β-amyloid and tau tangle pathology modifies the association between small vessel disease and cortical microinfarcts

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Abstract

Background and Purpose: There is increasing recognition of the importance of cortical microinfarcts to overall brain health, cognition, and Alzheimer's dementia. Cerebral small vessel pathologies are associated with microinfarcts, and frequently coexist with Alzheimer's disease (AD); however, the extent to which β-amyloid and tau pathology modulates microvascular pathogenesis is not fully understood. Study objective was to examine the relationship of small vessel pathologies, arteriolosclerosis and cerebral amyloid angiopathy (CAA), with cortical microinfarcts in persons with differing levels of β-amyloid or tau tangle burden.

Methods: Participants were 1,489 autopsied older persons (mean age-at-death=89 years; 67% women) from one of three ongoing clinical-pathologic cohort studies of aging. Neuropathological evaluation identified cortical β-amyloid and tau tangle burden using immunohistochemistry in 8 brain regions, provided semi-quantitative grading of cerebral vessel pathologies, and identified the presence of cortical microinfarcts. Logistic regression models, adjusted for demographics and atherosclerosis, and examined whether β-amyloid or tau tangle burden modified relations between small vessel pathologies and cortical microinfarcts.

Results: Cortical microinfarcts were present in 17% of older persons, moderate-to-severe CAA pathology in 36%, and arteriolosclerosis in 34%. In logistic regression models, we found interactions with β-amyloid and tau tangles, reflecting that the association between

arteriolosclerosis and cortical microinfarcts was stronger in the context of greater β-amyloid (estimate = 0.15; SE = 0.07; p = 0.02) and tau tangle burden (estimate = 0.13; SE = 0.06; p = 0.02). Interactions also emerged for CAA, suggesting that the association between CAA and cortical microinfarcts is more robust in the presence of higher β-amyloid (estimate = 0.27; SE = 0.07; p<0.001) and tangle burden (estimate = 0.16; SE = 0.06; p = 0.005).

Conclusions: These findings suggest that in the presence of elevated β-amyloid or tangle pathology, small vessel pathologies are associated with greater microvascular tissue injury; highlighting a potential link between neurodegenerative and vascular mechanisms.

INTRODUCTION

In older individuals, pathological changes affecting small vessels in the brain are important risk factors for ischemic tissue injury. Common subtypes of small vessel pathologies include arteriolosclerosis which is characterized by hyaline thickening of arterioles, and cerebral amyloid angiopathy (CAA) where accumulation of β-amyloid deposits occurs within the vessel wall. We and others have shown both arteriolosclerosis and CAA are associated with increased likelihood for microinfarct tissue injury [1–5]. Data from clinical pathological studies indicate that a 'pure' vascular burden is relatively uncommon, and that the majority of older individuals exhibit mixed pathologies at autopsy; with mixed AD and vascular being the most common [6,7]. Further, there is increasing recognition that cortical microinfarcts are common in the aging brain, especially in the context of Alzheimer's dementia, and are associated with worse cognitive performances [8–12].

While it is evident that there are numerous pathogenic changes in the aging brain, most commonly there is accumulation of β-amyloid and tau peptides associated with AD pathology, as well as vascular changes associated small vessel pathologies and ischemic tissue injuries [13,14]. However, the relationship between AD pathology and vascular changes remains complex, and it remains unclear whether β-amyloid and tau burden can influence or promote an ischemic environment. Mechanistically, studies using both human tissue and animal models have reported amyloid- and tau-dependent neurotoxicity may impact endothelial biology, altering the integrity of the microvasculature in the brain, thus impacting cerebral perfusion [15–19], an etiology associated with cortical microinfarcts [20,21]. Clarifying the relationship between AD pathology and vascular tissue injury will be important in understanding small vessel disease in the context of mixed pathologies.

In the present study we examined whether β-amyloid and tau burden influences cortical microinfarct pathogenesis specifically in persons with severe small vessel disease. Data came from 1,489 participants enrolled in one of 3 longitudinal clinical pathologic cohort studies of aging. A series of systematic logistic regression models were employed to examine whether β-amyloid or tau tangle modified the relationship between cerebral vessel pathologies (arteriolosclerosis and CAA) and cortical microinfarcts.

