Association of Aortic Stiffness with Biomarkers of Neuroinflammation, Synaptic Dysfunction, and Neurodegeneration

Elizabeth E. Moore, BS[a], Dandan Liu, PhD[a,b], Judy Li[a], Samantha J. Schimmel[a], Francis E. Cambronero, AB[a], James G. Terry, MS[c], Sangeeta Nair, DVM, MS[c], Kimberly R. Pechman, PhD[a], Marissa E. Moore, MS[a], Susan P. Bell, MBBS, MSCI[a,d,e], Joshua A. Beckman, MD, MS[e], Katie A. Gifford, PsyD[a], Timothy J. Hohman, PhD[a,f], Kaj Blennow, MD, PhD[g,h], Henrik Zetterberg, MD, PhD[g,h,i,j], John Jeffrey Carr, MD, MSc[c] & Angela L. Jefferson, PhD[a,e]

[a]*Vanderbilt Memory & Alzheimer's Center, Department of Neurology, Vanderbilt University Medical Center, Nashville, TN, USA*

[b]*Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN, USA*

[c]*Radiology & Radiological Sciences, Vanderbilt University Medical Center, Nashville, TN, USA*

[d]*Division of Geriatric Medicine, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA*

[e]*Division of Cardiovascular Medicine, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA*

[f]*Vanderbilt Genetics Institute, Vanderbilt University Medical Center, Nashville, TN, USA*

[g]*Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at University of Gothenburg, Mölndal, Sweden*

[h]*Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden*

[i]*Department of Neurodegenerative Disease, University College London Institute of Neurology, Queen Square, London, UK*

[j]*United Kingdom Dementia Research Institute at University College London, London, UK*

Address for Correspondence:
Angela L. Jefferson, PhD
Vanderbilt Memory & Alzheimer's Center
1207 17th Avenue South, Suite 204
Nashville, TN 37212
Phone: 615-322-8676
Fax: 615-343-1302
Email: angela.jefferson@vumc.org

Short Title: Aortic Stiffness & CSF Biomarkers
Total Word Count: 3121
Tables: 3
Figures: 4

Online Data Supplements (Figures): 2

Abstract

Objectives: To test the hypothesis that increased aortic stiffening is associated with greater cerebrospinal fluid (CSF) evidence of core Alzheimer's disease pathology (Aβ, phosphorylated tau (p-tau)), neurodegeneration (total tau (t-tau)), synaptic dysfunction (neurogranin), neuroaxonal injury (neurofilament light (NFL)), and neuroinflammation (YKL-40, sTREM2), we analyzed pulse wave velocity (PWV) data and CSF data among older adults.

Methods: Participants free of stroke and dementia from the Vanderbilt Memory and Aging Project, an observational community-based study, underwent cardiac magnetic resonance to assess aortic pulse wave velocity (PWV, m/sec) and lumbar puncture to obtain CSF. Linear regressions related aortic PWV to CSF Aβ, p-tau, t-tau, neurogranin, NFL, YKL-40, and sTREM2 concentrations adjusting for age, race/ethnicity, education, apolipoprotein (APOE) ε4 status, Framingham Stroke Risk Profile, and cognitive diagnosis. Models were repeated testing PWV interactions with age, diagnosis, APOE-ε4, and hypertension on each biomarker.

Results: 146 participants were examined (72±6 years). Aortic PWV interacted with age on p-tau (β=0.31, p=0.04), t-tau, (β=2.67, p=0.05), neurogranin (β=0.94, p=0.04), and sTREM2 (β=20.4, p=0.05). Among participants over age 73 years, higher aortic PWV related to higher p-tau (β=2.4, p=0.03), t-tau (β=19.3, p=0.05), neurogranin (β=8.4, p=0.01), and YKL-40 concentrations (β=7880, p=0.005). Aortic PWV had modest interactions with diagnosis on neurogranin (β=-10.76, p=0.03) and hypertension status on YKL-40 (β=-18020, p<0.001).

Conclusions: Among our oldest participants, age 74 years and older, greater aortic stiffening is associated with in vivo biomarker evidence of neuroinflammation, tau phosphorylation, synaptic dysfunction, and neurodegeneration, but not amyloidosis. Central arterial stiffening may lead to cumulative cerebral microcirculatory damage and blood flow delivery to tissue, resulting in neuroinflammation and neurodegeneration in more advanced age.

Keywords: arterial stiffness, pulse wave velocity, cardiac magnetic resonance, CSF biomarkers, aging

Clinical Perspective

What is new?

- Greater aortic stiffness quantified from cardiac magnetic resonance is associated with greater in vivo molecular evidence of neuroinflammation, synaptic dysfunction, phosphorylated tau, and total tau among the oldest-old participants.
- Aortic stiffness is not associated with amyloid-β, suggesting aortic stiffness does not affect brain health through an amyloidosis pathway.
- Central arterial stiffening may lead to cumulative cerebral microcirculatory damage and blood flow delivery to tissue, resulting in neuroinflammation and neurodegeneration in more advanced age.

What are the clinical implications?

- These findings provide greater understanding into how arterial stiffness effects brain health, identifying molecular pathologies that may contribute compromised brain structure and function.
- Understanding the molecular pathologies underlying associations between aortic stiffness and brain health may inform targeted interventions that mitigate the onset of more severe structural damage or cognitive decline.

1. Introduction

Age-related arterial stiffening, most commonly assessed with pulse wave velocity (PWV), is associated with cognitive impairment,[1] structural brain changes,[2] and cerebral small vessel disease.[3] As elastic arteries, like the aorta, stiffen with age, they are less able to buffer normal changes in blood pulsatility[4] throughout the cardiac cycle.[5] Thus, greater pulsatile energy is transmitted,[6] particularly in high flow organs receiving a large proportion of the blood supply, such as the brain. Increased pulsatility damages the cerebral microcirculation,[7] leading to reductions in blood flow delivery.[8] However, the molecular pathologies incited or exacerbated by microcirculatory changes, possibly accounting for subsequent cognitive impairment, remain unknown.

Previous research has investigated associations between vascular stiffening and core Alzheimer's disease (AD) pathology, namely amyloid-beta (Aβ) and phosphorylated tau (p-tau). Using a proxy measure of arterial stiffness[9,10] and tonometry,[11,12] increased stiffness is associated with greater cerebral Aβ[9,11,12] and p-tau deposition.[10] Beyond core AD pathology, increased PWV-induced damage to the microcirculation[7] and corresponding cerebral blood flow (CBF) reductions[8] may lead to neurodegeneration, synaptic dysfunction, and neuroaxonal injury. In animal work, cerebral hypoxia leads to diminished long term potentiation,[13] axonopathy,[14] and increased total tau[15] (t-tau, a marker of AD-type neurodegeneration), suggesting alternative pathways through which arterial stiffness may affect brain health. Additionally, given the activation of microglia[16] and astrocytes[17] following capillary damage and blood-brain barrier disruption, it is likely that increased blood pulsatility incites an inflammatory cascade. Despite growing evidence that these pathologies are

associated with vascular risk[18-21] and contribute to abnormal brain aging,[22-25] it remains unknown how arterial stiffness relates to core AD pathology and these concomitant pathways of injury.

The current study examines associations between arterial stiffness and cerebrospinal fluid (CSF) biomarker evidence of core AD pathology (Aβ, p-tau), AD-type neurodegeneration (t-tau), synaptic dysfunction (neurogranin), general neuroaxonal injury (neurofilament light (NFL)), and neuroinflammation (YKL-40, soluble triggering receptor expressed on myeloid cells 2 (sTREM2)) among older adults free of clinical dementia and stroke. Unlike most prior studies investigating arterial stiffness, we directly measure PWV in the aortic arch with cardiac magnetic resonance (CMR). Based on prior work examining arterial stiffness, AD pathology,[9,10] and CBF reductions,[8] we hypothesize that greater aortic stiffening will relate to increased *in vivo* molecular evidence of AD pathology, neurodegeneration, synaptic dysfunction, axonal injury, and neuroinflammation. Given literature suggesting arterial stiffness may have stronger associations with pathology among the oldest old,[9,11,12] and our prior work showing that cognitive diagnosis, apolipoprotein E (*APOE*) ε4 status, and hypertension modify the effects of PWV on brain health,[8,26] we also tested *PWV x age*, *PWV x diagnosis*, *PWV x APOE-ε4*, and *PWV x hypertension* interactions on each CSF biomarker.

