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ORIGINAL ARTICLE

Histopathological Analysis of Cerebrovascular Lesions Associated With Aging

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Abstract

Cerebrovascular disease (CVD) has been associated with cognitive impairment. Yet, our understanding of vascular contribution to cognitive decline has been limited by heterogeneity of definitions and assessment, as well as its occurrence in cognitively healthy aging. Therefore, we aimed to establish the natural progression of CVD associated with aging. We conducted a retrospective observational study of 63 cognitively healthy participants aged 19–84 years selected through the histological archives of the CHU de Québec. Assessment of CVD lesions was performed independently by 3 observers blinded to clinical data using the Vascular Cognitive Impairment Neuropathology Guidelines (VCING). We found moderate to almost perfect interobserver agreement for most regional CVD scores. Atherosclerosis ($\rho = 0.758$) and arteriolosclerosis ($\rho = 0.708$) showed the greatest significant association with age, followed by perivascular hemosiderin deposits ($\rho = 0.432$) and cerebral amyloid angiopathy (CAA; $\rho = 0.392$). Amyloid and tau pathologies were both associated with higher CVD load, but only CAA remained significantly associated with amyloid plaques after controlling for age. Altogether, these findings support the presence of multiple CVD lesions in the brains of cognitively healthy adults, the burden of which increases with age and can be quantified in a reproducible manner using standardized histological scales such as the VCING.

Key Words: Aging, Cerebrovascular disease, Dementia, Neuropathology.

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INTRODUCTION

Cerebrovascular disease (CVD) is regarded as the second most common cause of age-associated cognitive impairment. These vascular changes include, but are not limited to, damage to the vascular wall (i.e. atherosclerosis, arteriolosclerosis, cerebral amyloid angiopathy [CAA]), as well as resulting ischemic and hemorrhagic tissue damage (1, 2).

A strong association exists between CVD and other neurodegenerative disorders. According to reports from postmortem studies, cerebrovascular pathology is found in most dementia cases, and in as much as 80% of individuals with a clinical diagnosis of Alzheimer disease (AD) (3, 4). Indeed, pure vascular and pure AD cases appear relatively rare, particularly at older age (5–7). Cases of mixed etiologies are generally associated with worse cognitive performance than those with either pathology alone (8–12). One proposed hypothesis is that vascular pathology enhances β-amyloid generation and reduces elimination of abnormal protein deposits (13–15), thereby precipitating or hastening other neurodegenerative types of dementia.

Two difficulties hinder our understanding of the contribution of CVD to cognitive impairment. The first is that these histological changes are not specific and can also be found in the brains of cognitively healthy individuals with a prevalence that increases with age (16). The second is that identifying CVD reliably and consistently remains challenging.

Regarding the latter, current reading standards are based on a rough descriptive analysis of the various macroscopic vascular changes observed throughout the brain (17). In the absence of standardized guidelines for neuropathological assessment of CVD, quantitative reporting of cerebrovascular lesions is not routinely performed in classical practice. Research efforts are therefore limited by the substantial heterogeneity of neuropathological protocols and definitions (18, 19). A few years ago, the vascular cognitive impairment neuropathology guidelines (VCING) was suggested by Skrobot et al (20). Vascular cognitive impairment (VCI) here refers to cognitive decline caused by or associated with vascular factors and encompasses a wide clinical spectrum (21). The VCING scale is a consensus-based approach to the postmortem assessment and scoring of cerebrovascular lesions in relation to VCI, designed to include both vessel wall pathology and sec-

ondary tissue damage, with good reproducibility and clinical predictability. However, it has not yet been validated in cognitively intact individuals, and therefore a normative characterization of cerebrovascular changes associated with cognition is missing.

This further addresses the issue of specificity. We believe that arriving at such a better definition of "normally" expected age-associated vascular lesions is essential as it may (i) identify thresholds of pathological cerebrovascular aging and (ii) facilitate research toward validated biomarkers for in vivo assessment of cognitive impairment etiologies. This study aimed to validate a modified version of the VCING scale, which we supplemented with the gradation of atherosclerosis, to assess the natural progression of both large and small vessel lesions associated with cognitively intact aging. Its importance lies in the fact that increasing our knowledge of CVD would matter for future patient management, as it is reasonable to postulate that acting on this modifiable component of neurodegeneration could potentially prevent, postpone, or attenuate functional impairment associated with dementia.

MATERIALS AND METHODS

Ethics Approval and Consent to Participate

The methodological protocol was approved by the Centre Hospitalier Universitaire de Québec (#2019-4429) and the Centre intégré universitaire de santé et de services sociaux de la Capitale-Nationale (#2021-2055) ethics committees. No consent was needed.