METHODS

Participants

Participants enrolled in one of three ongoing cohort studies of aging; the Religious Orders Study (ROS), the Rush Memory and Aging Project (MAP), and the Minority Aging Research Study (MARS) [22]. ROS started in 1994 and recruits older Catholic priests, nuns, and brothers from across the United States, MAP started in 1997 and recruits older adults from across the Chicagoland area, and MARS started in 2004 and enrolls older African-Americans. Eligibility in all 3 studies requires enrollment without known dementia, and agreement to undergo annual clinical evaluation and interview. Participants in both ROS and MAP consent to brain donation at the time of death; for MARS, brain donation is optional. Follow-up across all studies exceeds 85%, with autopsy rates exceeding 95% for ROS, 80% for MAP, and 55% for MARS. Each study was approved by a Rush University Medical Center institutional review board. All participants signed an informed consent and Anatomical Gift Act for brain donation. Of note, all 3 studies share uniform structured neuropathologic evaluations. At the time of the analyses, 4,168 older persons were recruited and had completed baseline evaluations, of whom 1,893 died, and 1,545 were autopsied. Neuropathologic workup for diagnostic purposes was completed on 1,522; and 1,489 had complete data necessary for the current study. Age was calculated from birth date and date of death. Education was based on self-reported years of regular schooling. Sex was self-reported. Demographic and neuropathologic characteristics across the three cohorts are included (Supplemental Materials I). For dementia diagnosis, clinical data was reviewed by a neurologist with expertise in aging and dementia, blind to all neuropathologic data, as previously described [22].

Neuropathology

The average post-mortem interval was 9.25 hours (SD=8.51), and brain removal and processing followed a standard protocol, as previously described [23,24]. Briefly, one hemisphere was cut into 1-cm coronal slabs and fixed in 4% paraformaldehyde for 48–72 hours. Tissue from 11 brain regions including cortical, subcortical, brainstem, and midbrain was taken for diagnostic purposes. In addition, 8 cortical brain regions were taken for β-amyloid and tau tangle image analysis. To exclude potential sources of bias, all neuropathologic data was collected blind to demographic or clinical information.

Cortical β-amyloid load and tau tangle density—Tissue blocks from 8 brain regions; entorhinal, midfrontal (Brodmann [BA] 46/9), inferior temporal (BA 20), inferior parietal (BA 39/40), calcarine (BA 17), anterior cingulate (BA 24), and superior frontal (BA 6/8) cortices, and CA1/subiculum subfield of the hippocampus, were cut into 20μm sections. During the course of the study, immunohistochemistry was performed using three monoclonal antibodies against Aβ, 4G8 (1:9000; Covance Labs, Madison, WI), 6F/3D (1:50; Dako North America Inc., Carpinteria, CA), and 10D5 (1:600; Elan Pharmaceuticals, San Francisco, CA). For β-amyloid, cortical regions of interest were manually outlined (excluding meninges) and analyzed at 20x using an Olympus BX51 microscope attached to an MBF CX9000 camera coupled to a stage driver. The systematic random sampling (SRS) grid within the Stereo Investigator software was applied to the regions of interest. Images at

each sampling grid were taken, and using Image J software, a percentage of amyloid positivity was calculated using a custom positive-pixel algorithm. For analyses, percentage of amyloid positivity was averaged across, as previously described [25]. For assessment of neuritic plaque burden, manual counts were obtained by experienced neuropathologists (Supplemental Materials II). For assessment of PHF-tau tangle pathology, an antibody specific to phosphorylated tau, AT8 (1:2000, Thermoscientific) was used. Manual annotation of regions of interest were outlined by experienced raters trained to recognize neurofibrillary tangles. Using the StereoInvestigator software, the SRS and optical fractionator probe were applied to regions to obtain an estimate quantitative measure for the total number of neurofibrillary tangle cell counts within a defined area, which was then calculated as a density measure per square millimeter for analyses. Other tau pathologies (glial tau and tau neurites) were not included. During the duration of the study, multiple raters have collected neurofibrillary tangle data, in which standard protocols were followed (Supplemental Materials III). For analyses, a composite measures for β-amyloid burden and PHF-tau tangle density were only computed if 4 or more regions were non-missing, and obtained by averaging the mean percentage area per region, across all regions [25]. Square root transformation was also applied to tau-tangle density and cortical β-amyloid load.

Cerebrovascular pathology

Microscopic Infarcts: Microscopic infarcts were not visible to the naked eye and identified by microscopy in tissue blocks, including cortical, subcortical, midbrain, and cerebellum regions, that were paraffin-embedded, and stained with hematoxylin & eosin. Location and age were also documented [24]. For this study, we considered any ischemic lesion detected on histological examination, that was not identified on gross examination, to be a microscopic infarct. Typically, chronic microinfarct lesions would present with cystic cavitation with a few remaining macrophages surrounded by fibrillary gliosis. In addition, morphology of chronic microinfarcts could also include a 'scar' like lesion with very little or no cavitation with possible invagination of the pial surface. Age of all microscopic infarcts were evaluated and defined by experienced neuropathologists. For analyses, only chronic infarcts were considered, and all infarct variables dichotomous, and categorized into absent vs. present. Variables were further categorized into cortical or subcortical locations.

