additionally, larger studies with greater sample sizes and diversity in races and ethnicities should be employed to increase generalizability.

REFERENCES

1. Global Burden of Disease Collaborators. (2019). Global, regional, and national burden of Alzheimer's disease and other dementias, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol, 18(1), 88-106. https://doi.org/10.1016/S1474-4422(18)30403-4
2. 2022 Alzheimer's disease facts and figures. (2022). Alzheimers Dement, 18(4), 700-789. https://doi.org/10.1002/alz.12638
3. Tahami Monfared, A. A., Byrnes, M. J., White, L. A., & Zhang, Q. (2022). Alzheimer's Disease: Epidemiology and Clinical Progression. Neurol Ther, 11(2), 553-569. https://doi.org/10.1007/s40120-022-00338-8
4. Breijyeh, Z., & Karaman, R. (2020). Comprehensive Review on Alzheimer's Disease: Causes and Treatment. Molecules, 25(24). https://doi.org/10.3390/molecules25245789
5. Dubois, B., Villain, N., Frisoni, G. B., Rabinovici, G. D., Sabbagh, M., Cappa, S., . . . Feldman, H. H. (2021). Clinical diagnosis of Alzheimer's disease: recommendations of the International Working Group. Lancet Neurol, 20(6), 484-496. https://doi.org/10.1016/S1474-4422(21)00066-1
6. Serrano-Pozo, A., Das, S., & Hyman, B. T. (2021). APOE and Alzheimer's disease: advances in genetics, pathophysiology, and therapeutic approaches. Lancet Neurol, 20(1), 68-80. https://doi.org/10.1016/S1474-4422(20)30412-9
7. Samba, H., Guerchet, M., Ndamba-Bandzouzi, B., Mbelesso, P., Lacroix, P., Dartigues, J. F., & Preux, P. M. (2016). Dementia-associated mortality and its predictors among older adults in sub-Saharan Africa: results from a 2-year follow-up in Congo (the EPIDEMCA-FU study). Age Ageing, 45(5), 681-687. https://doi.org/10.1093/ageing/afw097
8. Guerchet, M., Mbelesso, P., Ndamba-Bandzouzi, B., Pilleron, S., Desormais, I., Lacroix, P., . . . group, E. (2014). Epidemiology of dementia in Central Africa (EPIDEMCA): protocol for a multicentre population-based study in rural and urban areas of the Central African Republic and the Republic of Congo. Springerplus, 3, 338. https://doi.org/10.1186/2193-1801-3-338
9. Hall, K. S., Gao, S., Emsley, C. L., Ogunniyi, A. O., Morgan, O., & Hendrie, H. C. (2000). Community screening interview for dementia (CSI 'D'); performance in five disparate study sites. Int J Geriatr Psychiatry, 15(6), 521-531. https://doi.org/10.1002/1099-1166(200006)15:6<521::aid-gps182>3.0.co;2-f
10. Sabbagh, M. N., Malek-Ahmadi, M., Kataria, R., Belden, C. M., Connor, D. J., Pearson, C., . . . Singh, U. (2010). The Alzheimer's questionnaire: a proof of concept study for a new informant-based dementia assessment. J Alzheimers Dis, 22(3), 1015-1021. https://doi.org/10.3233/JAD-2010-101185
11. Olsson, B., Lautner, R., Andreasson, U., Öhrfelt, A., Portelius, E., Bjerke, M., . . . Zetterberg, H. (2016). CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol, 15(7), 673-684. https://doi.org/10.1016/S1474-4422(16)00070-3
12. Leuzy, A., Mattsson-Carlgren, N., Palmqvist, S., Janelidze, S., Dage, J. L., & Hansson, O. (2022). Blood-based biomarkers for Alzheimer's disease. EMBO Mol Med, 14(1), e14408. https://doi.org/10.15252/emmm.202114408