Participants

Our sample was selected through the histological archives of the neuropathology department of the CHU de Québec (Hôpital de l'Enfant-Jésus, Quebec City), which includes both medicolegal and hospital autopsies. The cohort was composed of collected brains between 2003 and 2018 with consent for autopsy that met the selection criteria. Based on careful examination of available data from their respective medical files, all cases had no documented cognitive impairment or cognitive complaint prior to death and no history of symptomatic stroke. Only brain samples from adult participants (18 years or older) with no or minimal postmortem evidence of neurodegenerative disease were included in the present study. Exclusion criteria were the presence of substantial AD (defined by Braak neurofibrillary tangle stage >III), Lewy body (defined by Braak Lewy body stage >3) or other nonvascular neurodegenerative pathology. The clinical study and use of brain tissue received ethical approval from our local committee at the CHU de Québec.

Brain Sampling and Histological Procedures

We used available formalin-fixed, paraffin-embedded tissue blocks from all brain regions specified in the VCING protocol, which included frontal, temporal, parietal and occipital lobes, anterior and posterior hippocampus, caudate, putamen, internal capsule, globus pallidus, and thalamus. All these regions were originally selected because of their relevance to cerebral systems involved in cognition and their vascular supply from all major cerebral arteries. Tissue sections stained with hematoxylin and eosin (H&E) for all regions (from aleatory hemispheres) were prepared as part of our local protocol.

Some minor modifications were applied to the VCING protocol. First, we decided to use the striatal region to assess both caudate and putamen, and the lenticular nucleus region to assess both internal capsule and globus pallidus. This minor deviation allowed easier sampling without compromising evaluation integrity. Second, we used only 1 hippocampal region, usually the middle section when available, because of our laboratory routine protocol. Third, we restricted Luxol fast blue staining to frontal white matter (frontal lobe) and internal capsule (lenticular nucleus) sections and amyloid immunohistochemistry to hippocampal and occipital sections for practical considerations. Based on the literature, those anatomical regions were considered the most relevant with respect to their assessed pathology (20, 22). Finally, 1 limitation of the VCING scale was the omission of the atherosclerosis status on large size arteries (23). Therefore, we decided to analyze histological slides of coronal sections of major arteries from the circle of Willis (those mostly affected, including the internal carotid, middle cerebral, and basilar arteries) whenever available. The anterior and posterior cerebral arteries were only collected when presenting some evidence of atherosclerotic changes.

Neuropathological Assessment

The modified version of the VCING assessment scale used for the present study, including definitions and scoring keys, is presented in Supplementary Data Table S1 and Fig. S1. Briefly, most lesions were graded according to severity using histological semiquantitative scales ranging from 0 to 3 (0 = normal, 1 = mild changes, 2 = moderate changes, and 3 = severe changes), including atherosclerosis, arteriolosclerosis, perivascular space dilatation and perivascular hemosiderin leakage in all H&E-stained anatomical regions. The degree of severity retained was based on the worst observed lesion on the whole slide analysis except for arteriolosclerosis that was graded according to the overall impression. Because the nature of presumed hemosiderin was not verified with a specific stain for iron deposits such as Perl's Prussian blue, hemosiderin here refers to "degenerative material of uncertain identity, possibly hemosiderin." A similar scale was used for gradation of myelin loss (or, more aptly, myelin pallor) in the frontal and internal capsule regions. CAA was graded on a semiquantitative scale ranging from 0 to 4 in leptomeningeal and cortical vessels and on a dichotomic scale for capillaries (absent or present). Lacunar infarcts were reported on a 3-points scale (0 = absent, 1 = solitary, 2 = 2 to 4, 3 = 5 or more on the whole slide). All other cerebrovascular lesions (fibrinoid necrosis, microaneurysms, microinfarcts, large infarcts, microhemorrhages, and larger hemorrhages) were dichotomously reported as being either absent or present. The total vascular burden was obtained by the addition of all individual lesional scores. All histological slides were examined and rated independently by 2 different assessors (CDT and SS), blinded to age, final diagnosis, and report, between October 2018 and

March 2019. Any conflictual score was resolved by a consensus approach by the 2 observers after inter-rater agreement evaluation. A third assessor (MR; neuropathologist) was asked post hoc to assess and rate 15% (n = 10) of the cases to provide additional validation of interrater reliability. Scores for assessment of amyloid β plaques (Thal phases), neurofibrillary tangles (Braak stages), and neuritic plaques (CERAD score) were extracted from the original neuropathological reports realized by our 2 local neuropathologists (SS and PVG) following NIA-AA guidelines for the neuropathological assessment of AD (17). The monoclonal mouse antihuman β-amyloid antibody (1:200; Clone 6F/3D; Dako Cytomation, Glostrup, Denmark) was used for the detection of β-amyloid parenchymal and vascular deposition and the monoclonal mouse antihuman antibody PHF-Tau (1:2500; Clone AT8; Thermo Scientific, Rockford, IL) was used for the detection of hyperphosphorylated tau protein in AD-type neurofibrillary changes.