Cerebral Amyloid Angiopathy (CAA): Meningeal and parenchymal vessels from 4 neocortical regions (midfrontal, midtemporal, inferior parietal, and calcarine cortices) were evaluated on sections immunostained with three monoclonal antibodies against Aβ, 4G8 (1:9000; Covance Labs, Madison, WI), 6F/3D (1:50; Dako North America Inc., Carpinteria, CA), and 10D5 (1:600; Elan Pharmaceuticals, San Francisco, CA). CAA scores were scored 0–4 based on amyloid deposition, where 0 = no deposition, 1 = scant amyloid deposition, 2 = circumferential deposition in up to 10 vessels, 3 = circumferential deposition in up to 75% of vessels, and 4 = circumferential deposition in over 75% of vessels. A score for each region was created using the maximum of the meningeal and parenchymal score. A continuous summary score was created by averaging scores across regions [26].

Arteriolosclerosis: Small vessels in the basal ganglia were evaluated on hematoxylin and eosin stained sections. For analyses, grading used a semi-quantitative 4-level rating system

(0=none, 1=mild, 2=moderate, and 3=severe) based on the histological changes of the small arterioles, including intimal deterioration, smooth muscle degeneration, and hyaline concentric thickening with narrowing of the vascular lumen [27]. In a subset of participants, we assessed arteriolosclerosis pathology in both the subcortical frontal and parietal white matter using the same semi-quantitative grading scheme and examined the correlation between our basal ganglia arteriolosclerosis measures with other regional arteriosclerosis measures (Supplemental Materials IV). We show that our measure of arteriolosclerosis in the basal ganglia is moderately representative of arteriosclerosis measures in other brain regions closely linked to frontal and parietal lobes.

Atherosclerosis: Large vessel atherosclerosis was evaluated at the circle of Willis at the base of the brain, and included evaluation of the vertebral, basilar, posterior, middle, and anterior cerebral arteries, and their proximal branches. Visual examination included the number of atherosclerotic plaque, extent of involvement in each vessel, and the degree of vessel occlusion. For analyses, a semi-quantitative 4-level grading system (0–3) was used; where 0 (none) = no significant atherosclerosis observed, sparse number of plaques (1–2) without vessel occlusion; 1 (mild) = small number of plaques (3–7) in up to several arteries without occlusion, 2 (moderate) = moderate number of plaques (8–11), involvement of 50% - 75% of vessel length, or up to 75% vessel occlusion, and 3 (severe) = frequent number of plaques (>11), involvement of >75% of vessel, or >75% vessel occlusion [27].

Statistical Analyses

First, we used a single logistic regression model without an interaction term to examine main effects of arteriolosclerosis and CAA with cortical microinfarcts, adjusted for demographics, atherosclerosis, β-amyloid, and tau tangles burden. Next, we included an interaction term in models to examine association of arteriolosclerosis with cortical microinfarcts in persons with and without β-amyloid. We fitted a logistic regression model with cortical microinfarcts as the outcome, terms for beta-amyloid and arteriolosclerosis, as well as an interaction term between arteriolosclerosis and β-amyloid, and terms to adjust for demographics, tau burden, atherosclerosis and CAA. Next, we repeated analyses, but included an interaction term between CAA and β-amyloid, adjusting for demographics, tau burden, atherosclerosis, and arteriolosclerosis. To examine differences in the association of arteriolosclerosis with cortical microinfarcts in persons with and without tau tangle burden, we repeated similar analyses as previously stated, but replaced the interaction term for β-amyloid and arteriolosclerosis with tau tangle burden and arteriolosclerosis. Models that included an interaction term between arteriolosclerosis and PHF-tau tangles were adjusted for demographics, β-amyloid, atherosclerosis and CAA. Models that included an interaction term between CAA and tau tangles were adjusted for demographics, β-amyloid, atherosclerosis and arteriolosclerosis. All analyses were conducted with SAS/STAT software version 9.4 (SAS Institute Inc, Cary, NC). Statistical significance was determined at nominal α level 0.05.

Data Availability

Raw data are available by request through the Rush Alzheimer's Disease Center (RADC) Research Resource Sharing Hub https://www.radc.rush.edu/

RESULTS

Participants

1,489 participants were included. Characteristics of participants are presented in Table 1. Mean age-at-death was 89 years, with 67% women. Cortical microinfarcts were present in 17% of older persons. Those with cortical microinfarcts were older at death, more likely to be over 90 years, and more likely to have microinfarcts in subcortical regions (basal ganglia or thalamus nuclei). Moderate-to-severe small vessel pathologies were present in about 40% of older persons with cortical microinfarcts, and 35% of persons without cortical microinfarcts. Individuals with cortical microinfarcts mixed with moderate-severe arteriolosclerosis pathology were more likely to be older (90.7 years) compared to those without either pathology (88.8 years) (Z=3.1; p=0.001), with 64.7% having dementia at last evaluation. Similarly, those with cortical microinfarcts mixed with moderate-severe CAA were more likely to be older (90.9 years vs. 88.8 years) (Z=3.0; P=0.001) with 60.0% having dementia at last evaluation. Only 12% (n=179) of participants had both moderate-to-severe arteriolosclerosis and CAA pathology. The distribution of β-amyloid load was weakly bimodal, with values ranging from 0 to 5 units; the 95th percentile was 3.5 units. By contrast, the distribution of tau tangle burden was right skewed, with the 95th percentile being 4.5 units, and the largest value being 9 units. (Figure 1).

