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Longitudinal Changes in Blood Biomarkers of Clinical Alzheimer Disease in a Biracial Population Sample

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ABSTRACT

Background and Objectives: Recent studies suggest the utility of blood biomarkers in detecting changes in neurodegenerative disorders. The objective of our research is to test the hypothesis that the longitudinal changes in total tau (t-tau), neurofilament light (Nf-L), and glial fibrillary acidic protein (GFAP) is associated with structural MRI and the development of clinical Alzheimer's disease (AD), and cognitive decline.

Methods: Data came from a population-based sample with serum concentrations of t-tau, Nf-L, and GFAP and cognitive characteristics measured over 17 years. The inclusion criteria for this investigation were based on participants with blood samples, cognitive function testing, and clinical diagnosis for AD. The longitudinal changes in the serum biomarkers were examined using linear mixed models for log10 transformed concentrations.

Results: In 1,327 participants (60% Black participants and 60% women, the concentration of t-tau increased annually by 4.8% (95% CI= 4.0, 5.6) and Nf-L by 5.9% (95% CI= 5.4, 6.4). The longitudinal change in GFAP was higher among Black participants than White participants (4.4% vs. 3.5% per year, p=0.028). Baseline MRI characteristics were associated with the longitudinal changes in serum biomarkers of clinical AD. Specifically, a higher baseline third ventricular volume was associated with a higher rate of increase in the concentration of t-tau, and

white matter hyperintensities predicted a higher rate of increase in Nf-L. The rate of change in concentrations of t-tau, NF-L, and GFAP was significantly higher among those who developed clinical AD than those with no cognitive impairment. For each standard deviation unit decline in global cognition, longitudinal change in t-tau increased by 81% (95% CI= 76, 86), Nf-L by 113% (95% CI= 105, 120), and GFAP by 66% (95% CI= 62, 70).

Discussion: Blood biomarkers showed significant longitudinal changes corresponding to cognitive decline, clinical AD, and structural MRI characteristics. Our findings show that longitudinal changes in serum biomarkers were associated with several cognitive endophenotypes.

Glossary: t-tau= total tau; Nf-L= Neurofilament Light; GFAP= Glial Fibrillary Acidic Protein; AD= Alzheimer's disease; MRI= Magnetic Resonance Imaging; CI = Confidence Interval

Classification of Evidence: The study found Class II evidence that longitudinal changes in serum t-tau, Nf-L, and GFAP were associated with cognitive decline and the development of clinical AD in people over age 65.

INTRODUCTION

Blood-based biomarkers can serve as secondary outcomes in therapeutic trials for Alzheimer's disease and related dementias.[1-3] The use of blood biomarkers in clinical trials requires understanding the longitudinal changes in these biomarkers and how they are connected to brain characteristics and cognitive decline, leading to clinical AD. Several studies report the relation of plasma total tau with cognitive decline[4-7] and changes in hippocampal volume and neurofibrillary

tangles.[8] Several studies also show the relation of the neurofilament light chain (Nf-L) to the risk of neurodegeneration, leading to cognitive impairment and dementia.[9-11] The glial fibrillary acidic protein (GFAP) that has a central role in the glial cell activity and their higher concentrations predict cognitive impairment[12-13] leading to AD.[14] We found that serum concentrations of t-tau, Nf-L, and GFAP predicted the development of clinical AD and cognitive decline.[15] However, this is the first study that looks at the three biomarkers in the same cohort and examines the longitudinal changes in these blood biomarkers that might help us better understand clinical AD pathogenesis and alterations in brain morphology.

To address this research area, we investigated the longitudinal changes in serum blood biomarkers of t-tau, Nf-L, and GFAP over 17 years in a biracial population sample. We tested whether these changes differed between Black and White participants. Second, we tested whether baseline structural MRI characteristics predicted the longitudinal changes in these blood biomarkers. We examined the longitudinal change in these biomarkers among those who progressed to clinical AD and those with no cognitive impairment. Finally, we examined the rate of change in these biomarkers with the rate of change in global cognition, memory, and perceptual speed score tests.

METHODS

Study Participants

The Chicago Health and Aging Project (CHAP) enrolled participants based on a door-to-door census in four Chicago neighborhoods with substantial proportions of Black and White residents.[16] Inclusion criteria required participants to live in the geographical area, be over age 65, and consent to be enrolled in the population study. About one-third of participants were

selected for a clinical assessment for AD when they also provided blood samples stored below 80°C freezers. The selections were made evenly between Black and White participant groups. The CHAP study collected blood samples from 5,696 participants with 11,600 blood draws. Of those, 1,327 participants with 3,000 blood samples were selected for participants who provided multiple blood draws and underwent a clinical diagnosis for AD.