2. Methods

2.1 Cohort Selection

The Vanderbilt Memory and Aging Project[8] is a longitudinal study investigating vascular health and brain aging. Inclusion required participants be age ≥60 years, speak English, have adequate auditory and visual acuity, and have a reliable study partner. At eligibility, participants underwent medical history and record review, clinical interview (including functional questionnaire and Clinical Dementia Rating[27] with the study partner), and neuropsychological assessment for cognitive diagnosis by consensus, including normal cognition (NC), early mild cognitive impairment (eMCI),[28] or MCI[29] based on the National Institute on Aging/Alzheimer's Association Workgroup clinical criteria.[29] Participants were excluded for magnetic resonance imaging (MRI) contraindication, history of neurological disease (e.g., stroke), heart failure, major psychiatric illness, head injury with loss of consciousness >5 minutes, and systemic or terminal illness that could impact follow-up participation. At enrollment participants completed a comprehensive evaluation, including but not limited to fasting blood draw, physical examination, clinical interview with medication review, cardiac magnetic resonance (CMR), echocardiogram, and optional lumbar puncture. Participants were excluded from the current study for missing PWV, CSF biomarker, or covariate data (**Figure 1**).

The Vanderbilt University Medical Center Institutional Review Board approved the protocol. Written informed consent was obtained from participants prior to commencement of data collection. Due to participant consent limitations in data sharing, a subset of data is available to others for purposes of reproducing the results or

replicating procedures. These data, analytic methods, and study materials can be obtained by contacting the corresponding author.

2.2 CMR Imaging

CMR imaging was acquired at Vanderbilt University Medical Center using a 1.5T Siemens Avanto system (Siemens Medical Solutions USA, Inc., Malvern, PA) with a phased-array torso receiver coil. Velocity-encoded flow data were acquired from the ascending and descending thoracic aorta. Under the supervision of a board-certified radiologist (JCC), trained raters blinded to clinical information (JGT, SN) used the 2-dimensional flow sequence to draw contours on the ascending and descending aorta using QFLOW 5.6 Enterprise Solution (Medis, Leiden, Netherlands). The thoracic aorta centerline length (cm) from the ascending aorta to descending aorta was measured using OsiriX (PIXMEO SARL, Bernex, Switzerland). Transit time was calculated using a custom MATLAB script to calculate the difference in time (milliseconds) at half-max between the leading edges of the ascending and descending aortic flow curves. PWV (m/sec) was calculated as distance traveled across the aorta (m) divided by time delay in onset of velocity waves (seconds). Inter-reader reliability (coefficient of variation=6.6%) was determined by independent review of 34 scans by two readers (JGT, SN). Pulsatile wave transmission increases with decreasing arterial wall elasticity, so higher PWV indicates higher arterial stiffness. The direct measurement of PWV at the aortic arch, the vessel primarily responsible for buffering pulsatile flow, with CMR allows for local assessment of proximal aortic stiffness without the confound of the distal arterial tree with varied vascular wall properties and tortuosity.

2.3 Lumbar Puncture and Biochemical Analyses

At baseline, participants were invited to complete an optional fasting lumbar puncture procedure. CSF was collected with polypropylene syringes using a Sprotte 25-gauge spinal needle in an intervertebral lumbar space. Samples were immediately mixed and centrifuged, and supernatants were aliquoted in 0.5 mL polypropylene tubes and stored at -80°C. Samples were analyzed in batch using commercially available enzyme-linked immunosorbent assays (Fujirebio, Ghent, Belgium) to determine the levels of Aβ (INNOTEST b-AMYLOID$_{(1-42)}$), p-tau (INNOTEST PHOSPHO-TAU(181P)), and t-tau (INNOTEST hTAU). NFL was measured using a commercially available enzyme-linked immunosorbent assay (UmanDiagnostics). Samples were analyzed using in house enzyme-linked immunosorbent assays to determine the levels of neurogranin[30] and sTREM2.[32] CSF YKL-40 (also called chitinase 3-like 1) concentration was measured using the Human Chitinase 3-like 1 Quantikine ELISA Kit (R&D Systems, Inc., Minneapolis, MN). Board certified laboratory technicians processed data blinded to clinical information, as previously described.[33] Intra-assay coefficients of variation were <10 percent.[30-32]

2.4 Covariates

Covariates were collected at the enrollment visit and selected *a priori* for their potential to confound analytical models. Systolic blood pressure was the mean of two measurements. Hypertension was defined as current antihypertensive medication use, systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg. Diabetes

mellitus was defined as fasting blood glucose ≥126 mg/dL, hemoglobin A1C ≥6.5%, or oral hypoglycemic or insulin medication usage. Medication review determined anti-hypertensive medication use. Left ventricular (LV) hypertrophy was defined on echocardiogram as LV mass index >115 g/m^2 in men or >95 g/m^2 in women. Self-report or history of atrial fibrillation was corroborated by any one of the following sources: echocardiogram, CMR, documented prior procedure/ablation for atrial fibrillation, or medication usage for atrial fibrillation. Current cigarette smoking (yes/no within the year prior to baseline) was ascertained by self-report. Self-report prevalent cardiovascular disease (CVD) with medical record documentation included coronary heart disease, angina, or myocardial infarction (heart failure was a parent study exclusion). Framingham Stroke Risk Profile (FSRP) score assigned points by sex for age, systolic blood pressure (accounting for anti-hypertensive medication usage), diabetes, cigarette smoking, LV hypertrophy, CVD, and atrial fibrillation.[34] *APOE*-ε4 status was defined as positive (ε2/ε4, ε3/ε4, ε4/ε4) or negative (ε2/ε2, ε2/ε3, ε3/ε3).

2.5 Analytical Plan

Linear regressions with ordinary least square estimates related aortic PWV to each CSF biomarker. Models were adjusted for age, race/ethnicity, education, modified FSRP (excluding points assigned for age), *APOE*-ε4 status, and cognitive diagnosis. To test hypotheses related to age effects, models were repeated with a *PWV x age* interaction term and stratified by age groups split by median age (≤73 young-old, >73 older-old). Models also tested *PWV x diagnosis* and *PWV x APOE*-ε4 interactions on each CSF biomarker with follow-up models stratified by diagnosis (NC, MCI) and

APOE-ε4 status (carrier, non-carrier), respectively. In secondary analyses, the effect of hypertension was examined by relating a *PWV* × *hypertension* interaction to CSF outcomes and stratifying models by hypertensive status (yes, no).

To assess if CVD or atrial fibrillation accounted for any significant results, all significant models were repeated in sensitivity analyses excluding participants with these conditions. To determine if outliers were driving the results, additional models were calculated excluding predictor or outcome values >4 standard deviations from the group mean. Multiple comparison correction was performed using a false discovery rate. Significance was set *a priori* at $p<0.05$ and analyses were conducted using R 3.4.2 (www.r-project.org).

3. Results

3.1 Participant Characteristics

The sample included 146 adults age 60-90 years (72±6), 66% were male, and 94% self-identified as non-Hispanic White. PWV ranged 3.5 to 21.6 m/sec, and 16% had clinically elevated PWV defined as >10 m/sec.[35] See **Table 1** for participant characteristics for the total sample and stratified by age group (young-old, older-old).

3.2 PWV and CSF Biomarkers

Among all participants, PWV was unrelated to all CSF biomarkers (p-values>0.13, **Table 2**).