Small Vessel Pathologies and Microinfarct Burden

First, we examined the association between arteriolosclerosis and CAA pathology with cortical and subcortical microinfarcts, and fitted logistic regression models without an interaction term, adjusting for age, sex, education, atherosclerosis, β-amyloid, and tau tangle burden, with regional microinfarcts as the outcome. Persons with arteriolosclerosis pathology had 17% higher odds of having cortical microinfarcts (OR=1.17; 95% CI: 1.01–1.38; p=0.04) and 37% higher odds of having subcortical microinfarcts (OR=1.37; 95% CI: 1.16 – 1.62; p<0.01). Those with CAA had 16% higher odds of having cortical microinfarcts, but did not reach the threshold for statistical significance (OR=1.16; 95% CI: 0.99 – 1.35; p=0.06), and were not associated with subcortical microinfarcts (OR=0.98; 95% CI: 0.83 – 1.16; p=0.80). β-amyloid and tau tangle pathology were not related to cortical or subcortical microinfarcts (both p's>0.10).

Small Vessel Pathologies, β-Amyloid, and Microinfarct Burden

To examine whether β-amyloid burden modified the association between small vessel pathologies (arteriolosclerosis and CAA) and cortical microinfarcts, logistic regression models included an interaction term between β-amyloid and the presence of arteriolosclerosis (Table 2). β-amyloid burden modified the association between arteriolosclerosis and cortical microinfarcts. With each additional 1 unit of β-amyloid, arteriolosclerosis pathology was associated with 0.15-unit higher log-odds for cortical microinfarcts, suggesting the association of arteriolosclerosis with cortical microinfarcts occurs only in the presence of higher β-amyloid levels. Table 3 illustrates this interaction for persons with differing levels of β-amyloid. For example, a person with β-amyloid at the 75th percentile would have about 1.5 times the likelihood of microinfarcts compared to someone at the lowest quartile (Table 3).

Next, we included an interaction term between β-amyloid and CAA (Table 2). The interaction term was positive (0.27 units) and significant (p<0.001), suggesting that the association between CAA and cortical microinfarcts is stronger with more severe β-amyloid. The estimated probability for cortical microinfarcts was about 2-times higher in those with β-amyloid burden at the 75th percentile compared to the 25th percentile (Table 3). Figure 2A and 2B displays the estimated probability of cortical microinfarct tissue injury versus level of β-amyloid burden by each level of arteriolosclerosis and CAA pathology.

In contrast, β-amyloid burden did not modify the association between either arteriolosclerosis or CAA with subcortical microinfarcts (Table 2). In sensitivity analyses, we also ran similar logistic regression models and included an interaction term between neuritic plaques and either arteriolosclerosis or CAA on microinfarct burden (Supplemental Materials V). We find a significant interaction remained between CAA and neuritic plaque burden on cortical microinfarcts (estimate = 0.21; SE = 0.09; p = 0.02), while the significance between arteriolosclerosis and neuritic plaque burden on cortical microinfarcts became attenuated (estimate = 0.12; SE = 0.09; p = 0.15), an effect likely due to power since neuritic plaque data is collected on 4 brain regions vs. using β-amyloid data which is collected on 8 brain regions and is collected with greater sampling methods, thus getting a more granular measure of amyloid burden.

Small Vessel Pathologies, Tau Tangle Burden, and Microinfarct Burden

Next, we examined whether arteriolosclerosis or CAA increased likelihood of cortical microinfarcts in the presence of tau tangle burden. In the same approach, we included an interaction term between tau tangle burden and arteriolosclerosis in logistic regression models (Table 2). With every 1 additional unit of tau tangle load, arteriolosclerosis pathology was associated with 0.13-unit higher log-odds for cortical microinfarcts. Given the level of arteriolosclerosis pathology, those with tau tangle burden at the 75th percentile were associated with a higher odds ratio than those at the 25th percentile (Table 3).