Of the 5,696 participants with blood draws, 1,534 underwent clinical evaluations, of whom we selected all 454 participants had four or more blood draws and two or more cognitive assessments. The remaining 843 participants were randomly selected from 3,148 participants with three or fewer blood draws and two or more population assessments of cognitive tests. We compared the demographic characteristics of 1,327 participants selected for blood evaluations and 5,696 participants with blood samples. We found they had similar age, race and ethnicity, sex, and the ApoE4 allele frequency.

Standard Protocol Approvals, Registrations, and Patient Consents:

The Institutional Review Board of the Rush University Medical Center approved the study protocols, and all participants provided written consent for blood collection, population interviews, and clinical evaluations. Data that support study findings are available through our research data portal, https://riha.rush.edu/dataportal.html.

Quantification of Serum T-tau, Nf-L, and GFAP

After the blood draws during in-home assessments, study personnel collected and transported serum samples on dry ice to Rush Alzheimer's Disease Center Biorepository to be frozen in –80C ultra-low freezers. All serum samples for this analysis were collected and frozen between 1994 and 2012. In mid-2019, we extracted previously unthawed samples and shipped them to Quanterix Corporation (Billerica, MA) by FedEx on dry ice. We assayed three neuronal cytoskeletal biomarkers using an ultrasensitive immunoassay performed in duplicates using the bead-based HD platform and the Neurology 4Plex A kit. The coefficient of variation ranged from 7.3% for t-tau and 3.0% for Nf-L and GFAP. We used the mean concentration based on the average of duplicate measurements for all our analyses.

Covariates

Race and ethnicity were assessed using the 1990 US census questions. We asked the following question, "What do you feel is the racial category which best describes yourself?" with the possible responses, "White," "Black," "American Indian," "Eskimo," "Aleut," "Asian/Pacific Islander," or "Refused." We also asked, "Do you consider yourself to be of Hispanic origin?" with possible responses "Yes," "No," or "Refused." The study had a small fraction (<1%) of Hispanic origin, and participants described themselves as either "Black" or "White." We also asked participants their date of birth to determine their age, the number of years of formal schooling completed, and sex at the time of birth. We examined the APOE genotype at the Broad Institute using two SNPs: rs7412 and rs429358 using the hME Sequenom MassARRAY® platform. Genotyping success rate was 100% for SNP rs7412 and 99.8% for SNP rs429358.

Both SNPs were in Hardy-Weinberg equilibrium, and we created an indicator variable for participants with one or more copies of the APOE ε4 allele.

Clinical Diagnosis of AD and Dementia

The clinical diagnosis for AD and dementia followed NINCDS/ADRDA guidelines requiring a history of cognitive decline and impairment in at least two cognitive domains.[17] To minimize random variability and improve diagnostic decisions across clinicians and time, we developed educationally adjusted cut points on 11 tests[18] and used impairment ratings for five cognitive domains: orientation, attention, memory, language, and perception.[19] A neuropsychologist, who had access to all cognitive data, education, occupation, and ratings of sensorimotor problems and motivation, agreed or disagreed with each cognitive domain rating and supplied a revised rating in the event of disagreement. A board-certified neurologist with access to all clinical data then agreed or disagreed with the diagnosis algorithm and provided a revised diagnosis in case of a dispute. Dementia requires a history of cognitive decline and evidence of impairment in at least two cognitive domains, one of which must be memory to meet the criteria for AD.[17]

Global and Individual Tests of Cognition

A brief battery of four cognitive tests was administered to participants during in-home population assessments. We derived a global measure of cognitive function by averaging four tests – two tests of episodic memory, one test of executive function, and the MMSE after centering and scaling each to the baseline mean and standard deviation of the original cohort.[20] Individual cognitive tests were based on standardized tests of memory scores, executive function-based speed scores, and the MMSE.

Structural MRI Evaluations

All participants were scanned at the High-Tech Imaging Center, Palos Heights, IL using the same General Electric 1.5T scanner. Three imaging sequences used fluid-attenuated inversion recovery, SPGR with an echo-time minimum, and double-spin echo with a repetition time of 2100 ms.[21] The scans were digitally transferred to UC Davis for processing and analysis. Briefly, non-brain tissue was removed using an atlas-based method and nonlinearly registered by a cubic B-spline deformation template adapted for those over 60.[22] The B-spline deformation was modeled using a spatially smooth thin-plate spline interpolation. Structural MRI images were processed to remove the skull using an atlas-based method.[23,24] Gray, white, and CSF tissues segmentation algorithm was designed to enhance accuracy at likely tissue boundaries after correcting image intensity inhomogeneities. A multi-atlas hippocampal segmentation algorithm computed 25 hippocampal and ventricular volumes.[24] A registration-based method using Das et al.[26] consisted of an initial probabilistic segmentation of GM, WM, and CSF after intensity inhomogeneity correction using segmentation methods.[27] For each boundary voxel on the GM/WM boundary, the thickness was calculated as the distance moved under the registration transformation and propagated across the GM mask.

