Background and Purpose—Most previous studies addressed the cognitive impact of lacunar infarcts using radiologic correlations that are known to correlate poorly with neuropathological data. Moreover, absence of systematic bilateral assessment of vascular lesions and masking effects of Alzheimer disease pathology and macrovascular lesions may explain discrepancies among previous reports. To define the relative contribution of silent lacunes to cognitive decline, we performed a detailed analysis of lacunar and microvascular pathology in both cortical and subcortical areas of 72 elderly individuals without significant neurofibrillar tangle pathology or macrovascular lesions.

Methods—Cognitive status was assessed prospectively using the Clinical Dementia Rating (CDR) scale; neuropathological evaluation included Aβ-protein deposition staging and bilateral assessment of microvascular ischemic pathology and lacunes; statistical analysis included multivariate models controlling for age, amyloid deposits, and microvascular pathology.

Results—Thalamic and basal ganglia lacunes were negatively associated with CDR scores; cortical microinfarcts, periventricular and diffuse white matter demyelination also significantly affected cognition. In a multivariate model, cortical microinfarcts and thalamic and basal ganglia lacunes explained 22% of CDR variability; amyloid deposits and microvascular pathology explained 12%, and the assessment of thalamic and basal ganglia lacunes added an extra 17%. Deep white matter lacunes were not related to cognitive status in univariate and multivariate models.

Conclusions—In agreement with the recently proposed concept of subcortical ischemic vascular dementia, our autopsy series provides important evidence that gray matter lacunes are independent predictors of cognitive decline in elderly individuals without concomitant dementing processes such as Alzheimer disease. (Stroke. 2005;36:1184-1188.)

Key Words: aging ■ brain injuries ■ cognition ■ dementia ■ lacunar infarction ■ vascular diseases

Morphological substrates of cognitive decline associated with cerebrovascular disease are still strongly debated. The traditional view of a close relationship between a volume of cerebral infarcts >100 mL and dementia proposed by Tomlinson1 has been challenged by neuropathological studies that indicated that small microinfarcts and even macroscopic ischemic lesions can lead to dementia.2–6 More recently, focus has shifted to the identification of more homogeneous subtypes of vascular dementia such as subcortical ischemic vascular dementia, characterized clinically by a dysexecutive syndrome and memory deficits and neuropathologically by lacunes and deep white matter changes.7–10 Although the contribution of deep white matter changes to cognitive decline is now well-documented,11,12 the clinical significance of lacunes has been difficult to establish.13,14 In the Cardiovascular Health Study, 23% of individuals older than age 65 had MRI evidence of lacunes; however, 89% were essentially clinically silent from a cognitive point of view. In other studies, the prevalence of silent lacunes ranged from 11% to 24%.7,10 In a large neuropathological series, lacunes were present in approximately one-quarter of elderly cases without psychiatric or neurological disease.14 In contrast, the Nun study demonstrated that cognitive function was markedly influenced by thalamic, basal ganglia, and deep white matter lacunes in individuals with Alzheimer disease (AD) neuropathology.15 Moreover, a recent study reported intriguing results regarding the cognitive consequences of silent brain infarcts. At baseline, no relationship was found between the presence of silent brain infarcts and impaired cognition, yet cases with new silent brain infarcts during follow-up did have cognitive decline.13 Several methodological issues may explain difficulties encountered when attempting to define the role of lacunar
pathology in brain aging. Because lacunes occur diffusely within the brain, a valid evaluation of their repercussion on cognition presupposes their systematic bilateral assessment in regions known to be highly involved in dementia and prone to development of lacunes such as the thalamus, basal ganglia, and deep white matter. Moreover, the concomitant presence of other age-related dementing pathology may mask the cognitive consequences of lacunes. This includes AD pathology such as amyloid deposits and, most importantly, neurofibrillary tangles (NFT), which represent the strongest correlate of neuronal loss and cognition in AD, \(^{16,17}\) and also microvascular lesions such as cortical microinfarcts and periventricular and diffuse white matter demyelination, which have been shown to negatively influence cognitive performance in brain aging.\(^4\)

To address these issues, we report clinicopathological correlations in a series of prospectively investigated elderly individuals with various degrees of cognitive impairment but without significant NFT pathology or macrovascular lesions. To define the cognitive consequences of lacunar pathology, we compared the semi-quantitative assessment of cognitive findings to the semi-quantitative assessment of lacunes in the deep white matter, basal ganglia, and thalamus. The present analysis is based on multivariate models that control for the interaction with age, amyloid deposits, and significant microvascular pathology.