Including an interaction term for tau tangle pathology also demonstrated modification between CAA pathology and cortical microinfarcts (Table 2). Odds ratio for the effect of CAA on cortical microinfarcts was higher in the presence of tangle burden at the 75th percentile (OR=1.28; 95% CI: 1.08–1.51) versus tangle burden at the 50th (OR=1.10) or 25th percentile (OR=1.01) (Table 3. The estimated probability of cortical microinfarct tissue injury versus level of tau tangle burden by level of arteriolosclerosis and CAA pathology is displayed in Figure 2C and 2D. Unexpectedly, in models with the interaction term, we found that in the absence of arteriolosclerosis or CAA pathology, there was an inverse association between tau tangle pathology and cortical microinfarcts (Table 2). In sensitivity analyses, we found this association was driven by those individuals within the highest tau tangle percentile with few microinfarcts (Supplemental Materials VI).

Finally, including an interaction term for tau tangle pathology modified the association between arteriolosclerosis and subcortical microinfarcts, but not between CAA and subcortical microinfarcts. With every 1 additional unit of tau tangle load, arteriolosclerosis pathology was associated with 0.13-unit higher log-odds for subcortical microinfarcts (Table 2).

DISCUSSION

In the present study, we explored a potential interaction between AD pathology and small vessel disease on microvascular tissue injury. We examined neuropathologic data from 1,489 community-based older persons to examine whether elevated levels of β-amyloid deposits or tau tangle pathology modified the association between arteriolosclerosis or cerebral amyloid angiopathy and presumably downstream microinfarct tissue injury. We found that the association between arteriolosclerosis and CAA with cortical microinfarcts became more robust in the context of greater β-amyloid and tau tangle burden. While only the association between arteriolosclerosis and subcortical microinfarcts was greater with elevated tau tangle burden.

There is very limited data regarding direct or indirect involvement of amyloid and tau deposits on microinfarct pathogenesis. While multiple studies have shown that individuals with both AD and vessel brain pathologies do worse clinically, and having both pathologies is additive symptomatically [27–29], there is limited information regarding the mechanistic relationship of these pathologies in the context of ischemic tissue injury. To our knowledge, this is the first study to report accumulation of β-amyloid and tau tangle pathology modifies the likelihood of ischemic tissue injuries using data from human-based studies. There is increasing awareness of the contribution of microinfarct burden, especially cortical microinfarcts, to overall brain health, cognition and dementia [11,30–32]. Microinfarcts in pathology are identified microscopically, and are most commonly considered to be a result of an ischemic-hypoxic environment in the brain. We and others have previously reported small vessel pathologies are associated with microinfarct tissue injury [1,2]. Our current study extends these findings to provide evidence that higher levels of amyloid and tau may work to exacerbate the effect of vessel pathologies on ischemic tissue lesions, specifically cortical ischemic lesions, thus lowering an individual's threshold for developing vascular-related lesions. In the case of both arteriolosclerosis and CAA pathology, we found the higher burden of amyloid and tangles you have, the more likely you are to have microinfarcts in the cortex. While only those with arteriosclerosis and higher burden of tau tangles were more likely to have a greater burden of microinfarcts in subcortical regions. Furthermore, our data suggests that a higher number of cortical microinfarcts is largely driven in those individuals with at least moderate severity in vessel disease, and with β-amyloid/tau levels above the 50th percentile. Within and surrounding a cortical microinfarct lesion, there are extensive pathogenic changes, including microvasculature integrity, inflammation, and axonal damage, which likely contribute to the pathogenesis of microinfarcts [14,33]. Our arteriolosclerosis measure used in the current study documents SVD changes in the basal ganglia; however, we also demonstrate that half of the brains with basal ganglia pathology also have more widespread SVD changes in areas more closely connected to cortical lobes. It is plausible that in these individuals there is a more widespread insufficiency of blood flow contributing to microinfarct lesions throughout cortical and subcortical brain regions. By examining interactions between cerebral vessel pathologies and presence of AD proteins on cortical microinfarct burden, our study provides new mechanistic insight into additional factors that contribute to microinfarct pathogenesis. Overall, these findings have important implications

on SVD pathogenesis by unravelling complex relationships between AD pathogenesis, cerebral vessel pathologies, and ischemic brain tissue injury