Statistical Analysis

Means, standard deviations, and frequencies describe the baseline demographic and cognitive characteristics, such as age, the number of formal years of education completed, self-reported race and ethnicity, sex of the participant, and four neuropsychological test scores. The distribution of t-tau, Nf-L, and GFAP were positively skewed, and these biomarkers were log10 transformed for longitudinal regression analysis. All regression models adjusted for age at first

blood assay (centered at 80), education (centered at 12 years), female sex, Black race, chronic health conditions, and the *APOE* ε4 alleles.

The first analyses modeled the longitudinal person-specific changes in the blood serum biomarkers using a linear mixed-effects regression model with person-specific intercept and slope.[28] The time since baseline blood assessment, in years, captured the annual rate of change in log10 transformed blood biomarkers over time. We used interactions of the time since baseline measurement with an indicator for Black race, female sex, the APOE ε4 alleles, and education to test whether the annual change in log10 transformed blood biomarkers was different by these characteristics. We also included each baseline MRI characteristic – total brain volume, white matter hyperintensities, hippocampal, lateral, and third ventricular volumes and the interaction with the time variable used to test the association of these characteristics with the change in serum biomarkers. In the third set of analyses, we stratified participants by clinical AD status, participants who developed clinical AD, and participants with no cognitive impairment. Using a linear mixed-effects model, we examined the rate of change in log10 transformed blood biomarkers over time. Finally, we estimated the demographic-adjusted person-specific rate of change in cognitive function and the rate of change in the log10 transformed regression model. A regression model with a person-specific rate of change as the predictor and the person-specific rate of change in blood biomarkers was used to examine the relation of cognitive decline with change in blood biomarkers. All linear mixed-effects regression models were performed using the *nlme* library, and graphical representations were performed with the R program.[29]

Data Availability

The data used for publication within the article is available upon request for replication from the study data portal www.riha.rush.edu/dataportal.html.

RESULTS

The study sample consisted of 1,327 participants with 3,000 blood draws for a median of 3 draws and an average follow-up of 6.0 years (maximum 17 years). The average age of the participants at the first blood draw was 78.7 (SD= 6.3) years, with average education of 12.5 (SD=3.6) years (Table 1). The study sample consisted of 61% Black participants and 62% women. At the first blood draw, the average total brain volume was 853cc. The average white matter hyperintensity (WMH) was 7.29cc and did not differ between Black and White participants (p=0.25). However, after adjusting for total brain volume and demographic characteristics, the hippocampal volume was 0.23cc (95% CI= 0.10, 0.35) lower among Black participants than White participants.

The baseline demographic characteristics of participants selected for blood biomarkers (N=1,327) did not differ significantly from the entire cohort (N=10,802) and those who provided blood samples (N=5,696). The global cognitive function test scores were significantly higher among White participants than Black participants (Table 1). However, we found no significant differences in the rate of cognitive decline between White and Black participants. In our sample, Black participants have a higher prevalence of clinical AD. However, the incidence rates were similar between White and Black participants. The follow-up time was longer in White participants by about one year compared to Black participants, and the number of blood draws was similar between White and Black participants.

The log10 transformed geometric mean (95% CI) and raw mean concentrations of serum biomarkers are also shown in Table 1. The baseline concentration of t-tau did not differ between Black and White participants. However, Nf-L was significantly lower by 13.7% (95% CI= 7.5, 19.5) among Black participants than White participants, which reduced to 6.8% (95% CI= 0.4, 12.8) after adjusting for age. The concentration of GFAP among Black participants was also lower by 9.3% (95% CI= 3.5, 14.7) compared to White participants. However, this difference reduced to 1.2% (95% CI= -3.8, 7.3) after adjusting for age and was no longer statistically different between Black and White participants (p=0.51).

Longitudinal Change in Blood Biomarkers Over Time

After adjusting for age, sex, race and ethnicity, education, and the presence of the APOE ε4 alleles, the longitudinal change in concentration of serum t-tau was 0.020 pg/mL (95% CI= 0.017, 0.023) per year or about 4.8% (95% CI= 4.0, 5.6) increase per year (Table 2). The longitudinal change in concentration of serum Nf-L was 0.025 pg/mL (95% CI= 0.022, 0.027) per year or about 5.9% (95% CI= 5.4, 6.4) increase per year. Finally, the longitudinal change in concentration of GFAP was 0.017 pg/mL (95% CI= 0.015, 0.019) per year or about 4.0% (95% CI= 3.6, 4.4) increase per year. These findings show that the three blood biomarkers exhibit varying annual increase in serum concentrations.