3.3 PWV x Age Interactions and CSF Biomarkers

PWV interacted with age on CSF p-tau (p=0.04), t-tau (p=0.05), neurogranin (p=0.04), and sTREM2 (p=0.05). However, results were slightly attenuated when excluding participants with prevalent CVD, atrial fibrillation, or outliers (p-values>0.09). Stratified results revealed that among older-old participants (>73 years), higher PWV related to higher CSF p-tau (β=2.39, p=0.03), t-tau (β=19.31, p=0.05), neurogranin (β=8.35, p=0.01), and YKL-40 concentrations (β=7880, p=0.005). Notably, the neurogranin and YKL-40 results survived multiple comparison correction. When excluding participants with prevalent CVD, atrial fibrillation, or outliers, associations with YKL-40 persisted (p=0.04), but the remaining results were slightly attenuated (all p-values>0.18). Among the young-old participants (≤73 years), higher PWV related to lower YKL-40 (β=-7230, p=0.02) and sTREM2 concentrations (β=-169, p=0.04). See

Figure 2 for illustrations and **Table 3** for details. Results were similar when excluding participants with prevalent CVD, atrial fibrillation, or outliers (see Supplemental **Tables 1** and **2**) but do not survive multiple comparison correction.

Given the counterintuitive finding between PWV and CSF biomarkers of neuroinflammation in the young-old participants, post hoc analyses explored potential interactions. Within the young-old age group, PVW interacted with hypertension on CSF YKL-40 (p=0.02). *PWV x APOE-ε4* (p-values>0.71) and *PWV x diagnosis* (p-values>0.54) interactions were null for all inflammatory biomarkers. Stratified results suggested the counterintuitive findings in young-old individuals was present among normotensives (sTREM2 β=-307, p=0.04; YKL-40 β=-15914, p=0.01) and *APOE*-ε4 non-carriers (sTREM2 β=-167, p=0.04; YKL-40 β=-7230, p=0.02).

3.4 PWV x Diagnosis Interactions and CSF Biomarkers

PWV interacted with cognitive diagnosis on CSF neurogranin (p=0.03). Models stratified by diagnosis revealed that higher PWV (indicating greater aortic stiffness) was associated with higher CSF neurogranin (β=7.59, p=0.02) among NC participants (**Table 3**), but interaction and stratified results were attenuated when excluding participants with prevalent CVD or atrial fibrillation or when removing outliers (p-values>0.42, see Supplemental **Tables 1** and **2**). Results did not survive multiple comparison correction.

3.5 PWV x APOE-ε4 Interactions and CSF Biomarkers

PWV did not interact with *APOE*-ε4 on any CSF biomarker (p-values>0.40, **Table**

3).

3.6 PWV x Hypertension Interactions and CSF Biomarkers

PWV interacted with hypertension on CSF YKL-40 ($p<0.001$), which persisted when correcting for multiple comparisons (**Figure 3**). In stratified models, higher PWV was associated with higher CSF neurogranin ($\beta=6.15$, $p=0.03$) and CSF YKL-40 ($\beta=6469$, $p=0.006$) among hypertensive participants. Higher PWV related to lower CSF YKL-40 among normotensive participants ($\beta=-12{,}090$, $p=0.01$). See **Table 3** for details. These results persisted when excluding participants with prevalent CVD, atrial fibrillation, or outliers (Supplemental **Tables 1** and **2**).

3.7 Power Calculations

Due to the numerous significant *PWV x age* interactions, post hoc power calculations for the main models and *PWV* × *age* interaction models were conducted based on change in R^2 using an F distribution. Based on R^2 ranging from 0.10 to 0.25 (selected after a review of the total R^2 across all model results) and given the available sample sizes, the minimum detectable variance (change in R^2) explained by PWV in addition to covariates was calculated with 80% power and a Type I error of 0.05. The minimum detectable variance explained by the *PWV* × *age* interaction was calculated with the same approach. For example, we can detect 4.6% of variance in CSF YKL-40 explained by the PWV x age interaction term given a total model R^2 estimate of 0.15. 4.6% of variable in PWV is relatively small, suggesting that our study was possibly underpowered to detect more robust effects.

4. Discussion

Among community dwelling older adults, this study found increased central arterial stiffening was associated with increased *in vivo* molecular biomarker evidence of neuroinflammation, synaptic dysfunction, phosphorylated tau, and neurodegeneration, but not cerebral amyloidosis. Collectively, these findings offer new insights into novel pathways underlying connections between increased arterial stiffness and adverse brain outcomes and confirm prior observations focused on neurodegeneration and amyloidosis outcomes.

This study is among the first to report associations between a gold standard measurement of central arterial stiffening and *in vivo* biomarkers of neuroinflammatory processes and synaptic dysfunction among both adults over age 73 (i.e., the “older-old” participants) and older adults with hypertension. As arterial stiffness increases with age, the aorta is less able to buffer pulsatile energy. Harmful pressure is transmitted to the cerebral microcirculation,[6] contributing to microcirculatory damage,[7] remodeling, and subsequently lower CBF[8] (see **Figure 4** for theoretical model). Reduced CBF and subsequent oligemia incite neuroinflammatory cascades,[40,41] including activation of astrocytes and microglia, which release YKL-40[44] and sTREM2[45] to aid in tissue repair. Oligemia also leads to changes in neuronal signaling,[38] causing the retraction of dendritic spines[38,39] and the release of neurogranin into the CSF. These processes are exacerbated in older age and hypertension, as arteries continue to lose elasticity[46] and inflammatory processes increase.[47] Given that 72% of older adults are hypertensive and the same molecular biomarkers were implicated in both the older-old and hypertensive participants, results may represent a complex yet highly prevalent pathway to adverse

brain outcomes in aging. Future research using larger samples should investigate these potentially complex interactions, including whether these processes are overlapping or separate pathways of injury.

We also found increased arterial stiffness is associated with greater molecular biomarker evidence of phosphorylated tau and neurodegeneration (t-tau). While we are among the first to report an association between higher PWV and increased molecular biomarker evidence of phosphorylated tau, these results are not unexpected given prior research linking increased arterial stiffness with smaller cerebral grey matter volumes[51,52] and linking increased pulse pressure with increased p-tau deposition.[9,10] In animal models, reduced CBF leads to increased p-tau via enzyme modification[54] and neurodegeneration,[56] possibly accounting for associations reported here. These pathways may be exacerbated in the older-old who experience more cumulative burden of arterial stiffness, which aggravates the adverse effects on CBF and results in greater levels of tau phosphorylation and neurodegeneration.

We did not observe an association between central arterial stiffness and Aβ nor did we detect *APOE*-ε4 interactions. These null findings are consistent with each other given *APOE*-ε4 exacerbates Aβ deposition.[57] However, our lack of observations contrasts with some prior work linking CSF Aβ with pulse pressure,[9,10] a proxy measure of stiffness calculated from peripheral blood pressure in the brachial artery. The brachial artery has different elastic properties than the aorta, so pulse pressure may not reflect central (aortic) stiffness. Rather, pulse pressure may represent more of a proxy for vascular biological aging or a later stage of non-specific vascular dysfunction. *APOE*-ε4 is a known moderator of vascular damage, as supported by previous associations

showing increased arterial stiffness and decreased CBF associations are most robust in *APOE*-ε4 carriers.[8] However, it is plausible *APOE*-ε4 may not have the same modifying effect on downstream neuropathological processes, including neuroinflammation, synaptic dysfunction, and neurodegeneration, and exerts its detrimental effects earlier in disease pathogenesis. This hypothesis is consistent with work showing *APOE*-ε4 confers the greatest risk for AD earlier in aging. Collectively, our results suggest that arterial stiffness does not primarily affect brain health through core AD pathology but rather through concomitant pathways of injury.

Additional findings merit brief discussion. We observed a modest *PWV x diagnosis* interaction on neurogranin, such that associations between increased arterial stiffness and biomarker evidence of synaptic dysfunction were driven by cognitively normal participants. This finding is consistent with prior work showing higher aortic stiffness may affect brain health prior to clinical manifestation of cognitive changes.[8] Additionally, we found that among the young-old (≤73 years) and normotensive participants, increased arterial stiffness was associated with decreased biomarker evidence of neuroinflammation, contrary to expectation. It is plausible this result represents a selection bias because individuals with increased neuroinflammation in the setting of increased arterial stiffness would most likely also have hypertension and not be included in the normotensive participant subset for this analysis. However, these findings should be interpreted with caution, as they would not survive multiple comparison correction. Future studies are needed to better understand the nature of these associations.