The microvasculature is a complex network of arterioles, capillaries, and venules, and is central to maintaining adequate brain perfusion, as well as supplying the metabolic demands for optimal neuronal activity [34]. One postulated hypothesis regarding microinfarct etiology is that they are the result of local changes within the brain's environment [13]. Pathological stresses, for example accumulation of Aβ and tau peptides in AD, can drastically change the brain's microenvironment, disrupt the neurovascular link, leading to multiple downstream effects. Prior studies have suggested that both Aβ and tau peptides may promote vasoconstriction via structural and non-structural processes, thus leading to poor perfusion and reduced brain tissue oxygenation [16,18,35]. One proposed mechanism suggests that early Braak tau pathology is related to degradation of arterial smooth muscle cells and elastin, which over time can result in increasing levels of microvessel wall stress [16]. In addition, microvessels within a cluster of dystrophic neurites comprising of a 'neuritic' amyloid plaque have been reported to be 20–50% narrower than microvessels outside the plaque environment [36]. Furthermore, in brains of patients with AD, cortical microinfarcts tend to located close to vessels with CAA, as well as preferentially located in the arterial border zones, highlighting that cerebral hypoperfusion is an important pathophysiological mechanism that may contribute to the etiology of cortical microinfarcts [37]. The current study is cross-sectional, such that the order of the events cannot be determined. However, it is plausible that a greater burden of β-amyloid or tau protein aggregates may activate a cascade of pathological processes that reduces the integrity of the vasculature, promoting hypoperfusion, thus leading to an increased susceptibility to ischemic tissue injury. Alternatively, mechanisms related to protein aggregation or clearance could be disrupted leading to higher levels of AD pathological changes, especially in those individuals with ischemic brain injury. In line with this, recent studies have shown that ischemic stroke may impact tau phosphorylation via upregulation of microtubule kinase, a protein critical to hyperphosphorylation of the tau protein [38]. Future studies targeted to understanding how vascular and AD pathologies interact in vivo using neuroimaging will be important going forward. In addition, future work to identify molecular signatures that drive vascular damage and neurodegeneration, will be imperative in understanding complex relationships between vascular changes, AD pathology, and neurodegeneration.

Overall, findings from this study advances our knowledge regarding microvascular disease in the context of comorbid AD pathology. A major strength of this study is that findings are based on a large sample of autopsied individuals, which allows us to quantify associations more accurately, with comprehensive neuropathologic evaluation, including β-amyloid and PHF-tau tangle burden from 8 brain regions, and multiple vascular pathogenic markers. In addition, by examining continuous measures of amyloid and tangles pathology rather than a dichotomous measure of pathologic AD diagnosis allows us to examine relations across the spectrum of amyloid and tangle burden in more detail. We combined data from three different cohorts to increase the number of cases for statistical power. However, we do recognize there are statistical differences across the cohorts with age-at-death and education, but no differences with sex. For neuropathologic findings, there was a significant difference across cohorts for amyloid burden and atherosclerosis pathology. All other pathologic

findings were comparable across ROS, MAP, MARS cohorts. Limitations are also noted. As previously noted, the study is cross-sectional, and cannot infer cause or consequence. Although multiple vascular pathogenic markers were used in the current study, we did not systematically investigate capillary CAA or microhemorrhages with AD pathology on vasculopathic change. We used standard and commonly used antibodies for amyloid immunohistochemistry; however, the use of multiple amyloid antibodies during the course of the study was not taken into account, as well as, we did not dissociate our amyloid findings by different amyloid species, for example by β-amyloid-42 vs. −40. In addition, we used neurofibrillary tangles as a measure for tau burden; however, glial- and neuritic-tau are common pathological findings in the aging brain, therefore future studies using a total tau burden would provide more accuracy. Finally, our study sample was highly educated and mostly white non-Latino and therefore may limit generalizability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

Non-standard Abbreviations and Acronyms

Aβ	Amyloid-beta
AD	Alzheimer's Disease
BA	Brodmann
CAA	Cerebral amyloid angiopathy
MAP	Memory and Aging Project
MARS	Minority Aging Research Study
ROS	Religious Orders Study
SVD	Small vessel disease