Data Analysis

Inter-rater reliability was tested by calculating weighted Kappa's coefficient between SS and CDT and between SS and MR. We used Cohen's interpretation benchmarks to evaluate the extent of agreement between the 2 observers ($\leq$0 = no agreement, 0.01–0.20 = slight agreement, 0.21–0.40 = fair agreement, 0.41–0.60 = moderate agreement, 0.61–80 = substantial agreement, and 0.81–1.00 = almost perfect agreement). Spearman's rank correlation test (for ordinal variables) and Pearson's correlation test (for binary variables) were used to evaluate regional gradients of vascular load associated with age. A 0.005 significance threshold (p value) was fixed to correct for multiple comparisons. We also performed Spearman's rank correlation tests and multiple linear regression analysis a posteriori to evaluate for a possible association between perivascular space dilatation severity and postmortem delay. Finally, the associations of lesional and total vascular load with Thal phases, Braak stages, and CERAD scores were assessed by using partial Spearman's rank correlation tests for nonparametric analysis of 2 ordinal variables adjusted for age. A more permissive p value of $<$0.05 was considered statistically significant given the lower number of computed tests. All statistical analyses were conducted in R Studio 1.4.1106.

RESULTS

Participant Characteristics

Brain specimens from 63 cognitively intact adult participants ranging from 19 to 84 years old, or 9 cases per decade (third to ninth decades), were obtained from the CHU de Québec histological archives for inclusion in our study. The mean age was 53.95 (SD, 20.26) years and 49.21% were females (observed sex from clinical files). Ethnicity was not documented. The mean postmortem delay for brain fixation was 16.84 days (range 0–199 days). The cause or circumstance of death was available for all cases and was highly variable, the most frequent being sudden unexpected death in epilepsy (SUDEP; 23.8%), cardiac arrest (19.0%), neoplasm (11.1%), respiratory failure (4.8%), infection (4.8%), and suicide (4.8%; Supplementary Data Fig. S1). The final neuropathological diagnosis was normal in 28.5% of cases and minor senile changes in also 28.5% of cases. Neuropathological scoring ranged from 0 to 2 for amyloid beta plaques (Thal phases 0–3), from 0 to 1 for neurofibrillary tangles (Braak stages 0 to II) and from 0 to 1 for neuritic plaques (CERAD scores 0 to I), with a median of 0 for all scores. Perimortem hypoxemic changes, acute inflammation, incidental lesions, congenital malformations, and epileptogenic foci were also described in neuropathological reports from our case series. Characteristics of participants are presented in Table 1.

Inter-Rater Reliability

In general, analysis showed the modified VCING criteria to be reproducible. Interobserver agreement between SS and CDT ranged from 0.39 to 1.00 for most regional cerebrovascular pathology scores. Validation of interobserver agreement between SS and MR yielded similar results. Only the assessment of perivascular hemosiderin leakage showed generally lower agreement and no agreement in the occipital cortex, based on discrepancy of scores for an isolated slide from 1 participant. Globally, agreement was moderate for perivascular space dilatation and hemosiderin leakage, substantial for arteriolosclerosis and microhemorrhages, and almost perfect for atherosclerosis, fibrinoid necrosis, microaneurysms, CAA, myelin pallor, large infarcts, microinfarcts, lacunar infarcts, and large hemorrhages. Cohen's kappa coefficients for estimation of inter-rater reliability are presented in Supplementary Data Table S2 and Figure S3.

Prevalence of Neuropathological Findings

In our cohort of cognitively intact subjects, we observed mild degrees of atherosclerosis of the circle of Willis in 28 of 51 cases (54.9%) with available data, but none with at least 1 artery occluded more than 50%. All subjects with atherosclerosis were 30 years or older, with 88.8%–100% of individuals affected between the sixth and ninth decades (50 years or older). Only few cases presented with minimal degree of arteriolosclerosis in their twenties. Globally, prevalence increased with age in all regions across the brain, with almost 80% showing signs of arteriolosclerosis in the lenticular nucleus by the age of 50. No arteriolosclerosis-related secondary vessel lesions such as fibrinoid necrosis or microaneurysms were found in these normal subjects. At least some degree of perivascular changes, both space dilatation and hemosiderin leakage, were seen in the vast majority of subjects, in all age groups and all anatomical locations. Myelin pallor was found slightly more often in the internal capsule and frontal lobe sections of older adults but was also frequently present in youngest cases. Leptomeningeal (but not cortical nor capillary) CAA was occasionally found in hippocampal and occipital regions of older individuals by the sixth decade (n = 11; 17.5%). Finally, a few isolated microinfarcts (n = 8) and microhemorrhages (n = 3), mainly in the striatum, the lenticular nucleus, and the thalamus, were observed in a minority of cases (n = 9; 14.3%) ranging from 27 to 84 years old. No macroscopic infarct or lacune were detected, nor was macroscopic

TABLE 1. Participant Characteristics by Age Group (Years)