In terms of racial differences, the longitudinal change in the concentration of t-tau (p=0.49) and Nf-L (p=0.22) was not different between Black and White participants (Table 2). However, the annual increase in GFAP (4.4% vs. 3.5%, p=0.028) was higher among Black participants than White participants. These findings suggest that the changes in astroglial cell neurons might longitudinally vary between Black and White participants. We found no

significant association of longitudinal changes in the three biomarkers with demographic and genetic risk factors, such as age, sex, education, and the APOE ε4 alleles.

Baseline Structural MRI Characteristics and Change in Blood Biomarkers

In a sample of 435 participants, several baseline structural MRI characteristics predicted longitudinal changes in blood biomarkers (Table 3). Participants with higher third ventricular volume had a higher rate of longitudinal change in the concentration of t-tau. Specifically, for each cc with higher third ventricular volume, the annual increase in t-tau was higher by 0.015 pg/mL per year or 3.6% (95% CI= 0.8, 6.5) increase in the concentration of t-tau per year. We also found an association of baseline WMH with a higher increase in Nf-L concentration. For every 5cc higher WMH, the rate of longitudinal change in NF-L was higher by 0.010 pg/mL per year, 2.4% (95% CI= 1.2, 3.6) increase in longitudinal change in Nf-L per year. Our analysis did not find a significant association of structural MRI characteristics with the change in GFAP over time.

Examining change in Nf-L further by quartiles of WMH, participants with WMH above 19cc (4th quartile) had their annual Nf-L increase by 7.3% (95% CI=5.8, 8.9) (Figure 1). In contrast, those below 3cc (1st quartile) increased 3.8% (95% CI= 2.4, 5.2) per year. Participants with WMH between 3.0–7.8cc (2nd quartile) had their Nf-L increase by 6.6% (p=0.003), and those between 7.8-19cc (3rd quartile) increase by 5.0% (p=0.019), both of which were significantly higher than the longitudinal change in participants below 3cc WMH and combined into one group in Figure 1.

Longitudinal Change in Blood Biomarkers and Clinical AD and Dementia

Figure 2 shows the longitudinal changes in blood biomarkers for those who developed clinical AD and those with no cognitive impairment. The baseline t-tau was higher by 32%, and the longitudinal change was higher by 8% per year among those with clinical AD compared to those with no cognitive impairment. The differences and changes in Nf-L and GFAP for participants who developed clinical AD were strikingly higher than those with no cognitive impairment. The baseline Nf-L was higher by 62%, and the longitudinal change was higher by 88% per year among those who developed clinical AD compared to those with no cognitive impairment. In terms of GFAP, the baseline concentration was higher by 45%. The longitudinal change was higher by 80% per year among those who developed clinical AD than those with no cognitive impairment. The average increases in Nf-L and GFAP were much higher than the average increases seen in t-tau. The rate of change in longitudinal blood biomarkers among those who developed clinical AD did not change when adjusting for baseline WMH characteristics.

We found similar increases in the baseline differences and longitudinal changes in serum biomarkers with dementia. The baseline concentrations were higher among those who developed dementia – 35% for t-tau, 64% for Nf-L, and 48% for GFAP. The longitudinal change was higher by 9% per year for t-tau, 90% per year for Nf-L, and 83% per year for GFAP (data not shown).

Longitudinal Change in Blood Biomarkers and Cognitive Decline

Longitudinal changes in blood biomarkers were significantly associated with the longitudinal cognitive decline after adjusting for demographic characteristics and the APOE ε4 alleles (Figure 3). For each standard deviation unit decline in global cognition, longitudinal change in t-tau

increased by 81% (95% CI= 76, 86), Nf-L by 113% (95% CI= 105, 120), and GFAP by 66% (95% CI= 62, 70) (Table 4). The association of longitudinal change in blood biomarkers and corresponding longitudinal decline in memory test scores was also substantial, ranging from 96% (95% CI= 92, 99) for GFAP to 173% (95% CI= 159, 189) for Nf-L. We also found similar associations between longitudinal changes in blood biomarkers and longitudinal decline in speed test scores. These findings show that the longitudinal change in blood biomarkers corresponds to the longitudinal change in cognitive function. We observed that changes in Nf-L and GFAP showed a higher association with cognitive decline than t-tau.

Classification of Evidence

The primary research question examines the longitudinal change in serum biomarkers of t-tau, Nf-L, and GFAP and their association with cognitive decline and the development of clinical AD in people over 65. The study found Class II evidence that longitudinal changes in serum t-tau, Nf-L, and GFAP were associated with cognitive decline and the development of clinical AD in people over age 65.