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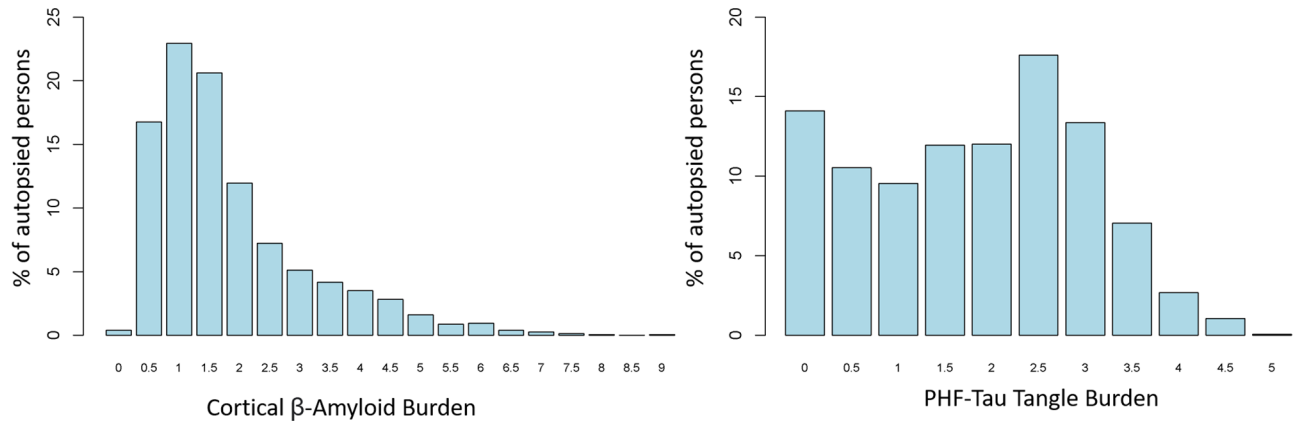
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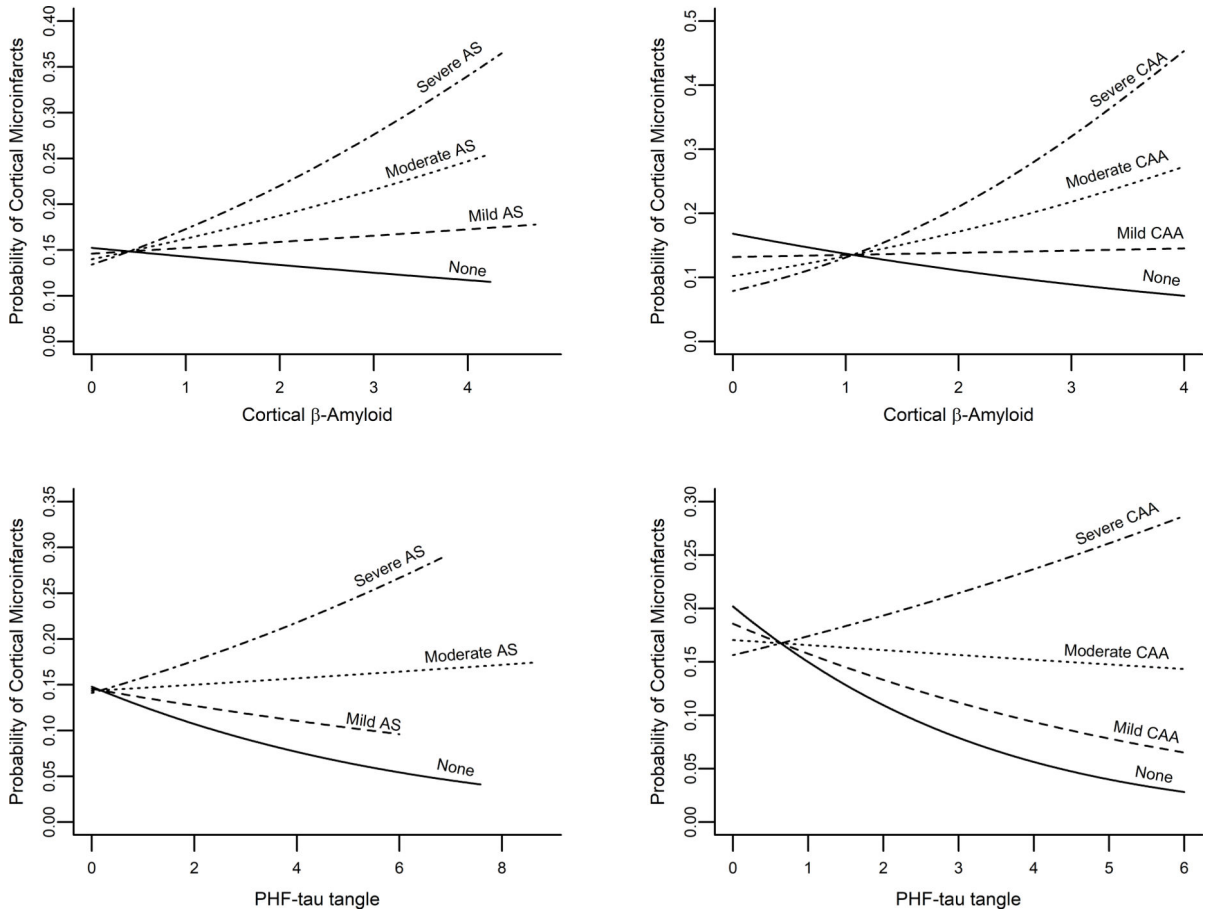[PubMed: 31429800]

Figure 1. Distribution of β-amyloid and tau tangle burden.
The histogram reveals a bimodal pattern in the distribution for cortical β-amyloid burden
and a right-skewed distribution for PHF-tau tangle burden.

Figure 2. Probability of cortical microinfarct tissue injury versus level of β-amyloid and PHF-tau tangle burden by level of arteriolosclerosis and CAA pathology.

Figures show the estimated probability of cortical microinfarcts for representative women with an average age-at-death at 89 years, 16 years of education, and no atherosclerosis.

Abbreviations: AS – Arteriolosclerosis; CAA – Cerebral Amyloid Angiopathy; PHF – Paired-Helical Filament

Table 1.