13. Blennow, K., & Zetterberg, H. (2018). Biomarkers for Alzheimer's disease: current status and prospects for the future. J Intern Med, 284(6), 643-663. https://doi.org/10.1111/joim.12816
14. West, T., Kirmess, K. M., Meyer, M. R., Holubasch, M. S., Knapik, S. S., Hu, Y., . . . Yarasheski, K. E. (2021). A blood-based diagnostic test incorporating plasma Aβ42/40 ratio, ApoE proteotype, and age accurately identifies brain amyloid status: findings from a multi cohort validity analysis. Mol Neurodegener, 16(1), 30. https://doi.org/10.1186/s13024-021-00451-6
15. Zetterberg, H., & Burnham, S. C. (2019). Blood-based molecular biomarkers for Alzheimer's disease. Mol Brain, 12(1), 26. https://doi.org/10.1186/s13041-019-0448-1
16. Chong, J. R., Ashton, N. J., Karikari, T. K., Tanaka, T., Saridin, F. N., Reilhac, A., . . . Chen, C. P. (2021). 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. Alzheimers Dement, 17(10), 1649-1662. https://doi.org/10.1002/alz.12332
17. O'Bryant, S. E., Gupta, V., Henriksen, K., Edwards, M., Jeromin, A., Lista, S., . . . groups, S.-B. a. B. w. (2015). Guidelines for the standardization of preanalytic variables for blood-based biomarker studies in Alzheimer's disease research. Alzheimers Dement, 11(5), 549-560. https://doi.org/10.1016/j.jalz.2014.08.099
18. Andreasson, U., Blennow, K., & Zetterberg, H. (2016). Update on ultrasensitive technologies to facilitate research on blood biomarkers for central nervous system disorders. Alzheimers Dement (Amst), 3, 98-102. https://doi.org/10.1016/j.dadm.2016.05.005

TABLES AND FIGURES

Table 1. Descriptive Characteristics of the Sample Population, Stratified by Neurological Status

Variable, n (%)	**Healthy Controls** (n = 38)	**Dementia Cases** (n = 43)	**Overall** (n = 81)
Demographics			
Male	16 (42%)	19 (44%)	35 (43%)
Body Mass index, kg/m^2 *	24.6 (4.1)	24.8 (4.4)	24.7 (4.3)
Age, years *	71.7 (7.9)	74.0 (8.1)	73.0 (8.0)
Age Groups, years			
50-64	6 (16%)	5 (12%)	11 (14%)
65-74	16 (42%)	13 (30%)	29 (36%)
75-84	16 (42%)	21 (49%)	37 (46%)
85+	0 (0%)	4 (9%)	4 (5%)
Years of Education *	9.2 (5.3)	7.3 (5.5)	8.2 (5.4)
Education Level			
Primary School (1-6 years)	2 (5%)	6 (14%)	8 (10%)
Secondary School (7-12 years)	12 (32%)	16 (37%)	28 (35%)
Some/Completed University (13-17 years)	14 (37%)	13 (30%)	27 (33%)
Beyond University (18+ years)	10 (26%)	8 (19%)	18 (22%)
APOE e4			
Presence of ≥ e4 Allele †	21 (26%)	19 (24%)	40 (50%)
e2/e4 Genotype	0 (0%)	2 (3%)	2 (3%)
e2/e3 Genotype			
e3/e3 Genotype			
e3/e4 Genotype	17 (21%)	13 (16%)	30 (38%)
e4/e4 Genotype	4 (5%)	4 (5%)	8 (10%)
Medical History			
Hypertension	17 (45%)	26 (60%)	43 (53%)
High Cholesterol	1 (3%)	1 (2%)	2 (2%)
Poor Nutrition	0 (0%)	1 (2%)	1 (1%)
Stroke	0 (0%)	2 (5%)	2 (2%)
Tobacco Abuse	5 (13%)	4 (9%)	9 (11%)
Alcohol Abuse	4 (11%)	12 (28%)	16 (20%)
Anxiety	2 (5%)	3 (7%)	5 (6%)
Depression	2 (5%)	5 (12%)	7 (9%)

*This variable is reported as mean (SD)

†These values may not sum to the total due to missing data

Table 2. Descriptive Data of Cognitive Tests and Biomarkers, stratified by Neurological Status