DISCUSSION

Our study builds on previous findings that serum t-tau, Nf-L, and GFAP predict the development of clinical AD and dementia and cognitive decline in the population sample.[15] Our study findings were significant and showed that the longitudinal change in blood biomarkers was substantial. They were associated with baseline structural MRI, development of clinical AD and dementia, and longitudinal changes in global and individual cognition tests, typical during preclinical and prodromal phases of development of clinical AD. These findings are novel since the longitudinal

changes in blood biomarkers in biracial studies are yet to be published. A recent study on the relation of change in serum Nf-L with familial AD[9] suggests changes in blood biomarkers can be substantial and provide valuable insights into disease development. Additional findings suggest that the rate of change in Nf-L in CSF may not be static, and these associations may attenuate with worse disease severity.[30]

The lack of association of the ApoE4 allele and demographic characteristics with longitudinal blood biomarkers changes was noteworthy. This finding suggests that longitudinal changes might not be directly associated with demographic or genetic risk factors as hypothesized but more tightly with the preclinical and prodromal changes of the disease as manifested by brain structure and cognitive functioning changes. The lack of racial differences in longitudinal changes in t-tau and NF-L suggest that the disease progression might not differ between Black and White patients. However, the underlying pathologies of brain structural change and cognitive decline may differ.[31,32] Of significance were the findings that ventricular volumes increase the longitudinal change of t-tau. In contrast, higher WMH increases the longitudinal change in Nf-L, suggesting the role of mixed and vascular pathology on the longitudinal change in Nf-L.

The higher rate of longitudinal change in GFAP among Black participants was concerning since these proteins provide astroglia cells that support and nourish cells in the brain and spinal cord and play a significant role in learning and cognition.[33] While pathological studies show a relationship between astroglia expression and degenerative or vascular diseases,[34,35] the relation of these glial proteins measured in the blood to differences between Black and White participants was novel, perhaps suggesting a higher amount of progressive atrophy in Black

patients related to the astroglia cells. These findings provide additional secondary outcomes to monitor disease activity in treatment trials or may be the source of disease modification.

Serum Nf-L also was higher in individuals with silent cerebral vascular lesions and WMH severity.[36,37] We showed a similar relationship between cerebral vascular lesions characterized by white matter injuries and a significantly faster longitudinal rate of change in Nf-L concentration. However, WMH did not influence t-tau or GFAP, suggesting that the pathobiological changes in serum Nf-L may be more specific to cerebrovascular disease. Alternatively, Nf-L may be a sensitive marker of structural brain changes detected with even subtle brain changes due to WMH. Enlargement of the third ventricular volume, which reflects an actual loss of neuronal tissue, results in higher concentrations of t-tau over time. Differences in these structural MRI characteristics to longitudinal change in blood biomarkers were potentially significant since they suggest differences in pathological mechanisms. These blood biomarkers may become elevated over time.

Our study limitations include restriction to three cytoskeletal biomarkers and a lack of Hispanic, Asian, and American Indian participants and neuropathological material. We assayed the blood biomarkers before the availability of various phospho-tau assays. However, we are in the process of assessing p-tau biomarkers in our sample. Therefore, they were not specific to the AD process and might be less informative on the disease pathogenesis. The inclusion of ultrasensitive assays of other neurodegeneration markers, especially ones representing non-cytoskeletal elements, may have provided further clues to support or deny their potential as markers of AD pathogenesis. Autopsy material availability may have provided more pathogenetic relevance to the longitudinal change in blood biomarkers.

Nonetheless, the inability to look at whether the factors that lead to the systematic disadvantage of Black patients contribute to or account for differences in disease biomarkers over time is also a potential limitation. Although having more blood draws was a strength, it also means that we did not include participants who died earlier or lost to follow-up. In our sample, 309 (23%) had one blood evaluation, 583 (44%) had two blood evaluations, 264 (20%) had three blood evaluations, and 171 (13%) had four or more blood evaluations. Hence, the sample has a combination of low and high blood counts.

The preclinical course of AD may be longer than has been demonstrated,[38] and a middle life or earlier study may provide a better understanding of the pathogenesis of the disease, especially in those with MCI. Another fluid biomarker development perspective limitation is the lack of a replication cohort. The external validity is limited to heterogenous populations with cognitive impairment not supported by AD biomarkers, becoming less relevant for clinical trials. The Nf-L and tau could be differentially sensitive to the same neurodegenerative process.[39] The ROI-driven method to assess the relationships between the fluid biomarker change and volume change has limitations. Interrogating this relationship through parametric statistical mapping (e.g., VBM analysis) could yield a more complete and possibly, different picture. Another limitation is that it is hard to interpret these differences due to the lack of brain autopsies when the population is potentially heterogeneous. The study population with cognitive impairment defined by the NINDS/ADRDA criteria can be called amnestic multidomain dementia rather than clinical AD.