Our study has several strengths, including gold-standard methods for non-invasively assessing central arterial stiffening at the level of the aorta, stringent quality control procedures, and utilization of a core laboratory for processing CSF biomarker and CMR measurements with blinded raters. Additionally, this is among the first study to examine diverse molecular biomarkers reflecting neuropathological processes beyond core AD pathology. Limitations include the cross-sectional, observational design, and the predominantly White, well-educated, and relatively healthy sample, limiting generalizability to other races, ethnicities, ages, and medical conditions. Several associations were influenced by statistical outliers, suggesting associations would likely be more robust in a cohort more representative of the general population with increased vascular risk factors and prevalent cardiovascular disease. Multiple comparisons raise the possibility of a false positive finding and emphasize the need for replication. While samples sizes were relatively small and possibly underpowered, this study is among the largest studies to date examining arterial stiffness and molecular biomarkers. Though many results would not survive multiple comparison correction with a false discovery rate, associations between PWV and CSF YKL-40 and neurogranin in the oldest-old participants survive correction, supporting our hypothesis that aortic stiffness contributes to neuroinflammation and synaptic dysfunction in older age.

5. Conclusions

Among older-old adults, greater central arterial stiffening was associated with increased molecular biomarker evidence of neuroinflammation, synaptic dysfunction, tau phosphorylation, and neurodegeneration. Associations among the oldest-old reflect the multifactorial nature of associations between arterial stiffness and brain health. Results support neuroinflammation and synaptic dysfunction as potential concomitant pathways to injury beyond tau phosphorylation and neurodegeneration. As additional molecular biomarkers for neuropathological processes are discovered and validated, our understanding of mechanisms underlying adverse brain outcomes of arterial stiffness in aging adults at risk for cognitive impairment and dementia will continue to expand, including potential therapeutic targets.

6. Acknowledgements

The authors wish to thank the dedicated Vanderbilt Memory and Aging Project participants and their families. The authors would also like to thank the dedicated and skilled laboratory technicians at the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital, Sweden, who performed all the CSF analyses.

7. Sources of Funding

Alzheimer's Association IIRG-08-88733 (ALJ); R01-AG034962 (ALJ); R01-NS100980 (ALJ); K24-AG046373 (ALJ); R01-AG056534 (ALJ); Paul B. Beeson Career Development Award in Aging K23-AG045966 (KAG); F30-AG064847 (EEM); T32-GM007447 (EEM); K01-AG049164 (TJH); UL1-TR000445 (Vanderbilt Clinical Translational Science Award); S10-OD023680 (Vanderbilt's High-Performance Computer Cluster for Biomedical Research); Vanderbilt Memory and Alzheimer's Center. HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), and the UK Dementia Research Institute at UCL.

8. Disclosures

HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS),

which is a part of the GU Ventures Incubator Program (all outside submitted work). The other authors report no disclosures.

8. References

1. Elias MF, Robbins MA, Budge MM, Abhayaratna WP, Dore GA, Elias PK. Arterial pulse wave velocity and cognition with advancing age. *Hypertension*. 2009;53:668-673
2. Maillard P, Mitchell GF, Himali JJ, Beiser A, Tsao CW, Pase MP, Satizabal CL, Vasan RS, Seshadri S, DeCarli C. Effects of arterial stiffness on brain integrity in young adults from the framingham heart study. *Stroke*. 2016;47:1030-1036
3. Henskens LH, Kroon AA, van Oostenbrugge RJ, Gronenschild EH, Fuss-Lejeune MM, Hofman PA, Lodder J, de Leeuw PW. Increased aortic pulse wave velocity is associated with silent cerebral small-vessel disease in hypertensive patients. *Hypertension*. 2008;52:1120-1126
4. Saphirstein RJ, Gao YZ, Jensen MH, Gallant CM, Vetterkind S, Moore JR, Morgan KG. The focal adhesion: A regulated component of aortic stiffness. *PLoS One*. 2013;8:e62461
5. Avolio A. Arterial stiffness. *Pulse (Basel, Switzerland)*. 2013;1:14-28
6. Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, Vasan RS, Levy D. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: The framingham heart study. *Hypertension*. 2004;43:1239-1245
7. Coutinho T, Turner ST, Kullo IJ. Aortic pulse wave velocity is associated with measures of subclinical target organ damage. *JACC. Cardiovascular imaging*. 2011;4:754-761
8. Jefferson AL, Cambronero FE, Liu D, Moore EE, Neal JE, Terry JG, Nair S, Pechman KR, Rane S, Davis LT, Gifford KA, Hohman TJ, Bell SP, Wang TJ, Beckman JA, Carr JJ. Higher aortic stiffness is related to lower cerebral blood flow and preserved cerebrovascular reactivity in older adults. *Circulation*. 2018;138:1951-1962
9. Nation DA, Edmonds EC, Bangen KJ, Delano-Wood L, Scanlon BK, Han SD, Edland SD, Salmon DP, Galasko DR, Bondi MW. Pulse pressure in relation to tau-mediated

neurodegeneration, cerebral amyloidosis, and progression to dementia in very old adults. *JAMA Neurol*. 2015;72:546-553

10. Nation DA, Edland SD, Bondi MW, Salmon DP, Delano-Wood L, Peskind ER, Quinn JF, Galasko DR. Pulse pressure is associated with alzheimer biomarkers in cognitively normal older adults. *Neurology*. 2013;81:2024-2027
11. Hughes TM, Kuller LH, Barinas-Mitchell EJ, McDade EM, Klunk WE, Cohen AD, Mathis CA, Dekosky ST, Price JC, Lopez OL. Arterial stiffness and beta-amyloid progression in nondemented elderly adults. *JAMA Neurol*. 2014;71:562-568
12. Hughes TM, Kuller LH, Barinas-Mitchell EJ, Mackey RH, McDade EM, Klunk WE, Aizenstein HJ, Cohen AD, Snitz BE, Mathis CA, Dekosky ST, Lopez OL. Pulse wave velocity is associated with beta-amyloid deposition in the brains of very elderly adults. *Neurology*. 2013;81:1711-1718
13. Payne RS, Goldbart A, Gozal D, Schurr A. Effect of intermittent hypoxia on long-term potentiation in rat hippocampal slices. *Brain research*. 2004;1029:195-199
14. Valeriani V, Dewar D, McCulloch J. Quantitative assessment of ischemic pathology in axons, oligodendrocytes, and neurons: Attenuation of damage after transient ischemia. *J Cereb Blood Flow Metab*. 2000;20:765-771
15. Koike MA, Garcia FG, Kitazawa M, Green KN, Laferla FM. Long term changes in phospho-app and tau aggregation in the 3xtg-ad mice following cerebral ischemia. *Neurosci Lett*. 2011;495:55-59
16. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science (New York, N.Y.)*. 2005;308:1314-1318
17. Endoh M, Maiese K, Wagner J. Expression of the inducible form of nitric oxide synthase by reactive astrocytes after transient global ischemia. *Brain research*. 1994;651:92-100

18. Kester MI, van der Flier WM, Mandic G, Blankenstein MA, Scheltens P, Muller M. Joint effect of hypertension and apoe genotype on csf biomarkers for alzheimer's disease. *J Alzheimers Dis*. 2010;20:1083-1090

19. Skillback T, Farahmand B, Bartlett JW, Rosen C, Mattsson N, Nagga K, Kilander L, Religa D, Wimo A, Winblad B, Rosengren L, Schott JM, Blennow K, Eriksdotter M, Zetterberg H. Csf neurofilament light differs in neurodegenerative diseases and predicts severity and survival. *Neurology*. 2014;83:1945-1953