	19–29 (n = 9)	30–39 (n = 9)	40–49 (n = 9)	50–59 (n = 9)	60–69 (n = 9)	70–79 (n = 9)	80–89 (n = 9)
Female (%)	5 (55.6)	4 (44.4)	5 (55.6)	5 (55.6)	5 (55.6)	3 (33.3)	4 (44.4)
Mean age (SD)	24.1 (2.85)	33.6 (2.8)	43.2 (3.4)	55.4 (2.8)	63.4 (3.1)	76.0 (2.7)	81.9 (1.5)
Thal phase[†] (SD)	0.0 (0.0)	0.0 (0.0)	0.4 (0.7)	0.7 (1.0)	0.9 (1.0)	0.8 (1.1)	1.0 (1.4)
Braak stage[†] (SD)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.8 (0.8)	1.1 (0.8)	1.2 (0.8)	1.6 (0.9)
CERAD score[†] (SD)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.2 (0.4)	0.2 (0.4)

[†]The "ABC" neuropathological assessment for Alzheimer pathology was obtained according to the NIA-AA scoring criteria (17).

hemorrhage. The first cases with some evidence of amyloid pathology at autopsy were encountered from the fifth decade (40–49 years) and the presence of tau pathology was observed by the sixth decade (50–59 years). The frequency and regional distribution of cerebrovascular lesions and AD pathology for each age group are presented in Figure 1.

Correlations Between Vascular Lesions and Age

Cerebral atherosclerosis (0.758, $p = 1.20 \times 10^{-10}$) and arteriolosclerosis (0.708, $p = 8.64 \times 10^{-11}$) showed the highest correlation with age. In arteriolosclerosis, the strongest regional gradients were observed in deeper brain structures, including the striatum, the lenticular nucleus, and the thalamus, followed by the hippocampus. Global perivascular hemosiderin leakage (0.432, $p = 0.0004$) and CAA (0.392, $p = 0.001$) scores were also significantly associated with age, but to a lesser degree. There was a trend in the severity of perivascular retraction (0.267, $p = 0.034$) and myelin pallor (0.322, $p = 0.01$) associated with increasing age, but which did not reach statistical significance when corrected for multiple comparisons. We did not find any association between perivascular space dilatation severity and postmortem delay (Supplementary Data Tables S4 and S5). Spearman's rank correlation coefficients for regional gradients of vascular load associated with age are presented in Figure 2. More detailed results are available in Supplementary Data Table S3.

Association With AD Pathology in Cognitively Intact Individuals

Higher levels of total CVD, atherosclerosis, and CAA burdens were significantly correlated with amyloid-β plaques severity according to Thal phases. Similarly, total CVD burden, atherosclerosis, arteriolosclerosis, myelin pallor, and CAA were significantly associated with tau pathology as assessed by Braak stages, with higher levels of neurofibrillary tangles at postmortem examination being associated with superior levels of vascular changes. Levels of total CVD burden and CAA were also positively correlated with neuritic plaques burden as assessed by CERAD score. However, the only pathological features that remained significantly correlated after correcting for age were CAA and diffuse amyloid-β plaques (Thal phases). The results from correlation analyses of lesional vascular load and AD pathology are presented in Table 2 and Figure 3.

DISCUSSION

The objective of this study was to describe and quantify cerebrovascular burden associated with cognitively intact aging using a modified version of a validated neuropathological assessment scale. We found that several large and small vessel changes and secondary parenchymal lesions typically accumulate with age, the burden of which can be reproducibly estimated with a slightly modified version of the VCING scale. Notably, the presence and severity of atherosclerosis of the circle of Willis, arteriolosclerosis of the deep gray matter structures, CAA, and perivascular hemosiderin deposits were significantly correlated with age. We also found a higher prevalence of atherosclerosis and CAA in individuals with some evidence of AD pathology (both amyloid and tau) and increased severity of total arteriolosclerosis in individuals with neurofibrillary tangles.

Reproducibility of the VCING Scale

The first step of our study was to validate the inter-rater reliability of the previously proposed VCING scale. We found moderate to almost perfect agreement for the vast majority of assessed pathologies and areas. In the reproducibility substudy of the original publication (20), the authors found similar reliability rates, with most regional scoring showing almost perfect agreement. The VCING scale and its slightly modified version are therefore adequate assessment tools to estimate the postmortem CVD burden in a reproducible manner. Yet, we believe that a fully accessible visual reference system could still improve inter-rater agreement among neuropathologists.

Vessel Wall Pathology

Several of our findings of age-related vessel wall pathology are in keeping with previous neuropathological studies. However, most autopsy studies focused on older cohorts of individuals and few of them have examined the vascular changes in young to middle-aged adults.