This manuscript addresses several questions related to the neurodegenerative process of late-life cognitive decline and clinical AD. Specifically, we show that convenient, cost-efficient measures of blood biomarkers showed increases in their concentrations over time and were

associated with longitudinal changes in cognition observed during the same observational period. We also showed that the neuroimaging biomarkers of structural MRI predicted the longitudinal change in these blood biomarkers, adding further evidence to the clinical significance of longitudinal assessment of blood biomarkers. Our findings suggest that serum biomarkers provide relational associations with the cognitive and brain health characteristics during the development of clinical AD in older at-risk populations.

Appendix 1. Authors

Name	Location	Contribution
Kumar B Rajan, PhD	Rush University, Chicago	Drafted manuscript and performed all biostatistical analysis
Elizabeth A. McAninch, MD	Stanford University, Palo Alto	Critical review and modifications to the manuscript
Neelum T. Aggarwal, MD	Rush University, Chicago	Lead MRI acquisition and critical review of the manuscript
Lisa L. Barnes, Ph.D.	Rush University, Chicago	Critical review and modifications to the manuscript
Robert S. Wilson, Ph.D.	Rush University, Chicago	Critical review and modifications to the manuscript
Jennifer Weuve, ScD	Boston University, Boston	Critical review and modifications to the manuscript
Charles DeCarli, MD	University of California, Davis	Lead MRI processing and critical review of the manuscript
Denis A. Evans, MD	Rush University, Chicago	Collected data and critical review of the manuscript

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Table 1. Baseline Characteristics of 1,327 Participants by Race and Ethnicity

	All Participants N=1,327	Black Participants N=811	White Participants N=516
Age at blood draw, years	78.7 (6.3)	77.8 (6.1)	80.0 (6.1)
Education, y	12.5 (3.6)	11.4 (3.5)	14.0 (3.2)
Female sex, %	824, 62%	508, 63%	316, 61%
Diabetes, %	304, 23%	232, 29%	72, 14%
Stroke, %	151, 11%	97, 12%	54, 10%
Hypertension, %	783, 59%	552, 68%	231, 45%
APOE ε4 allele, %	450, 34%	302, 37%	148, 29%
Global cognition	0.194 (.785)	0.067 (.756)	0.393 (.788)
Total brain volume, cc	852.8 (96.0)	837.9 (94.6)	877.2 (93.4)
Hippocampal volume, cc	5.75 (0.82)	5.66 (0.78)	5.89 (0.87)
WMH, cc[1]	7.3 (0.3, 119.7)	7.0 (0.5, 91.0)	7.9 (0.5, 129.2)
Lateral ventricle, cc	38.7 (21.2)	34.9 (20.1)	44.9 (21.5)
Third ventricle, cc	1.4 (0.5)	1.3 (0.5)	1.5 (0.5)
Cortical thickness, mm	123.2 (20.4)	122.2 (20.1)	124.8 (20.7)
Total tau, pg/mL[1]	0.40 (0.23, 0.68)	0.41 (0.23, 0.69)	0.40 (0.23, 0.68)
Nf-L, pg/mL[1]	25.7 (18.6, 37.1)	24.5 (17.8, 37.2)	26.9 (20.4, 39.8)
GFAP, pg/mL[1]	234 (227, 241)	226 (217, 235)	249 (238, 260)
Raw mean concentrations			
Total tau, pg/mL	0.78 (2.82)	0.76 (2.67)	0.83 (3.04)
Nf-L, pg/mL	36.0 (50.2)	33.4 (38.6)	40.2 (64.2)

GFAP, pg/mL	278.5 (251.8)	271.9 (278.6)	288.7 (251.9)

Note 1: Mean (SD) and N (%) are presented in the table

Note 2: Abbreviations: WMH= White matter hyperintensities, Nf-L = Neurofilament light, GFAP= Glial Acidic Fibrillary Protein

Note 3: [1]Geometric mean (95% CI) from log10 transformed measurements

Note 4: Global cognition is a standardized composite score of episodic memory, executive functioning, and the MMSE scores.