20. Ma WH, Wang XL, Du YM, Wang YB, Zhang Y, Wei DE, Guo LL, Bu PL. Association between human cartilage glycoprotein 39 (ykl-40) and arterial stiffness in essential hypertension. *BMC cardiovascular disorders*. 2012;12:35

21. Glodzik L, Rusinek H, Pirraglia E, McHugh P, Tsui W, Williams S, Cummings M, Li Y, Rich K, Randall C, Mosconi L, Osorio R, Murray J, Zetterberg H, Blennow K, de Leon M. Blood pressure decrease correlates with tau pathology and memory decline in hypertensive elderly. *Neurobiol Aging*. 2014;35:64-71

22. Zetterberg H, Skillback T, Mattsson N, Trojanowski JQ, Portelius E, Shaw LM, Weiner MW, Blennow K. Association of cerebrospinal fluid neurofilament light concentration with alzheimer disease progression. *JAMA Neurol*. 2016;73:60-67

23. Craig-Schapiro R, Perrin RJ, Roe CM, Xiong C, Carter D, Cairns NJ, Mintun MA, Peskind ER, Li G, Galasko DR, Clark CM, Quinn JF, D'Angelo G, Malone JP, Townsend RR, Morris JC, Fagan AM, Holtzman DM. Ykl-40: A novel prognostic fluid biomarker for preclinical alzheimer's disease. *Biological psychiatry*. 2010;68:903-912

24. Kester MI, Teunissen CE, Crimmins DL, Herries EM, Ladenson JH, Scheltens P, van der Flier WM, Morris JC, Holtzman DM, Fagan AM. Neurogranin as a cerebrospinal fluid biomarker for synaptic loss in symptomatic alzheimer disease. *JAMA Neurology* 2015;72:1275-1280

25. Suarez-Calvet M, Kleinberger G, Araque Caballero MA, Brendel M, Rominger A, Alcolea D, Fortea J, Lleo A, Blesa R, Gispert JD, Sanchez-Valle R, Antonell A, Rami L, Molinuevo JL, Brosseron F, Traschutz A, Heneka MT, Struyfs H, Engelborghs S, Sleegers K, Van Broeckhoven C, Zetterberg H, Nellgard B, Blennow K, Crispin A, Ewers M, Haass C. Strem2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage alzheimer's disease and associate with neuronal injury markers. *EMBO molecular medicine*. 2016;8:466-476

26. Cambronero FE, Liu D, Neal JE, Moore EE, Gifford KA, Terry JG, Nair S, Pechman KR, Osborn KE, Hohman TJ, Bell SP, Sweatt JD, Wang TJ, Beckman JA, Carr JJ, Jefferson AL. Apoe genotype modifies the association between central arterial stiffening and cognition in older adults. *Neurobiol Aging*. 2018;67:120-127

27. Morris JC. The clinical dementia rating (cdr): Current version and scoring rules. *Neurology*. 1993;43:2412-2414

28. Aisen PS, Petersen RC, Donohue MC, Gamst A, Raman R, Thomas RG, Walter S, Trojanowski JQ, Shaw LM, Beckett LA, Jack CR, Jr., Jagust W, Toga AW, Saykin AJ, Morris JC, Green RC, Weiner MW, Alzheimer's Disease Neuroimaging I. Clinical core of the alzheimer's disease neuroimaging initiative: Progress and plans. *Alzheimers Dement*. 2010;6:239-246

29. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH. The diagnosis of mild cognitive impairment due to alzheimer's disease: Recommendations from the national institute on aging-alzheimer's association workgroups on diagnostic guidelines for alzheimer's disease. *Alzheimers Dement*. 2011;7:270-279

30. Kvartsberg H, Duits FH, Ingelsson M, Andreasen N, Ohrfelt A, Andersson K, Brinkmalm G, Lannfelt L, Minthon L, Hansson O, Andreasson U, Teunissen CE, Scheltens P, Van der Flier WM, Zetterberg H, Portelius E, Blennow K. Cerebrospinal fluid levels of the synaptic protein neurogranin correlates with cognitive decline in prodromal alzheimer's disease. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*. 2015;11:1180-1190

31. Mattsson N, Tabatabaei S, Johansson P, Hansson O, Andreasson U, Mansson JE, Johansson JO, Olsson B, Wallin A, Svensson J, Blennow K, Zetterberg H. Cerebrospinal fluid microglial markers in alzheimer's disease: Elevated chitotriosidase activity but lack of diagnostic utility. *Neuromolecular medicine*. 2011;13:151-159

32. Henjum K, Almdahl IS, Arskog V, Minthon L, Hansson O, Fladby T, Nilsson LN. Cerebrospinal fluid soluble trem2 in aging and alzheimer's disease. *Alzheimer's research & therapy*. 2016;8:17

33. Palmqvist S, Zetterberg H, Blennow K, Vestberg S, Andreasson U, Brooks DJ, Owenius R, Hagerstrom D, Wollmer P, Minthon L, Hansson O. Accuracy of brain amyloid detection in clinical practice using cerebrospinal fluid beta-amyloid 42: A cross-validation study against amyloid positron emission tomography. *JAMA Neurol*. 2014;71:1282-1289

34. D'Agostino RB, Wolf PA, Belanger AJ, Kannel WB. Stroke risk profile: Adjustment for antihypertensive medication. The framingham study. *Stroke*. 1994;25:40-43

35. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, Filipovsky J, Huybrechts S, Mattace-Raso FU, Protogerou AD, Schillaci G, Segers P, Vermeersch S, Weber T. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *J Hypertens*. 2012;30:445-448

36. Huang KP, Huang FL, Jager T, Li J, Reymann KG, Balschun D. Neurogranin/rc3 enhances long-term potentiation and learning by promoting calcium-mediated signaling. *J Neurosci*. 2004;24:10660-10669

37. Kaleka KS, Gerges NZ. Neurogranin restores amyloid beta-mediated synaptic transmission and long-term potentiation deficits. *Experimental neurology*. 2016;277:115-123

38. Jourdain P, Nikonenko I, Alberi S, Muller D. Remodeling of hippocampal synaptic networks by a brief anoxia-hypoglycemia. *J Neurosci*. 2002;22:3108-3116

39. Shu L, Chen B, Chen B. Brain ischemic insult induces cofilin rod formation leading to synaptic dysfunction in neurons. 2018:271678x18785567

40. Snyder B, Shell B, Cunningham JT, Cunningham RL. Chronic intermittent hypoxia induces oxidative stress and inflammation in brain regions associated with early-stage neurodegeneration. *Physiological reports*. 2017;5

41. Cruz Hernández JC, Bracko O, Kersbergen CJ, Muse V, Haft-Javaherian M, Berg M, Park L, Vinarcsik LK, Ivasyk I, Rivera DA, Kang Y, Cortes-Canteli M, Peyrounette M, Doyeux V, Smith A, Zhou J, Otte G, Beverly JD, Davenport E, Davit Y, Lin CP, Strickland S, Iadecola C, Lorthois S, Nishimura N, Schaffer CB. Neutrophil adhesion in brain capillaries reduces cortical blood flow and impairs memory function in alzheimer's disease mouse models. *Nature neuroscience*. 2019;22:413-420

42. Didier N, Romero IA, Creminon C, Wijkhuisen A, Grassi J, Mabondzo A. Secretion of interleukin-1beta by astrocytes mediates endothelin-1 and tumour necrosis factor-alpha effects on human brain microvascular endothelial cell permeability. *Journal of neurochemistry*. 2003;86:246-254

43. Gelderblom M, Leypoldt F, Steinbach K, Behrens D, Choe CU, Siler DA, Arumugam TV, Orthey E, Gerloff C, Tolosa E, Magnus T. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. *Stroke*. 2009;40:1849-1857

44. Bonneh-Barkay D, Wang G, Starkey A, Hamilton RL, Wiley CA. In vivo chi3l1 (ykl-40) expression in astrocytes in acute and chronic neurological diseases. *Journal of neuroinflammation*. 2010;7:34