Characteristics of participants

	All (n=1,489)	With cortical microinfarcts (n=256)	Without cortical microinfarcts (n=1,233)	*p*-Value
Demographics, Mean (SD) or N (%)				
Age-at-death	88.9 (6.79)	89.9 (6.95)	88.7 (6.74)	0.004
90+	788 (53%)	142 (55.5%)	547 (44.4%)	<0.001
Sex, Women	1004 (67%)	168 (65.6%)	834 (67.6%)	0.64
Education	16.2 (3.63)	18.8 (3.67)	16.3 (3.63)	0.08
Neuropathologic, Mean (SD) or N (%)				
Pathologic diagnosis of AD	958 (64.3%)	166 (64.8%)	791 (64.2%)	0.72
Cortical β-Amyloid burden	1.59 (1.13)	1.72 (1.11)	1.56 (1.14)	0.04
PHF-tau Tangle burden	1.61 (1.34)	1.68 (1.39)	1.60 (1.14)	0.26
Moderate-to-severe CAA	517 (35.5%) [*]	106 (42.2%)	412 (34.2%)	0.02
Moderate-to-severe arteriolosclerosis	504 (34.1%) [*]	100 (39.5%)	404 (33.1%)	0.05
Moderate-to-severe atherosclerosis	504 (34.1%) [*]	86 (34.0%)	418 (34.0%)	0.99
Subcortical Microinfarcts	218 (14.6%)	66 (25.8%)	152 (12.3%)	<0.001

[*] Data missing for n=32 for CAA; n=12 for arteriolosclerosis; n=6 for atherosclerosis. Abbreviations: SD – standard deviation, N- number, AD – Alzheimer's disease, PHF – paired helical filament, CAA – cerebral amyloid angiopathy

Table 2.

Interaction of cerebral vessel pathology with β-amyloid/ PHF-tau tangle burden in relation to probability of microinfarct burden.

Model Set	Predictors	Interaction	Cortical microinfarcts	Subcortical microinfarcts
1	Arteriolosclerosis		−0.10 (0.14,0.48)	0.35 (0.14,0.01)
	β-amyloid		−0.12 (0.11,0.26)	0.09 (0.12,0.48)
		Arteriolosclerosis x β-amyloid	**0.15 (0.07,0.02)**	0.02 (0.07,0.73)
2	CAA		−0.29 (0.14,0.05)	−0.05 (0.15,0.76)
	β-amyloid		−0.24 (0.11,0.03)	0.04 (0.11,0.73)
		CAA x β-amyloid	**0.27 (0.07, <0.001)**	0.02 (0.08,0.84)
3	Arteriolosclerosis		−0.06 (0.12,0.64)	0.11 (0.13,0.39)
	PHF-tau		−0.22 (0.10,0.03)	−0.34 (0.13,0.01)
		Arteriolosclerosis x PHF-tau	**0.13 (0.06,0.02)**	**0.13 (0.07,0.04)**
4	CAA		−0.10 (0.12,0.40)	0.09 (0.13,0.49)
	PHF-tau		−0.36 (0.13,0.01)	−0.01 (0.13,0.93)
		CAA x PHF-tau	**0.16 (0.06,0.005)**	−0.08 (0.07,0.27)

Log-odds ratio estimates were derived from logistic regression models in which cortical microinfarct burden was the outcome. Models included an interaction term between vessel pathologies, either CAA or arteriolosclerosis, and β-amyloid or tau tangle burden. All model sets were adjusted for age, sex, education, and atherosclerosis. In addition, model set 1 was further adjusted for CAA and tangle burden. Model set 2 for arteriolosclerosis and tangles. Model set 3 for CAA and β-amyloid. Model set 4 for arteriolosclerosis and β-amyloid. Cell value represents log-odds estimate (SE, *p*-value).

Table 3.

Estimated probability for the association between cerebral vessel pathology and cortical microinfarcts at differing percentiles of β-amyloid/tau tangle burden.

Vessel Pathology		β-Amyloid Burden (percentile)	Tau Tangle Burden (percentile)	Cortical Microinfarct OR (95% CI)
Arteriolosclerosis	X	25th		0.98 (0.79–1.22)
		50th		1.17 (1.00–1.37)
		75th		1.32 (1.09–1.59)
CAA	X	25th		0.87 (0.69–1.08)
		50th		1.18 (1.00–1.38)
		75th		1.46 (1.20–1.78)
Arteriolosclerosis	X		25th	1.03 (0.85–1.25)
			50th	1.10 (0.93–1.30)
			75th	1.23 (1.05–1.46)
CAA	X		25th	1.01 (0.84–1.21)
			50th	1.10 (0.93–1.29)
			75th	1.28 (1.08–1.51)

Odds ratio (OR) estimates were derived from logistic regression models in which cortical microinfarct burden was the outcome. Models included an interaction term between small vessel pathologies, either CAA or arteriolosclerosis, with β-amyloid or tau tangle burden.