Table 2. Annual Rate of Percent Change in Serum Concentration of Total Tau, Neurofilament Light, and Glial Fibrillary Acidic Protein in 1,327 Participants from a Biracial Population Sample

	All Participants Estimate (95% CI)	Black Participants Estimate (95% CI)	White Participants Estimate (95% CI)	P-value Black vs White Participants
Tau, pg/mL	4.8% (4.0, 5.6)	4.0% (2.9, 5.1)	5.4% (4.9, 5.9)	0.49
Nf-L, pg/mL	5.9% (5.4, 6.4)	6.1% (5.4, 6.9)	5.6% (4.9, 6.2)	0.22
GFAP, pg/mL	4.0% (3.6, 4.4)	4.4% (3.9, 4.8)	3.5% (2.9, 4.1)	0.028

Note 1: The longitudinal change in blood biomarkers was significant at $p<0.0001$.

Note 2: The rate of change in serum concentrations is based on log10 transformed values adjusting for age, sex, education, APOE ε4 alleles, and race and ethnicity. Coefficients for Black and White participants are from a regression model that includes the interaction of Black race with time.

Table 3. Annual Rate of Percent Change in Blood Concentrations of Total Tau, Neurofilament Light, and Glial Fibrillary Acidic Protein and Neuroimaging Biomarkers in 742 Participants

	Total Tau	NF-L	GFAP
	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)
Total brain volume, 10cc	4.9 (-6.0, 17.2)	-1.1 (-7.2, 5.3)	-0.7 (-4.6, 6.3)
WMH, 5cc	0.1 (-0.5, 0.7)	2.4 (1.3, 3.2)	0.1 (-0.3, 0.5)
Hippocampal volume, cc	-1.0 (-2.8, 0.9)	-1.0 (-2.4, 0.4)	0.3 (-0.6, 1.2)
Lateral ventricles, 5cc	0.2 (-3.7, 4.1)	0.1 (-0.3, 0.4)	-0.1 (-0.3, 0.2)
Third ventricles, cc	3.6 (0.8, 6.5)	-0.7 (-2.5, 1.1)	-0.3 (-1.9, 1.3)

Note: Each structural MRI characteristic was independently modeled to predict the rate of change in serum concentrations based on log10 transformed values adjusting for age, sex, education, ApoE4 alleles, and race and ethnicity.

Table 4. Percent Increase in Serum Concentration of Total Tau, Neurofilament Light, and Glial Fibrillary Acidic Protein Corresponding to One Standard Deviation Unit Decline in Global and Individual Tests of Cognition in 1,327 Participants

	Global Cognition Coefficient (SE)	Memory Score Coefficient (SE)	Speed Score Coefficient (SE)
Tau, pg/mL	81% (76, 86)	123% (111, 132)	122% (118, 127)
Nf-L, pg/mL	113% (105, 120)	173% (159, 189)	171% (164, 179)
GFAP, pg/mL	66% (62, 70)	97% (90, 105)	95% (92, 99)

Note: Log10 transformed serum biomarkers are presented after adjusting for age, sex, education, APOE E4, and race and ethnicity. We found no racial differences in the association of serum biomarkers with global cognition.

Figure 1. Association of Quartiles of Baseline White Matter Hyperintensities with Longitudinal Change in Neurofilament Light

Legend: The second and the third quartile of WMH were combined into one since their associations were similar and primarily overlapped. The association of baseline WMH with the annual rate of change in serum concentration of neurofilament light was adjusted for age, female sex, Black race, education, and the ApoE4 allele.

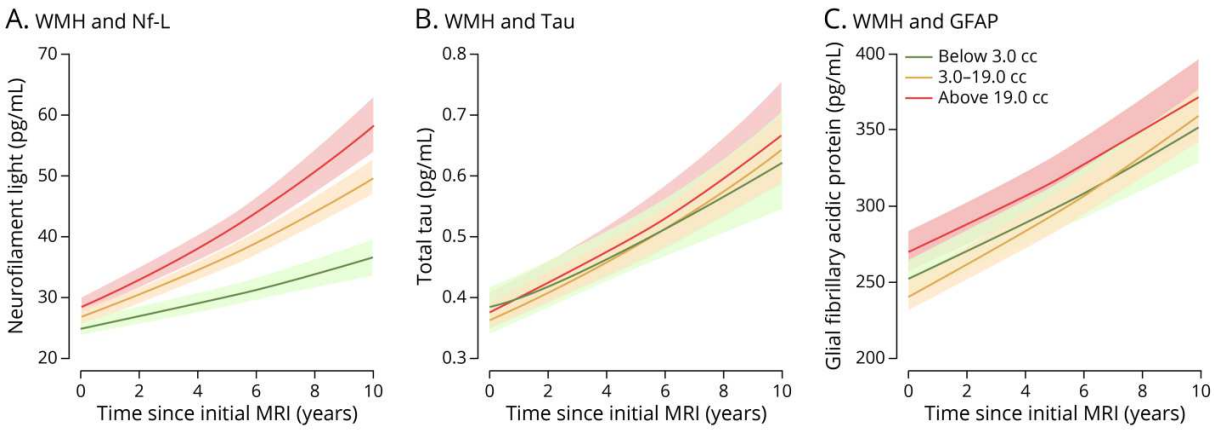


Figure 2. Predicted 10-Year Change in Concentrations of Total Tau, Neurofilament Light, and Glial Fibrillary Acidic Protein Among Those Diagnosed with Clinical AD and No Cognitive Impairment in a Biracial Population Sample

Legend: The models adjusted for age, female sex, Black race, education, and the ApoE4 allele with log10 transformed serum biomarkers concentrations as outcome measures.