45. Brendel M, Kleinberger G, Probst F, Jaworska A, Overhoff F, Blume T, Albert NL, Carlsen J, Lindner S, Gildehaus FJ, Ozmen L, Suarez-Calvet M, Bartenstein P, Baumann K, Ewers M, Herms J, Haass C, Rominger A. Increase of trem2 during aging of an alzheimer's disease mouse model is paralleled by microglial activation and amyloidosis. *Front Aging Neurosci*. 2017;9:8

46. Schlatmann TJ, Becker AE. Histologic changes in the normal aging aorta: Implications for dissecting aortic aneurys4m. *The American journal of cardiology*. 1977;39:13-20

47. Kim EJ, Park CG, Park JS, Suh SY, Choi CU, Kim JW, Kim SH, Lim HE, Rha SW, Seo HS, Oh DJ. Relationship between blood pressure parameters and pulse wave velocity in normotensive and hypertensive subjects: Invasive study. *J Hum Hypertens*. 2007;21:141-148

48. Bojesen SE, Johansen JS, Nordestgaard BG. Plasma ykl-40 levels in healthy subjects from the general population. *Clinica chimica acta; international journal of clinical chemistry*. 2011;412:709-712

49. Xu T, Zhong C, Wang A, Guo Z, Bu X, Zhou Y, Tian Y, HuangFu X, Zhu Z, Zhang Y. Ykl-40 is a novel biomarker for predicting hypertension incidence among prehypertensive subjects: A population-based nested case-control study in china. *Clinica chimica acta; international journal of clinical chemistry*. 2017;472:146-150

50. Dorans KS, Mills KT, Liu Y, He J. Trends in prevalence and control of hypertension according to the 2017 american college of cardiology/american heart association (acc/aha) guideline. *J Am Heart Assoc*. 2018;7

51. Palta P, Sharrett AR, Wei J, Meyer ML, Kucharska-Newton A, Power MC, Deal JA, Jack CR, Knopman D, Wright J, Griswold M, Tanaka H, Mosley TH, Heiss G. Central arterial stiffness is associated with structural brain damage and poorer cognitive performance: The aric study. *J Am Heart Assoc*. 2019;8:e011045

52. Hughes TM, Wagenknecht LE, Craft S, Mintz A, Heiss G, Palta P, Wong D, Zhou Y, Knopman D, Mosley TH, Gottesman RF. Arterial stiffness and dementia pathology: Atherosclerosis risk in communities (aric)-pet study. *Neurology*. 2018;90:e1248-e1256

53. Osborn KE, Liu D, Samuels LR, Moore EE, Cambronero FE, Acosta LMY, Bell SP, Babicz MA, Gordon EA, Pechman KR, Davis LT, Gifford KA, Hohman TJ, Blennow K, Zetterberg H, Jefferson AL. Cerebrospinal fluid beta-amyloid42 and neurofilament light relate to white matter hyperintensities. *Neurobiol Aging*. 2018;68:18-25

54. Qiu L, Ng G, Tan EK, Liao P, Kandiah N, Zeng L. Chronic cerebral hypoperfusion enhances tau hyperphosphorylation and reduces autophagy in alzheimer's disease mice. *Scientific reports*. 2016;6:23964

55. Wong SM, Jansen JFA, Zhang CE, Hoff EI, Staals J, van Oostenbrugge RJ, Backes WH. Blood-brain barrier impairment and hypoperfusion are linked in cerebral small vessel disease. *Neurology*. 2019;92:e1669-e1677

56. Chen ZL, Strickland S. Neuronal death in the hippocampus is promoted by plasmin-catalyzed degradation of laminin. *Cell*. 1997;91:917-925

57. Risacher SL, Kim S, Shen L, Nho K, Foroud T, Green RC, Petersen RC, Jack CR, Jr., Aisen PS, Koeppe RA, Jagust WJ, Shaw LM, Trojanowski JQ, Weiner MW, Saykin AJ.

The role of apolipoprotein e (apoe) genotype in early mild cognitive impairment (e-mci). *Front Aging Neurosci*. 2013;5:11

Figure 1. Inclusion and Exclusion Criteria

Figure 1. Missing data categories are mutually exclusive. CSF=cerebrospinal fluid; PWV=pulse wave velocity.

Figure 2. PWV x Age Interactions on CSF Biomarkers

Figure 2. Lines reflect fitted values of each CSF biomarker corresponding to pulse wave velocity (based on a 73-year-old, non-Hispanic White, *APOE*-ε4 non-carrier man with normal cognition, 16 years of education, and a Framingham Stroke Risk Profile score of 12). Shading reflects the 95% confidence interval. Age groups were determined based on the median age of this sample. A: Interaction p=0.05. B: Interaction p=0.05. C: Interaction p=0.04. D: Interaction p=0.15. E: Interaction p=0.05. APOE=apolipoprotein E; CSF=cerebrospinal fluid; PWV=pulse wave velocity; p-tau=phosphorylated tau; sTREM2=soluble triggering receptor expressed on myeloid cells 2.

Figure 3. PWV x Hypertension Interaction on CSF YKL-40

Figure 3. Interaction p<0.001. Lines reflect fitted values of CSF YKL-40 corresponding to PWV (based on a 73-year-old, non-Hispanic White, *APOE*-ε4 non-carrier man with normal cognition, 16 years of education, and a Framingham Stroke Risk Profile score of 12). Shading reflects the 95% confidence interval. Hypertension was defined as Hypertension was defined as current antihypertensive medication use, systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg. APOE=apolipoprotein E; CSF=cerebrospinal fluid; PWV=pulse wave velocity.

Figure 4. Theoretical model of synaptic dysfunction, and neuroinflammation induced by aortic stiffness among older adults

Figure 4. As arterial stiffness increases with age, harmful pulsatile energy is transmitted to the cerebral microcirculation, leading to endothelial cell damage and reduced CBF. Reduced CBF leads to hypoxia, resulting in glutamate excitotoxicity and dendritic instability. Glutamate excitotoxicity triggers astrocyte activation and the release of YKL-40, and dendritic instability causes the release of neurogranin as dendritic spines retract. CBF=cerebral blood flow.

Table 1. Participant Characteristics

	Total n=146	Age ≤73 n=78	Age >73 n=68	p-value
Demographic & Health Characteristics				
Age, years	72±6	67±4	78±3	**<0.001**
Sex, % male	66	67	66	0.37
Race, % White non-Hispanic	94	91	97	0.08
Education, years	16±3	16±3	16±3	0.63
Diagnosis, % MCI	36	35	38	0.14
Montreal Cognitive Assessment, total	26±3	26±3	25±3	**0.001**
APOE-ε4, % carrier	31	36	25	0.16
Framingham Stroke Risk Profile, total*	11.8±3.8	9.9±3.1	14.0±3.4	**<0.001**
Systolic blood pressure, mmHg	142±17	140±16	144±17	0.14
Anti-hypertensive medication usage, %	46	41	51	0.21
Diabetes, %	16	18	15	0.60
Current Cigarette Smoking, %	1	1	0	0.35
Prevalent CVD, %	3	4	3	0.76
Atrial fibrillation, %	4	3	6	0.31
Left ventricular hypertrophy, %	3	3	4	0.54
Hypertension, %	70	67	74	0.37
Aortic pulse wave velocity, m/sec	7.9±2.6	7.6±2.4	8.3±2.8	0.08
CSF Fluid Biomarkers, pg/mL				
$A\beta_{42}$	719±246	739±238	695±255	0.32
Phosphorylated tau	61±26	59±27	64±25	0.12
Total tau	428±230	404±238	454±218	**0.04**
Neurogranin	198±77	195±80	201±75	0.65
Neurofilament light	1070±592	926±457	1249±688	**<0.001**
YKL-40	193,252±65,031	178,693±63,356	209,952±63,320	**<0.001**
sTREM2	3668±1812	3307±1708	4082±1852	**0.005**

Note. Values presented as mean±standard deviation or frequency; *a modified score was included in models excluding points for age (6±3); Hypertension was defined as antihypertensive medication use, systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg; Aβ=amyloid beta; APOE=apolipoprotein E; CSF=cerebrospinal fluid; CVD=cardiovascular disease; MCI=mild cognitive impairment; sTREM2=soluble triggering receptor expressed on myeloid cells 2.