**Materials and Methods**

We surveyed a 5-year autopsy population of 629 cases from the Geriatric and Psychiatric Hospitals, Geneva University School of Medicine. All patients were from the Geneva area and older than 65 years; there were 270 men (81.0±7.1 years) and 359 women (82.8±7.3 years). In these institutions, all patients older than 65 undergo cognitive screening using the Mini-Mental State Examination\(^19\) and clock drawing test.\(^20\) If, as a result, dementia is suspected, neuropsychological evaluation is performed using tests appropriate to each situation. All cases are also rated according to the clinical dementia rating scale (CDR), a validated scale that is widely used for the clinical staging of dementia.\(^20\) It assigns cognitive function to five levels defined as no dementia (CDR 0), questionable dementia (CDR 0.5), mild dementia (CDR 1), moderate dementia (CDR 2), and severe dementia (CDR 3). Cases with stroke history or other central nervous system disorders (ie, tumors, inflammation, Parkinson disease, Lewy body disease) were excluded. Such ascertainment was based on review of written and computerized medical records.

Brains were fixed in 15% formaldehyde for a minimum of 4 weeks and cut into 1-cm coronal slices. Cases were classified according to Braak\(^21\) and Thal\(^22\) using highly specific, fully characterized antibodies to microtubule-associated \(\tau\) protein and to core-amyloid \(A\beta\)-protein as described elsewhere.\(^4\) Antibodies were a monoclonal anti-\(\tau\) antibody (AT8, 1/1000; Immunogenetics) and a monoclonal anti-\(A\beta\) antibody (4G8, 1/1000; Signet Laboratories). Tissues were incubated overnight at 4°C and sections were then processed by the PAP method using 3,3'-diaminobenzidine as a chromogen.\(^6\) For NFT, cases were classified as either transentorhinal (I and II), limbic (III and IV), or neocortical Braak stages (V and VI). \(A\beta\)-protein deposition staging was performed according to the 4-phase nomenclature proposed by Thal.\(^23\) In phase 1, diffuse \(A\beta\) deposits are found in the basal temporal neocortex. In phase 2, diffuse \(A\beta\) deposits occur within external entorhinal layers, whereas punctate amyloid deposits (“fleecy amyloid”) are seen in internal entorhinal layers and the CA1 field of the hippocampus. The third phase is characterized by increased \(A\beta\) deposition in the molecular layer of the dentate gyrus, entorhinal cortex, and temporal neocortex, and in the parvopyramidal layer of the presubiculum. In phase 4, diffuse \(A\beta\) deposits and core-only senile plaques (SP) are observed in the CA3–4 hippocampal fields. This sequence of \(A\beta\) deposition is also followed by neuritic plaques, which progressively invade the medial temporal lobe in sites receiving afferent input from NFT-containing neurons. To avoid the masking effect of substantial NFT-related pathology, only cases with very early Braak NFT stages I and II were considered in the present study (223 cases excluded with NFT stage >II). For the same reasons, cases with significant macrovascular pathology other than lacunes were excluded. The final sample included 72 patients aged 63 to 100 years who did not meet any of the aforementioned exclusion criteria and for whom presence and severity of dementia was assessed in all cases using the CDR during the 3 months before death (Table). All procedures involving use of postmortem human brain were conducted after written consent of the patients or their family and were approved by the Ethics Committee.