Atherosclerosis of the Circle of Willis

We found mild degree of atherosclerosis in 54.9% of our sample and in 88.8%–100% of individuals aged 50 and older. None of them presented with moderate to severe pathology. Other examined case series and cohorts showed similar rates of atherosclerosis, with 47% of normal individuals in 1 study

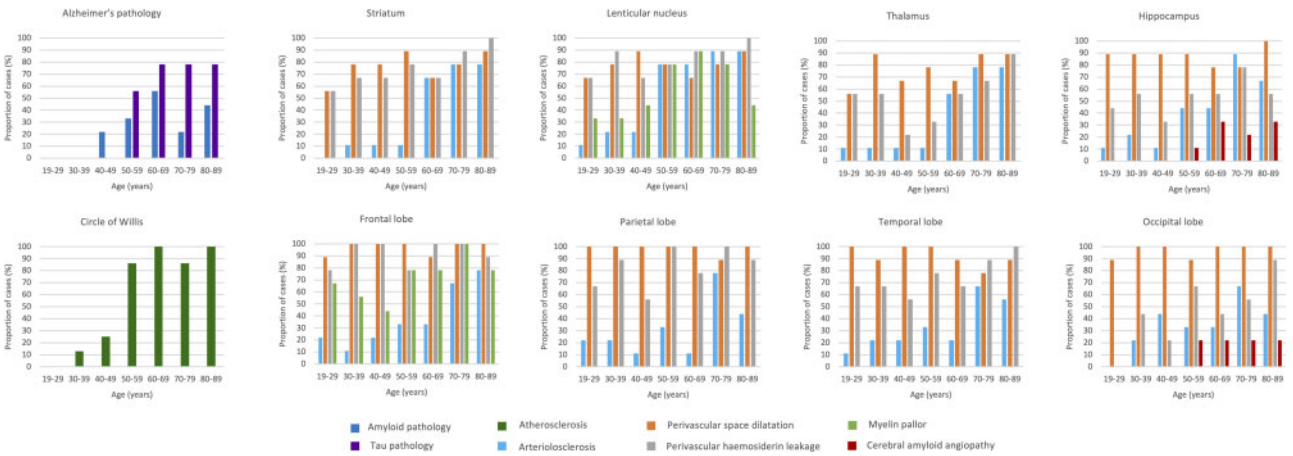

FIGURE 1. Relative frequency of Alzheimer and cerebrovascular pathology in the normal aging brain. Figure shows the frequency of AD-related and vascular pathology in each age group and individual brain area. Hemosiderin refers to "degenerative material of uncertain identity, possibly hemosiderin."

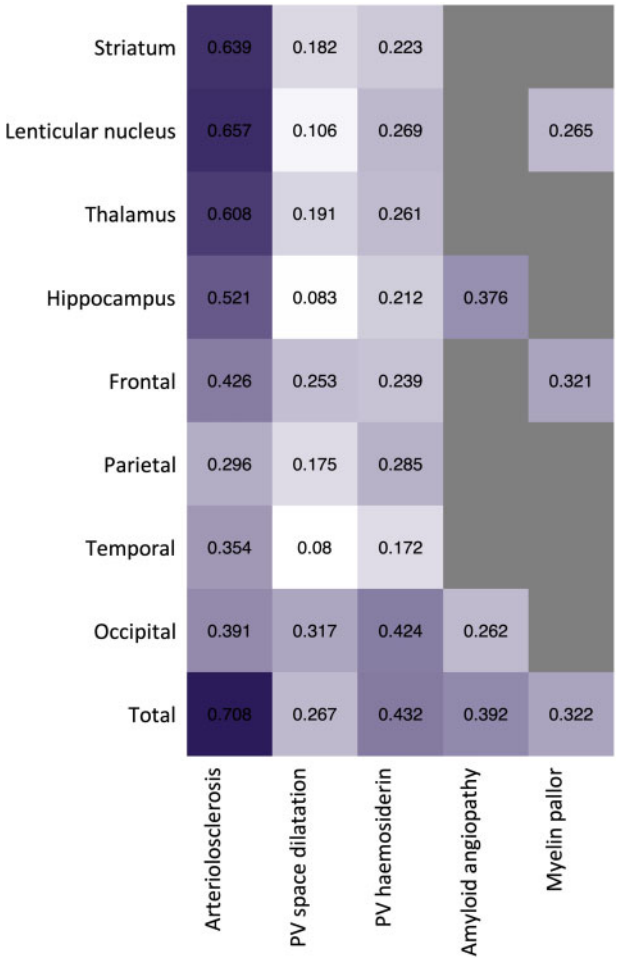

FIGURE 2. Spearman's rank correlation for regional gradients of vascular load associated with age. Heat map illustrates the relative strength of association between each subtype of regional vascular pathology and chronological age. Hemosiderin refers to "degenerative material of uncertain identity, possibly hemosiderin."