Variable, Mean (SD)	**Controls** (n = 38)	**Dementia** (n = 43)	**Overall** (n = 81)	**β_1 (95% CI) ***	**p-value**
Cognitive Tests					
CSID					
Overall Score	31.1 (4.2)	19.7 (5.6)	25.0 (7.6)	-11.0 (-13.2, -8.8)	<.001
CSID Semantic Domain Score	9.9 (0.3)	8.7 (1.8)	9.3 (1.5)	-1.2 (-1.8, -0.6)	0.003
CSID Executive Domain Score	14.9 (2.0)	10.0 (3.8)	12.3 (3.9)	-4.6 (-6.0, -3.3)	<.001
CSID Memory Domain Score	20.7 (4.3)	10.6 (5.1)	15.3 (6.9)	-9.9 (-12.0, -7.8)	<.001
AQ Test					
Overall Score	4.3 (5.4)	19.1 (3.9)	12.1 (8.8)	14.7 (12.6, 16.9)	<.001
AQ Semantic Domain Score	0.2 (1.0)	2.3 (1.7)	1.3 (1.7)	2.0 (1.4, 2.7)	<.001
AQ Executive Domain Score	1.4 (1.7)	6.3 (1.8)	4.0 (3.0)	4.8 (4.0, 5.6)	<.001
AQ Memory Domain Score	2.7 (3.0)	10.4 (2.7)	6.8 (4.8)	7.8 (6.5, 9.1)	<.001
Blood Biomarkers					
Aβ-42/40	0.106 (0.009)	0.099 (0.008)	0.102 (0.009)	-0.006 (-0.009, -0.002)	0.002
Aβ-42, ng/ml	51.0 (10.8)	47.8 (11.8)	49.3 (11.4)	-3.8 (-8.9, 1.4)	0.152
Aβ-40, ng/ml	486.0 (105.1)	483.4 (117.0)	484.6 (111.0)	-11.7 (-62.5, 39.0)	0.647
p-tau 181, ng/ml	1.5 (1.4)	1.6 (1.4)	1.6 (1.4)	0.02 (-0.6, 0.7)	0.939

*Results from linear regression models are adjusted for age, sex, and education.

Table 3. Association Between Cognitive Tests and Biomarkers, Overall and Stratified by Neurological Status

Test	Biomarker	Population	β_1 (95% CI) *	p-value
Overall CSID Score	p-tau 181, ng/ml	Overall	-0.63 (-1.99, 0.73)	0.36
		Dementia	-0.95 (-2.44, 0.53)	0.20
		Controls	-0.50 (-1.73, 0.74)	0.42
	Aβ-42/40 ǂ	Overall	3.77 (1.96, 5.58)	<.001
		Dementia	1.89 (0.68, 4.44)	0.14
		Controls	2.08 (0.62, 3.54)	0.01
Overall AQ Score	p-tau 181, ng/ml	Overall	0.97 (-0.65, 2.58)	0.23
		Dementia	0.92 (-0.18, 2.02)	0.10
		Controls	1.01 (-0.63, 2.65)	0.22
	Aβ-42/40 ǂ	Overall	-4.58 (-6.73, -2.43)	<.001
		Dementia	-1.37 (-3.32, 0.59)	0.17
		Controls	-2.24 (-4.28, -0.20)	0.03

*Results from linear regression models are adjusted for age, sex, and education.

ǂ Aβ-42/40 is modeled in 0.01 increments.

Table 4. Association Between Cognitive Test Domains and Biomarkers

Test	**p-tau 181, ng/ml**		**Aβ-42/40 ŧ**	
	β_1 (95% CI) *	**p-value**	**β_1 (95% CI) ***	**p-value**
CSID				
Overall Score	-0.63 (-2.0, 0.7)	0.36	3.77 (2.0, 5.6)	<.001
Semantic Domain Score	-0.30 (-0.6, -0.02)	0.03	0.38 (-0.01, 0.8)	0.06
Executive Domain Score	-0.51 (-1.2, 0.2)	0.15	1.78 (0.9, 2.7)	<.001
Memory Domain Score	-0.20 (-1.5, 1.1)	0.75	3.47 (1.8, 5.1)	<.001
AQ				
Overall Score	0.97 (-0.6, 2.6)	0.23	-4.58 (-6.7, -2.4)	<.001
Semantic Domain Score	0.20 (-0.1, 0.5)	0.23	-0.78 (-1.2, -0.3)	<.001
Executive Domain Score	0.29 (-0.3, 0.8)	0.29	-1.27 (-2.0, -0.5)	0.001
Memory Domain Score	0.39 (-0.5, 1.3)	0.39	-2.4 (-3.7, -1.2)	<.001

*Results from linear regression models are adjusted for age, sex, and education.

ŧ Aβ-42/40 is modeled in 0.01 increments.

Supplement 1. Assessing Interaction between Biomarkers and Covariates

Test	Biomarker	p-value for Interaction			
		Age	**Sex**	**Education**	**APOE e4 Allele**
CSID Total Score	p-tau 181, ng/ml	0.57	0.10	0.17	0.18
	Aβ-42/40 ǂ	0.76	0.43	0.41	0.27
Overall AQ Score	p-tau 181, ng/ml	0.80	0.21	0.30	0.18
	Aβ-42/40 ǂ	0.45	0.24	0.33	0.07

ǂ Aβ-42/40 is modeled in 0.01 increments.

Figure 1. Flow Diagram of Participant Recruitment