Lacunes were defined as small definitive ischemic necrosis, 1 mm to 1.5 cm, located in white matter, basal ganglia, or thalamus. On histological examination, lacunes consist of cavitations of cerebral tissue with scattered fat-laden macrophages and surrounding gliosis. Younger lesions show mild to severe rarefaction of neurons with reduced number of oligodendrocytes and early signs of cavitation. To visualize lacunes and significant microvascular pathology (ie, cortical microinfarcts, periventricular and diffuse white matter demyelination), tissue blocks, from the anterior hippocampus, inferior temporal cortex (area 20), frontal cortex (area 9), and parietal cortex (area 40) bilaterally and basal ganglia and thalamus were cut into 20-\(\mu\)m-thick sections and stained with the Globus silver impregnation technique\(^4\) (Figure 1). To assess diffuse white matter and periventricular demyelination, coronal slices at the level of the anterior border of the corpus callosum were embedded in paraffin, cut into 20-\(\mu\)m-thick sections, and stained with Luxol-van Gieson.

Lacunes and microvascular pathology assessed semi-quantitatively in 10 sections per area were scored as follows: 0 (absence of such lesions), 1 (<3 lesions per slide), 2 (3 to 5 lesions per slide), and 3 (>5 lesions per slide). A total score was obtained by adding the scores of each area. Severity of diffuse white matter and periventricular demyelination in each hemisphere was estimated in Luxol-van Gieson–stained sections using a semi-quantitative scale: 0 = absent, 1 = mild, 2 = moderate, and 3 = severe. Scores for each hemisphere were added to obtain a total score. Thus, 6 scores were determined in each case and used for statistical analysis: a microinfarct score (all microinfarcts regardless of location), 2 demyelination scores (diffuse white matter and periventricular white matter), and 3 lacunar scores according to region of interest (deep white matter, basal ganglia, and thalamus). All neuropathological analyses were performed by 2 independent investigators (E.K., and C.B.), blind to the clinical findings, with a high inter-rater reliability (\(k=0.90\) to 0.95).

Maximal likelihood ordered logistic regression with proportional odds was used to evaluate the association between CDR scores (the dependent variable) and neuropathological parameters (\(A\beta\)-protein deposition staging, lacune and microvascular pathology scores as the independent variables) in a univariate model. Subsequently, the same method was applied in a multivariate model to take into account the effect of age and the interaction between neuropathological variables. \(A\beta\)-protein deposition staging, an ordinal scale with 4 levels, and core-only senile plaques (SP) are observed in the CA3–4 hippocampal fields. This sequence of \(A\beta\) deposition is also followed by neuritic plaques, which progressively invade the medial temporal lobe in sites receiving afferent input from NFT-containing neurons. To avoid the masking effect of substantial NFT-related pathology, only cases with very early Braak NFT stages I and II were considered in the present study (223 cases excluded with NFT stage >II). For the same reasons, cases with significant macrovascular pathology other than lacunes were excluded. The final sample included 72 patients aged 63 to 100 years who did not meet any of the aforementioned exclusion criteria and for whom presence and severity of dementia was assessed in all cases using the CDR during the 3 months before death (Table). All procedures involving use of postmortem human brain were conducted after written consent of the patients or their family and were approved by the Ethics Committee.

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Lacunes and microvascular pathology assessed semi-quantitatively in 10 sections per area were scored as follows: 0 (absence of such lesions), 1 (<3 lesions per slide), 2 (3 to 5 lesions per slide), and 3 (>5 lesions per slide). A total score was obtained by adding the scores of each area. Severity of diffuse white matter and periventricular demyelination in each hemisphere was estimated in Luxol-van Gieson–stained sections using a semi-quantitative scale: 0 = absent, 1 = mild, 2 = moderate, and 3 = severe. Scores for each hemisphere were added to obtain a total score. Thus, 6 scores were determined in each case and used for statistical analysis: a microinfarct score (all microinfarcts regardless of location), 2 demyelination scores (diffuse white matter and periventricular white matter), and 3 lacunar scores according to region of interest (deep white matter, basal ganglia, and thalamus). All neuropathological analyses were performed by 2 independent investigators (E.K., and C.B.), blind to the clinical findings, with a high inter-rater reliability (\(k=0.90\) to 0.95).