Table 2. PWV and CSF Biomarkers

CSF Biomarker	β	95% Confidence Intervals	p-value
$A\beta_{42}$	6.92	-6.69, 20.54	0.32
Phosphorylated tau	0.63	-1.02, 2.28	0.45
Total tau	3.79	-10.57, 18.16	0.60
Neurogranin	3.77	-1.14, 8.68	0.13
Neurofilament light	3.30	-33.35, 39.95	0.86
YKL-40	1658	-2452, 5769	0.43
sTREM2	-3.3	-117.6, 111.1	0.96

Note. n=146. Models adjusted for age, race/ethnicity, education, Framingham Stroke Risk Profile (removing points assigned for age), *APOE*-ε4 status, and diagnosis. Note, an additional 5 participants were excluded from models examining neurofilament light. Aβ=amyloid beta; APOE=apolipoprotein E; CSF=cerebrospinal fluid; CVD=cardiovascular disease; PWV=pulse wave velocity; sTREM2=soluble triggering receptor expressed on myeloid cells 2.

Table 3. PWV x Age, Diagnosis, *APOE*-ε4, and Hypertension Interactions on CSF Biomarkers with Stratification

	***PWV x Age* Interaction n=146**			**Age ≤73 n=78**			**Age >73 n=68**		
	β	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
$A\beta_{42}$	0.69	-1.82, 3.20	0.59	8.11	-11.03, 27.24	0.40	8.38	-12.29, 29.05	0.42
Phosphorylated tau	0.31	0.01, 0.61	**0.04**	-2.08	-4.56, 0.41	0.10	2.39	0.20, 4.58	**0.03**
Total tau	2.67	0.06, 5.28	**0.05**	-20.50	-41.99, 0.98	0.06	19.31	0.34, 38.27	**0.05**
Neurogranin	0.94	0.04, 1.83	**0.04**	-3.36	-10.76, 4.05	0.37	8.35	1.82, 14.87	**0.01***
Neurofilament light	4.35	-2.34, 11.04	0.20	-36.51	-77.15, 4.12	0.08	31.83	-30.99, 94.65	0.31
YKL-40	556	-197, 1309	0.15	-7230	-13170, -1293	**0.02**	7880	2487, 13270	**0.005***
sTREM2	20.4	-0.4, 41.2	**0.05**	-168.7	-331.8, -5.6	**0.04**	123.5	-42.6, 289.5	0.14
	***PWV x Diagnosis* Interaction n=133**			**NC n=80**			**MCI n=53**		
	β	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
$A\beta_{42}$	14.26	-12.13, 40.65	0.29	6.70	-12.60, 25.99	0.49	11.11	-8.32, 30.55	0.26
Phosphorylated tau	-2.89	-6.22, 0.44	0.09	1.30	-0.67, 3.28	0.19	-0.28	-3.55, 2.98	0.86
Total tau	-26.49	-55.68, -2.70	0.07	9.78	-5.68, 25.24	0.21	-5.62	-36.37, 25.12	0.71
Neurogranin	-10.76	-20.44, -1.08	**0.03**	7.59	1.07, 14.12	**0.02**	-0.23	-8.71, 8.24	0.96
Neurofilament light	-58.19	-131.30, 14.87	0.12	24.68	-12.02, 61.37	0.18	-16.98	-97.57, 63.60	0.67
YKL-40	-2294	-1,0080, 5489	0.56	3352	-2285, 8989	0.24	2249	-3925, 8424	0.47
sTREM2	-195.4	-420.4, 29.7	0.09	107.8	-57.2, 272.8	0.20	-92.9	-265.4, 79.7	0.28
	***PWV x APOE-ε4* Interaction n=146**			***APOE*-ε4 Carrier n=45**			***APOE*-ε4 Non-Carrier n=101**		
	β	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
$A\beta_{42}$	-7.78	-38.56, 22.99	0.62	3.15	-22.57, 28.87	0.81	8.93	-7.80, 25.67	0.29
Phosphorylated tau	-1.03	-4.75, 2.69	0.59	-0.43	-4.25, 3.39	0.82	0.86	-0.96, 2.68	0.35
Total tau	-13.75	-46.17, 18.66	0.40	-9.93	-44.54, 24.68	0.56	7.06	-8.34, 22.46	0.37
Neurogranin	-4.70	-15.77, 6.38	0.40	-1.06	-10.76, 8.63	0.83	4.73	-1.17, 10.63	0.11

Neurofilament light	-28.80	-111.20, 53.65	0.49	-13.28	-83.28, 56.72	0.70	9.72	-35.08, 54.52	0.67
YKL-40	-1547	-10840, 7747	0.74	1985	-5212, 9183	0.58	1501	-3626, 6629	0.56
sTREM2	-53.8	-312.0, 204.8	0.68	28.9	-215.7, 273.6	0.81	-1.6	-136.1, 132.9	0.98
	***PWV x Hypertension* Interaction n=146**			**Hypertensive n=102**			**Normotensive n=44**		
	β	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
$A\beta_{42}$	-3.53	-36.46, 29.40	0.83	8.01	-7.73, 23.74	0.31	-4.14	-40.23, 31.95	0.82
Phosphorylated tau	-2.48	-6.45, 1.48	0.22	1.52	-0.33, 3.36	0.11	-2.47	-6.66, 1.71	0.24
Total tau	-19.65	-54.27, 14.97	0.26	10.88	-4.81, 26.57	0.17	-22.88	-61.10, 15.33	0.23
Neurogranin	-8.38	-20.17, 3.41	0.16	6.15	0.50, 11.80	**0.03**	-4.69	-16.25, 6.87	0.42
Neurofilament light	-28.68	-116.50, 59.17	0.52	13.23	-29.49, 55.96	0.54	-35.30	-127.50, 56.91	0.44
YKL-40	-18020	-27370, -8668	**<0.001***	6469	1898, 11040	**0.006***	-12090	-21490, -2693	**0.01**
sTREM2	-180.3	-453.5, 92.9	0.19	53.4	-84.0, 190.9	0.44	-231.2	-479.4, 17.0	0.07

Note: Models adjusted for age, race/ethnicity, education, Framingham Stroke Risk Profile (removing points assigned for age), *APOE*-ε4 status, and diagnosis. An additional 5 participants were excluded from models examining neurofilament light. Hypertension was defined as antihypertensive medication use, systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg. *indicates p-values that would survive a false discovery rate correction for multiple comparisons. Aβ=amyloid beta; APOE=apolipoprotein E; CI=confidence interval; CSF=cerebrospinal fluid; MCI=mild cognitive impairment; NC=normal cognition; PWV=pulse wave velocity; sTREM2=soluble triggering receptor expressed on myeloid cells 2.

Supplemental Table 1. PWV x Age, Diagnosis, and Hypertension Interactions on CSF Biomarkers with Stratification, Excluding Participants with Prevalent CVD or Atrial Fibrillation.