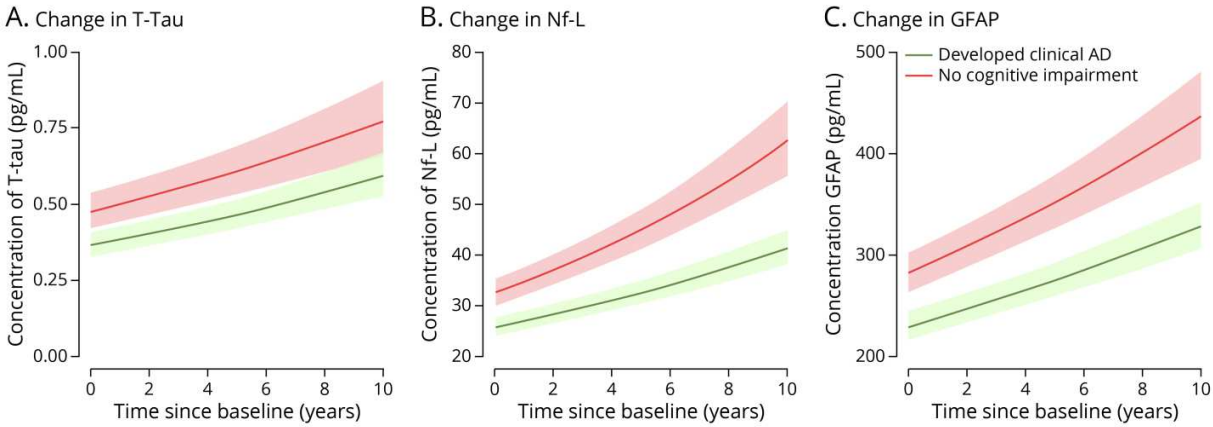
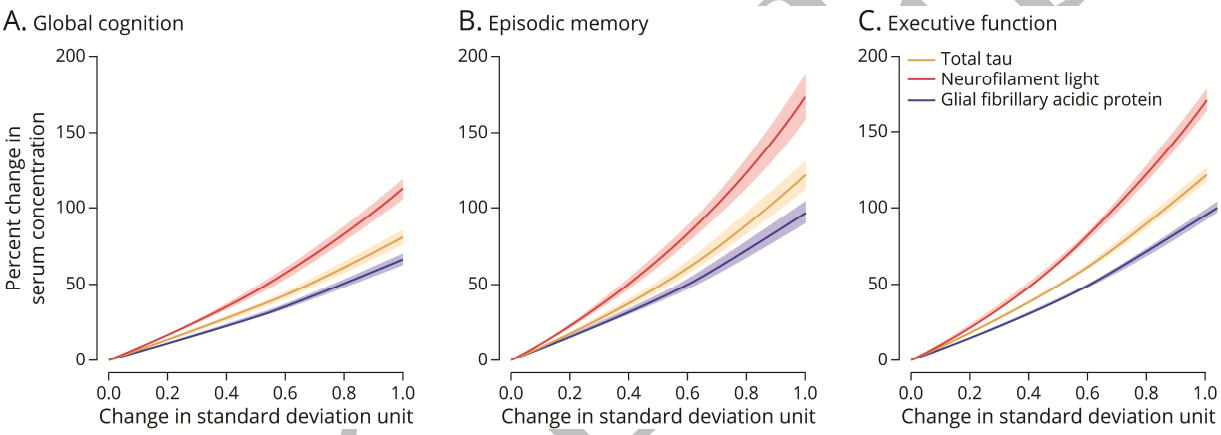


Figure 3. Association of Percent Increase in Concentration of Total Tau, Neurofilament Light, and Glial Fibrillary Acidic Protein with Change in Standardized Units of Global Cognition, Episodic Memory, and Executive Functioning in a Biracial Population Sample

Legend: The relation of change in serum concentrations of total tau, neurofilament light, and glial fibrillary acidic protein with cognitive function was adjusted for age, female sex, Black race, education, and the ApoE4 allele.



Longitudinal Changes in Blood Biomarkers of Clinical Alzheimer Disease in a Biracial Population Sample

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