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Results

The study sample included 17 cases with no dementia (CDR 0), 20 cases with questionable dementia (CDR 0.5), and 35 cases with varying severity of dementia (CDR 1 to 3) (Table). Lacunes were present in 21 cases in the basal ganglia, in 18 cases in deep white matter, and in 9 cases in the thalamus. Cortical microinfarcts were found in 29 cases, periventricular demyelination in 43 cases, and diffuse white matter demyelination in 62 cases. Mild αβ-protein deposition was present in most cases (45.8% were stage A, 27.8% stage B), but 26% displayed substantial αβ-protein deposition within the medial temporal lobe (22.2% stage C, 4.2% stage D).

There were 16 cases with lacunes and without cortical microinfarcts: 3 cases with no dementia (CDR =0), 3 cases with questionable dementia, 6 cases with moderate dementia (CDR =2), and 4 cases with severe dementia (CDR =3). The 3 cases with no dementia had small amounts of lacunes (score of ≤1) in both deep white matter and basal ganglia. All but one of these cases also had white matter lesions.

In a univariate model, αβ-protein deposition staging explained 7% \((P=0.002; R^2=0.07)\) and age only 3% \((P=0.02; R^2=0.03)\) of the variability in CDR scores. Among the different types of microvascular pathology, cortical microinfarcts \((P<0.001)\), diffuse white matter \((P=0.03)\), and periventricular demyelination \((P=0.01)\) were all significant correlates of cognitive function and explained, respectively, 11% \((R^2=0.11)\), 6% \((R^2=0.06)\), and 6% \((R^2=0.06)\) of the variability in cognitive function as measured by the CDR.

Lacunes in the thalamic and basal ganglia significantly predicted cognitive status \((P=0.02)\) and explained 5% \((R^2=0.05)\) and 6% \((R^2=0.06)\) of CDR variability, respectively. However, there was no significant relationship between deep white matter lacunes and CDR score \((P=0.19)\).

We performed a multivariate model including the following variables: age, αβ-protein deposition staging, thalamic and basal ganglia lacune scores, microinfarct scores, and periventricular and diffuse demyelination scores. It explained 29% of the clinical variability. In this analysis, only αβ-protein deposition staging, cortical microinfarcts, and basal ganglia and thalamic lacunes scores were significantly related to CDR. The latter 3 vascular variables were used to develop a combined vascular score \((\text{thalamo score} + \text{basal ganglia score} + \text{microinfarct score})/4\); the microinfarct score was divided by 4 to ensure that each of the 3 subscores had the same weight). This combined score explained 22% of the CDR variability (Figure 2). Importantly, in a forward stepwise regression model, the best predictors of cognitive status were in order of decreasing strength cortical microinfarcts, basal ganglia lacunes, thalamic lacunes, and αβ-protein deposition staging. In this model, lacunes explained 17% of the clinical variability.

Discussion

To our knowledge, this is the first study addressing the cognitive impact of silent lacunar infarcts and microvascular changes in an autopsy series of prospectively assessed elderly individuals. Its strengths include the detailed analysis of lacunes and different types of microvascular lesions in cortical areas bilaterally and control for the most important confounding variables (substantial NFT pathology corresponding to limbic and neocortical Braak stages and macroinfarcts) and use of multivariate models for statistical analysis, which can estimate the predictive value of each neuropathological measure taking into account the strength of their interaction. As usual, limitations related to the size of...
the study population and neuropathological sampling methods must also be taken into account. However, in view of the relative scarcity of cases with pathological changes confined to lacunes, demyelination, and microinfarcts, our series represents an unusually large autopsy cohort. Moreover, although we cannot exclude that additional pathology may have been present mainly in neocortical areas, we have minimized this bias by examining ten sections per area.