(mean age 69.6 years) (24) and higher occurrence with increasing age (60–70 years, 79%; 71–80 years, 96%; and 81 years and older, 96%) in another one (25). Those rates were similar between males and females. Higher frequencies of moderate to severe atherosclerosis were reported in most related studies, ranging from <25% to 34% (24, 26), but these discrepancies are most probably explained by the more advanced age of these cohorts (mean age ranging from 69.6 to 87.7 years). Other vascular risk factors associated with atherosclerosis such as high blood pressure, dyslipidemia, and smoking could have explained some variations, but were not available for our sample. The positive association between circle of Willis atherosclerosis grade and age was also confirmed elsewhere (24, 25).

Arteriolosclerosis

Our results suggest an increasing load of arteriolosclerosis with age throughout the brain, with basal ganglia and thalamus being the earliest and most affected regions. In our sample, almost 80% of cases showed signs of arteriolosclerosis in the lenticular nucleus by the age of 50. In line with our findings, other authors consistently reported over 70% of individuals beyond age 50% and 80% of individuals beyond age 80 having some postmortem evidence of arteriolosclerosis (16, 27–29), all cognitive status confounded, with more than 20% showing moderate to severe vessel wall changes (26, 28, 29). A similar temporal and spatial distribution of arteriolosclerosis was also previously described (25).

Cerebral Amyloid Angiopathy

We reported the presence of leptomeningeal CAA (only examined in hippocampal and occipital regions) in 30.6% of our cases beyond age 50, and 44.4% beyond age 80. This is much lower than previously reported in other autopsy case series, which found CAA positivity in 55% (30) to more than 75% of nondemented subjects (mean age >80 years) (31, 32).

TABLE 2. Spearman's Rank Correlation Coefficients for Total and Lesional Vascular Load Associated with AD Pathology

	Amyloid plaques (Thal phase)†				Neurofibrillary tangles (Braak stage)†				Neuritic plaques (CERAD score)†			
	ρ	p value	Adjusted ρ‡	p value	ρ	p value	Adjusted ρ‡	p value	ρ	p value	Adjusted ρ‡	p value
Total vascular burden	0.353	0.00457	0.125	0.3333688	0.446	0.000248	−0.075	0.5598953	0.289	0.02182	0.114	0.3767743
Circle of Willis atherosclerosis	0.504	0.000163	0.245	0.08619	0.703	< 0.0001	0.255	0.07361828	0.264	0.0608	−0.048	0.7428738
Arteriolosclerosis	0.235	0.06416	−0.074	0.5674396	0.484	< 0.0001	−0.054	0.6767099	0.193	0.1287	−0.039	0.7641459
Perivascular space dilatation	0.172	0.1773	0.089	0.4928867	0.168	0.1882	0.000	0.9998462	0.203	0.1098	0.142	0.2714188
Haemosiderin§ leakage	0.229	0.07143	0.066	0.6079329	0.234	0.06546	−0.128	0.3202897	0.167	0.1911	−0.128	0.3202897
Myelin pallor	0.122	0.3395	−0.006	0.9616499	0.344	0.005715	0.172	0.1817107	0.122	0.3393	0.026	0.8432326
CAA	0.429	0.0004556	0.324	0.01028808	0.345	0.005579	0.101	0.4363229	0.265	0.03553	0.165	0.1991828

†The "ABC" neuropathological assessment for Alzheimer's pathology was obtained according to the NIA-AA scoring criteria (17).
‡Adjusted for age.
§Hemosiderin here refers to "degenerative material of uncertain identity, possibly hemosiderin."

The incidence of CAA varied by brain region. The most frequently affected region was the occipital lobe, followed by parietal, temporal, frontal, and finally hippocampal cortices (30–32). As expected, leptomeningeal vessels in our study seemed consistently more affected by CAA than cortical and capillary vessels (25, 30), and CAA was either undetectable or very sparse in subcortical white matter (33). However, other groups reported involvement of intraparenchymal vessels in 66.2% of mixed demented and nondemented cases (mean age 83.5 years), and as much as 62% in a sample of cognitively normal subjects (mean age 83.9 years), while we found no cortical nor capillary involvement in our younger sample (mean age 54.0 years). The sparing of intraparenchymal vessels in our normal subjects may also be artificially explained by suboptimal sampling of only 2 brain regions (occipital lobe and hippocampus). Also, we used an amyloid immunohistochemistry visual analysis while other scientists use a cell suspension assay technique which can detect lower levels of early CAA.

Secondary Tissue Damage

Neuropathological studies addressing the prevalence, severity, and distribution of brain tissue damage of vascular origin across the normal aging spectrum are much rarer, and currently available evidence is mostly based on premortem MRI studies of older populations.