	***PWV x Age* Interaction n=137**			**Age ≤73 n=74**			**Age >73 n=63**		
	β	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
$A\beta_{42}$	1.20	-1.74, 4.13	0.42	4.45	-16.70, 25.59	0.68	4.81	-21.42, 31.03	0.71
Phosphorylated tau	0.19	-0.15, 0.53	0.27	-2.64	-5.36, 0.08	0.06	1.04	-1.68, 3.75	0.45
Total tau	1.70	-1.33, 4.74	0.27	-23.79	-47.55, -0.04	**0.05**	10.39	-13.51, 34.30	0.39
Neurogranin	0.48	-0.53, 1.49	0.35	-5.57	-13.70, 2.57	0.18	2.38	-5.41, 10.17	0.54
Neurofilament light	3.60	-4.21, 11.41	0.36	-31.04	-75.65, 13.57	0.17	31.15	-48.19, 110.50	0.43
YKL-40	419	-442, 1281	0.34	-7976	-14380, -1571	**0.02**	7758	1054, 14460	**0.02**
sTREM2	20.7	-3.6, 45.1	0.09	-203.5	-383.7, -23.4	**0.03**	113.1	-98.8, 325.0	0.29
	***PWV x Diagnosis* Interaction n=124**			**NC n=73**			**MCI n=51**		
	β	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
$A\beta_{42}$	18.87	-13.60, 51.34	0.25	0.24	-26.01, 26.50	0.99	9.54	-11.56, 30.64	0.37
Phosphorylated tau	-1.19	-5.21, 2.82	0.56	-0.48	-3.01, 2.05	0.71	-0.45	-4.00, 3.10	0.80
Total tau	-15.13	-50.82, 20.56	0.40	-2.38	-22.64, 17.88	0.82	-6.41	-39.94, 27.12	0.70
Neurogranin	-4.71	-16.22, 6.81	0.42	0.68	-7.57, 8.94	0.87	-1.20	-10.41, 8.00	0.79
Neurofilament light	-60.97	-150.90, 28.98	0.18	37.00	-11.75, 85.75	0.13	-8.23	-95.91, 79.46	0.85
YKL-40	-2667	-11950, 6620	0.57	3736	-3372, 10840	0.30	1872	-4858, 8601	0.58
sTREM2	-186.0	-463.6, 91.6	0.19	108.8	-114.2, 331.7	0.33	-93.3	-281.3, 94.6	0.32
	***PWV x Hypertension* Interaction n=137**			**Hypertensive n=95**			**Normotensive n=42**		
	β	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
$A\beta_{42}$	-1.75	-38.43, 34.93	0.93	5.41	-14.16, 24.98	0.58	-4.19	-41.65, 33.28	0.82
Phosphorylated tau	-0.89	-5.13, 3.35	0.68	-0.28	-2.47, 1.90	0.80	-2.22	-6.65, 2.21	0.31

Total tau	-7.96	-45.52, 29.59	0.68	-1.97	-20.84, 16.91	0.84	-20.27	-60.59, 20.04	0.31
Neurogranin	-3.04	-15.44, 9.35	0.63	-0.32	-6.90, 6.26	0.92	-4.36	-16.62, 7.90	0.47
Neurofilament light	-36.43	-133.90, 61.08	0.46	25.82	-26.07, 77.71	0.33	-29.88	-125.90, 66.13	0.53
YKL-40	-16191	-26550, -5834	**0.002***	6369	692, 12050	**0.03**	-9990	-19450, -535	**0.04**
sTREM2	-191.2	-493.7, 111.4	0.21	54.7	-114.0, 223.4	0.52	-234.8	-497.7, 28.1	0.08

Note: Models adjusted for age, race/ethnicity, education, Framingham Stroke Risk Profile (removing points assigned for age), *APOE*-ε4 status, and diagnosis. Hypertension was defined as antihypertensive medication use, systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg. An additional 5 participants were excluded from models examining neurofilament light. *indicates p-values that would survive a false discovery rate correction for multiple comparisons. Aβ=amyloid beta; APOE=apolipoprotein E; CI=confidence interval; CSF=cerebrospinal fluid; CVD=cardiovascular disease; MCI=mild cognitive impairment; NC=normal cognition; PWV=pulse wave velocity; sTREM2=soluble triggering receptor expressed on myeloid cells 2.

Supplemental Table 2. PWV x Age, Diagnosis, and Hypertension Interactions on CSF Biomarkers with Stratification, Excluding Outliers.

	***PWV x Age* Interaction**			**Age ≤73**			**Age >73**		
	β	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
$A\beta_{42}$	0.59	-2.18, 3.36	0.67	8.11	-11.03, 27.24	0.40	2.77	-22.28, 27.83	0.83
Phosphorylated tau	0.15	-0.17, 0.47	0.36	-2.08	-4.56, 0.41	0.10	0.94	-1.64, 3.53	0.47
Total tau	1.35	-1.23, 3.93	0.30	-19.04	-37.00, -1.08	**0.04**	10.03	-12.67, 32.73	0.38
Neurogranin	0.28	-0.66, 1.23	0.56	-3.36	-10.76, 4.05	0.37	2.27	-5.15, 9.70	0.54
Neurofilament light	1.78	-3.77, 7.33	0.53	-36.51	-77.15, 4.12	0.08	-3.52	-48.43, 41.39	0.88
YKL-40	321	-503, 1145	0.44	-7230	-13170, -1293	**0.02**	6828	274, 13380	**0.04**
sTREM2	17.4	-5.6, 40.3	0.14	-168.7	-331.8, -5.6	**0.04**	100.5	-101.5, 302.6	0.32
	***PWV x Diagnosis* Interaction**			**NC**			**MCI**		
	β	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
$A\beta_{42}$	20.79	-8.55, 50.13	0.16	-0.72	-23.94, 22.51	0.95	11.11	-8.32, 30.55	0.26
Phosphorylated tau	-0.88	-4.51, 2.75	0.63	-0.63	-2.88, 1.63	0.58	-0.28	-3.55, 2.98	0.86
Total tau	-9.54	-38.67, 19.60	0.52	-3.51	-21.43, 14.42	0.70	-2.34	-28.71, 24.03	0.86
Neurogranin	-3.42	-13.80, 6.97	0.52	0.41	-6.92, 7.73	0.91	-0.23	-8.71, 8.24	0.96
Neurofilament light	-64.66	-123.40, -5.96	**0.03**	15.03	-16.17, 46.23	0.34	-37.92	-94.38, 18.54	0.18
YKL-40	1006	-7577, 9589	0.82	11.42	-6688, 6711	1.00	2249	-3925, 8424	0.47
sTREM2	-161.3	-412.2, 89.6	0.21	73.0	-126.8, 272.8	0.47	-92.9	-265.4, 79.7	0.28
	***PWV x Hypertension* Interaction**			**Hypertensive**			**Normotensive**		
Excluding Outliers	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
$A\beta_{42}$	-1.51	-35.52, 32.51	0.93	6.02	-12.05, 24.08	0.51	-4.14	-40.23, 31.95	0.82
Phosphorylated tau	-1.08	-5.06, 2.89	0.59	0.13	-1.91, 2.17	0.90	-2.47	-6.66, 1.71	0.24
Total tau	-6.64	-38.31, 25.04	0.68	0.32	-17.14, 17.77	0.97	-13.18	-42.48, 16.11	0.37

Neurogranin	-2.92	-14.47, 8.62	0.62	0.76	-5.31, 6.82	0.81	-4.69	-16.25, 6.87	0.42
Neurofilament light	-18.17	-86.67, 50.32	0.60	-3.51	-42.64, 35.61	0.86	-46.63	-96.61, 3.35	0.06
YKL-40	-17167	-26820, -7516	**<0.001***	5889	641, 111,400	**0.03**	-12090	-21490, -2693	**0.01**
sTREM2	-147.3	-428.7, 134.2	0.30	25.4	-132.1, 182.9	0.75	-231.2	-479.4, 17.0	0.07

Note: Models adjusted for age, race/ethnicity, education, Framingham Stroke Risk Profile (removing points assigned for age), *APOE*-ε4 status, and diagnosis. Hypertension was defined as antihypertensive medication use, systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg. An additional 5 participants were excluded from models examining neurofilament light. In the entire sample, 1 PWV, 1 t-tau, and 3 NFL outliers were excluded. *indicates p-values that would survive a false discovery rate correction for multiple comparisons. Aβ=amyloid beta; APOE=apolipoprotein E; CI=confidence interval; CSF=cerebrospinal fluid; CVD=cardiovascular disease; MCI=mild cognitive impairment; NC=normal cognition; PWV=pulse wave velocity; sTREM2=soluble triggering receptor expressed on myeloid cells 2.