Consistent with our previous observations in an autopsy sample including elderly individuals with isolated microvascular pathology, cortical microinfarcts and periventricular and diffuse white matter demyelination were all significant determinants of cognitive decline in this series. Not surprisingly, this association was substantially weaker in the present cohort of cases with mixed vascular pathology compared with our previous results. Most importantly, the relationship between CDR scores and both diffuse white matter and periventricular demyelination was no longer significant after controlling for lacunes using multivariate models. This is not surprising because lacunes and demyelination often co-exist (95% of our cases with lacunes also had white matter demyelination); thus, once one variable is taken into account, in a multivariate model, the other adds little information. More importantly, our neuropathological data suggest that lacunes were the stronger correlate of cognitive status and that additional white matter demyelination had little added effect on intellectual function. This contrasts with results of several structural imaging studies that reported that white matter signal hyperintensities but not lacunes were related to cognitive measures in brain aging. One possible explanation for this discrepancy resides in poor radiologic–pathologic correlation. Two recent contributions comparing MRI to postmortem data demonstrated poor association between the presence of whiter matter hyperintensities and demyelination. White matter lesions depicted on MR images correspond to variable combinations of myelin and axonal loss and scattered microinfarcts, astrogliosis, and dilatation of periventricular spaces. Moreover, the radiologic concept of lacune covers a wide spectrum of histological changes such as complete infarcts, areas of focal gliosis, and perivascular space. In agreement with the recently developed concept of subcortical ischemic vascular dementia, our series provides important autopsy evidence that thalamic and basal ganglia lacunes are independent predictors of cognitive decline in the elderly.14,29,30

The present data also support the strategic importance of location in defining the cognitive impact of lacunes. In univariate and multivariate models, thalamic and basal ganglia lacunes but not deep white matter lacunes significantly predicted CDR scores. It has long been considered that cognitive deterioration in patients with lacunes may result from disruption of subcortical–frontal circuits. The present findings are partly consistent with this hypothesis because they indicate that damage in subcortical gray matter may decisively influence cognitive performances. However, they clearly demonstrate that the frequent development of lacunes within deep white matter in brain aging is not sufficient to induce dementia.

It is noteworthy that assessment of vascular pathology in the present series can predict at best 22% of CDR variability. This value is comparable to that previously reported for NFT Braak staging in a large autopsy series, indicating that assessment of microvascular changes and lacunes may represent a valid predictor of cognitive decline, in the absence of substantial NFT pathology, as strong as NFT staging in elderly individuals without vascular pathology. The relative weakness of the relationship between vascular pathology and cognitive status may reflect methodological issues related to semi-quantitative assessment of microvascular changes and lacunes. This possibility is further supported by recent findings showing that rigorous stereological assessment of NFT in vascular pathology-free cases leads to >85% prediction of clinical variability. Alternatively, other neuropathological variables such as neuronal or synaptic loss may decisively contribute to cognitive impairment in this context. The analysis of large autopsy series including various cognitive parameters and stereological assessment of microvascular changes, lacunes, as well as neuronal and synaptic loss is warranted to define precisely the structural background of dementia in cases with predominant vascular pathology.

These new data may be also relevant with respect to current efforts at neuropathological standardization in the dementia field. The current debate regarding the definition of these conditions mostly reflects the difficulty in evaluating the relative clinical impact of the various types of vascular lesions in pure vascular cases and defining the synergistic effect of vascular and AD-type changes in mixed conditions. Our previous work in cases with isolated microscopic ischemic pathology led to the conclusion that cortical microinfarcts are a strong predictor of cognitive changes in brain aging. The present observations complete this first study by providing an estimate of the cognitive consequences of gray matter lacunes in cases without AD neuronal pathology. These findings may be of particular importance for the...
definition of neuropathological criteria for vascular and mixed dementia and should serve as a basis for future clinical-pathological studies exploring the combined effect of clinically relevant macroscopic and microscopic ischemic vascular and degenerative changes in mixed conditions.

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