Perivascular Space Dilatation

Perivascular spaces are integral part of the glymphatic system and play a role in cerebral interstitial water clearance (25). In our study, at least some degree of nonspecific perivascular space dilatation was seen in most subjects of all age groups and in all brain regions. This finding raises the hypothesis that apparent dilated perivascular spaces are either physiological or exacerbated by postmortem changes due to tissue processing such as formalin fixation and paraffin embedment. Nevertheless, there was a trend between increased severity of total perivascular retraction and age. Findings from in vivo MRI studies are in line with our results and show a positive association of perivascular space count and volume with age in both deep white matter and basal ganglia in normal adults (from third to eighth decade), with however large intersubject variations (34, 35). Enlarged perivascular spaces are generally thought to be associated with concomitant small vessel disease (25), but cortical and subcortical perivascular enlargement was present even in the absence of arteriolosclerosis in our sample.

Perivascular Hemosiderin Leakage

Similarly, hemosiderin leakage was found in most subjects of all age group and in a variety of anatomical areas. There was also a significant association between hemosiderin burden and age. Because of scarce histopathological studies, we compared our findings to evidence from MRI studies, which have reported cerebral microbleeds, the main MRI correlate of parenchymal hemosiderin deposits (36, 37), frequen-

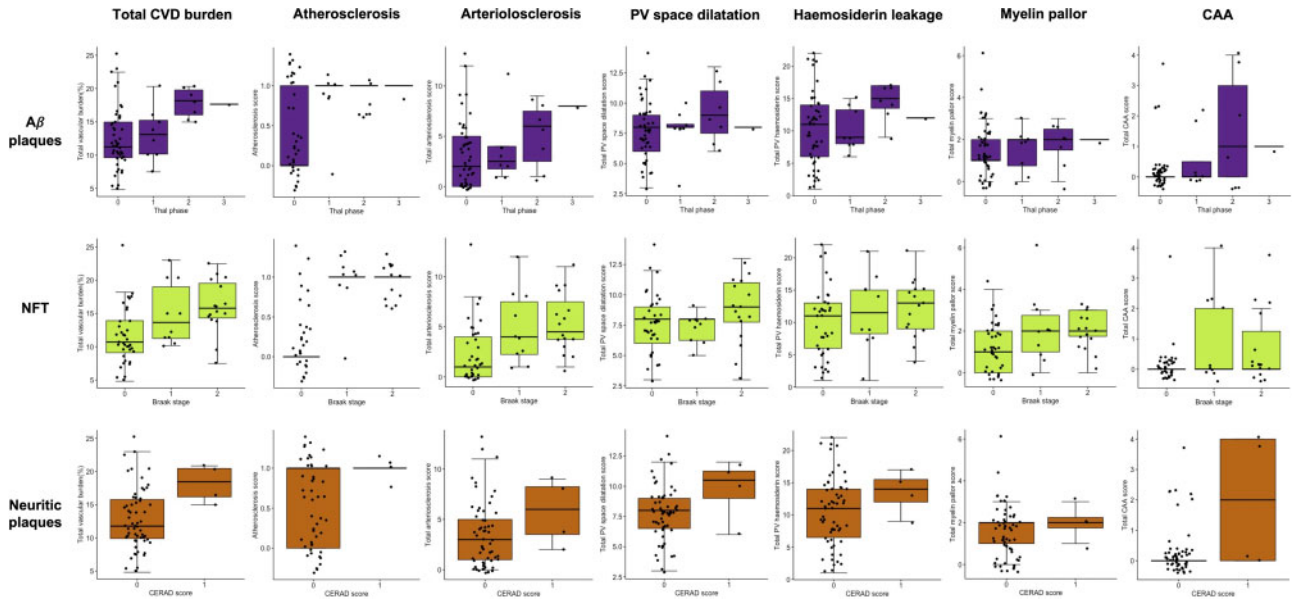

FIGURE 3. Total and lesional vascular burden according to AD pathology. Boxplots represent lesional vascular scores plotted against AD-related pathology scores. Note increase in vascular burden with increasing levels of amyloid and tau deposition in the brain. [a]Hemosiderin refers to "degenerative material of uncertain identity, possibly hemosiderin." Aβ, amyloid beta; CAA, cerebral amyloid angiopathy; CVD, cerebrovascular disease; NFT, neurofibrillary tangles; PV, perivascular.

cies ranging from 3% to 38% in the aging population (38–42). Only 1 group reported histological detection of focal hemosiderin deposition in the putamen in 99% of individuals aged 65 years and over (43). We found hemosiderin leakage in the putamen of 88.9% of our cases, but overall presence of perivascular hemosiderin in 100% of them. Altogether, these findings suggest a very high prevalence of perivascular tissue changes in normal adults, an increasing burden with age, and a far superior sensitivity of postmortem examination for detection of hemosiderin as compared to MRI susceptibility-weighted images and other gradient-echo pulse sequences.

Myelin Pallor

Seventy-eight percent of our participants showed histological evidence of myelin pallor with Luxol fast blue staining of internal capsule and frontal lobe white matter. We hypothesized that these findings were explained by different age-related mechanisms. Indeed, myelin pallor in younger adults is presumably due to incomplete ongoing myelination, particularly in the frontal lobe, which represents the last brain region to reach complete maturation (44). At the opposite, myelin pallor in older adults is most probably due to myelin damage and axonal loss. Nonetheless, there was a trend, but no significant association, between global myelin pallor and increasing age. We found only 1 postmortem analysis of white matter integrity in healthy aging (45). The authors reported a significant age-related anterior and posterior white matter volume loss, but much smaller changes than those reported in neuroimaging studies (46–49).

Ischemic and Hemorrhagic Parenchymal Lesions

We identified 1 or 2 isolated microinfarcts in the basal ganglia, thalamus, or frontal subcortical white matter of 11.1% of our participants, aged 27–82. We found even lower rates of one or 2 isolated basal ganglia microhemorrhages in 2 older cases (64 and 84 years old), and no macroscopic lesions. In previous study cohorts of aging, the prevalence of microscopic and macroscopic infarcts in older adults (mean age 85.0 years) without cognitive impairment ranged from 22% to 46% (50, 51). Macroscopic cortical and subcortical infarcts were described in 22% of older individuals without cognitive impairment, compared with 32.4% and 45.8% of those with mild cognitive impairment and dementia (52). There is no published data for these histological lesions in younger adults. However, the presence on MRI of silent brain infarcts is estimated to be around 3% in the fifth decade and 19% in the eighth decade (53).

Interaction with AD Pathology

Globally, our sample of normal adults presented similar age-associated accumulation of AD pathology (both senile plaques and neurofibrillary tangles) than previously described in nondemented people, but to a lesser degree (31, 51, 52, 54, 55). As described in another autopsy study (55), amyloid plaques were seen in some subjects by the fifth decade, but neurofibrillary tangles appeared later than expected in our sample.

Mostly consistent with conclusions from other postmortem studies (24, 56, 57), we reported a higher degree of total CVD, atherosclerosis, and CAA in individuals with β-amyloid pathology, and more severe total CVD, atherosclerosis, arteriolosclerosis, myelin pallor, and CAA in individuals with tau

pathology. On the other hand, selected findings from these and additional studies differ from our results. For example, in a mixed sample of cognitively normal, subcortical ischemic vascular disease, and AD participants (mean age around 84 years), neither atherosclerosis nor arteriolosclerosis were associated with AD pathology (57). Moreover, in a sample of AD subjects (98% males, mean age 75.1 years), none of the vascular measures were correlated with senile plaques and neurofibrillary tangles burden (58).

A causal relationship cannot be derived from our cross-sectional analysis. Yet, a plausible hypothesis is that vascular wall pathologies might directly influence pathological protein deposition through several mechanisms promoting hypoperfusion and supplementary ischemic injury, and affecting blood brain barrier integrity. This could result in downstream effects such as local production of β-amyloid or deposition from the systemic circulation, and alteration of parenchymal clearance, in addition to secondary ischemic and hemorrhagic tissue damage.

However, our results also suggest that the higher prevalence and burden of concomitant vascular pathology in cognitively healthy subjects with minimal AD changes seem to be mainly driven by increasing age. Only CAA appears to be truly associated with the severity of diffuse amyloid plaques after controlling for age. These results will need further validation in larger clinical cohorts covering the whole spectrum of cognitive decline, and with greater distribution of AD pathology, to allow more definite conclusions to be drawn.

Study Limitations

Potential limitations of our study must be acknowledged. Among these, there was limited demographic and clinical (e.g. vascular risk factors) data available, and no formal cognitive assessment was performed in these apparently cognitively healthy subjects. However, the clinical files were carefully explored and no notion of cognitive deficit was reported in the medical history and available clinical examinations. The chronological evolution and distribution of cerebrovascular lesions cannot be precisely determined due to our cross-sectional and retrospective study design. Finally, there was a high prevalence of epilepsy and SUDEP due to inclusion of younger participants. While chronic epilepsy is associated with repeated ischemic injury of certain hippocampal areas (59), there was no evidence of hippocampal sclerosis, hence no to low hypoxic lesions burden in our series. Additionally, no significant cerebrovascular findings were reported in autopsy studies of SUDEP cases (60, 61). However, a recent cohort study found an increased risk of incident cardiovascular disease (including strokes) in epileptic patients taking enzyme-inducing antiseizure medication, which becomes significant by ∼10 years from first exposure (62). Hence, we cannot exclude with certainty a contribution of epilepsy or its associated treatment in the prevalence of vascular changes reported in our cohort.

In summary, this study demonstrates the presence of a large number of cerebrovascular lesions in the brains of cognitively healthy adults, the burden of which increases with age, independently of clinical strokes and neurodegenerative diseases. The routine use of standardized and validated vascular histological staging scales such as the VCING on normal and diseased brains is greatly encouraged to better characterize and understand the clinical implications of CVD and further identify thresholds of pathological states associated with cognitive impairment.

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