Cortical Microinfarcts and Demyelination Significantly Affect Cognition in Brain Aging

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Background and Purpose—Microvascular lesions are common in brain aging, but their clinical impact is debated. Methodological problems such as the masking effect of concomitant pathologies may explain discrepancies among previous studies. To evaluate the cognitive consequences of such lesions, we prospectively investigated elderly individuals with various degrees of cognitive impairment but without significant neurofibrillary tangle pathology or macrovascular lesions.

Methods—This was a clinicopathological study of 45 elderly individuals. Cognitive status was assessed prospectively with the Clinical Dementia Rating (CDR) scale; neuropathological evaluation included Aβ-protein deposition staging and bilateral semiquantitative assessment of cortical microinfarcts, focal cortical and white matter glioses, and diffuse white matter and periventricular demyelination.

Results—In a univariate logistic regression model, cortical microinfarcts explained 36.1% of the variability in CDR; periventricular demyelination, 10.6%; and diffuse white matter demyelination, 4.6%. After controlling for age and Aβ-protein deposition, cortical microinfarcts were the best predictor of cognitive status (19.9% of CDR variability), whereas periventricular and diffuse white matter demyelination accounted for 9.7% and 5.4% of CDR variability, respectively. Altogether, these 3 types of microvascular lesions explained 27.9% of the clinical variability. Focal cortical and white matter glioses were not related to clinical outcome.

Conclusions—Our data imply that cortical microinfarcts and both periventricular and deep white matter demyelination contribute significantly to the progression of cognitive deficits in brain aging. In contrast, the neuropathological evaluation of focal cortical and white matter gliosis has no clinical validity. (Stroke. 2004;35:410-414.)

Key Words: aging ■ brain ischemia ■ cognition ■ dementia, vascular ■ microvascular injury

The morphological substrates of dementia associated with cerebrovascular disease are still poorly defined. The traditional view of a strong relationship between a volume of cerebral infarcts >100 mL and cognitive decline was challenged by Tomlinson and collaborators,1 who proposed the concept of “strategic macroinfarcts.” Consistent with this viewpoint, several neuropathological studies have indicated that even small macroinfarcts can lead to dementia or can significantly worsen cognitive impairment in patients with definite Alzheimer’s disease (AD).2-8 However, other clinicopathological studies in AD cases have suggested that small macrovascular lesions do not contribute to the overall rate of cognitive decline.9,10 Moreover, the location of macrovascular lesions is not a sufficient determinant of their clinical impact, as demonstrated by functional imaging studies (for review, see elsewhere11,12).

The possible impact of isolated microvascular lesions on cognition remains even more controversial, and data regarding this issue are very scarce. Although some studies point to a possible causal relationship with dementia2-4,13 this point of view has been challenged.10 Three main methodological issues may explain the difficulty to define the role of microvascular pathology in cognitive deterioration. Microvascular lesions are highly heterogeneous and include several pathological changes with possibly distinct patterns of clinical impact such as microinfarcts, focal cortical and white matter glioses, and diffuse white matter demyelination (PVD). Be-
cause they are diffusely developed within the brain, a valid evaluation of their effect on cognition presupposes their systematic bilateral assessment in cortical regions known to be highly involved in dementia such as the hippocampus and neocortical association areas.\textsuperscript{14} Moreover, the concomitant presence of age-related AD pathology such as amyloid deposits and, most importantly, neurofibrillary tangles (NFT), which represent the strongest correlate of neuronal loss and cognition in AD,\textsuperscript{15,16} can mask the consequences of microvascular pathology in the very frequent cases with mixed pathology.\textsuperscript{5,17} To address these issues, we report clinicopathological correlations in a large series of prospectively investigated elderly individuals with various degrees of cognitive impairment but without significant NFT pathology or macrovascular lesions. The present analysis included bilateral assessment of all types of microvascular lesions and is based on multivariate models that control for the interaction between microvascular pathology, age, and amyloid deposits.

**Materials and Methods**

The sample included 45 patients 63 to 100 years of age who died and were autopsied at the geriatric and psychiatric hospitals of the University of Geneva School of Medicine (Switzerland). The presence and severity of dementia were assessed in all cases with the Clinical Dementia Rating Scale (CDR) during the 3 months before death. The CDR is a validated scale widely used for clinical staging of dementia.\textsuperscript{18} It assigns cognitive function to 5 levels defined as no (CDR 0), questionable (CDR 0.5), mild (CDR 1), moderate (CDR 2), and severe (CDR 3) dementia. Sex and age distributions of the cases according to CDR score are listed in the Table. Cases with stroke history or other central nervous system disorders (ie, tumors, inflammation, Parkinson’s disease, Lewy body disease) were excluded from the present study.

Brains obtained at autopsy were fixed in 15% formaldehyde for at least 4 weeks and cut into 1-cm-thick coronal slices. All cases were classified neuropathologically according to Braak and Braak\textsuperscript{19} and Thal and collaborators\textsuperscript{20} using highly specific and fully characterized antibodies to the microtubule-associated tau protein\textsuperscript{21} and to the core-amyloid A\textsubscript{β}-protein.\textsuperscript{22} The antibodies used in the present study were a monoclonal anti-tau antibody (AT8, 1/1000, Immunogenetics) and monoclonal anti-A\textsubscript{β} antibody (4G8, 1/1000, Signet Laboratories). Tissues were incubated overnight at 4°C. After incubation, sections were processed by the PAP method with 3,3'-diaminobenzidine as a chromogen.\textsuperscript{23} For NFT, all cases were classified as belonging to the transentorhinal (I and II), limbic (III and IV), or neocortical Braak stages (V and VI).\textsuperscript{19} A\textsubscript{β}-protein deposition staging was performed according to the amyloid nomenclature proposed by Thal and collaborators.\textsuperscript{20} 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. For the same reasons, cases with macrovascular pathology such as cortical macroinfarcts and lacunes identified in the macroscopic examination were excluded.

To visualize microinfarcts and focal cortical and white matter gliosis, tissue blocks from the anterior hippocampus, inferior tem-

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**Table**: Demographic Data in the Present Series

<table>
<thead>
<tr>
<th>CDR</th>
<th>Mean (SD) Age, y</th>
<th>Cases (Women/Men), n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>78.2 (10.6)</td>
<td>13 (8/5)</td>
</tr>
<tr>
<td>0.5</td>
<td>83.7 (9.9)</td>
<td>16 (10/6)</td>
</tr>
<tr>
<td>1</td>
<td>87.2 (5.3)</td>
<td>5 (2/3)</td>
</tr>
<tr>
<td>2</td>
<td>89.8 (6.1)</td>
<td>5 (4/1)</td>
</tr>
<tr>
<td>3</td>
<td>86.5 (6.9)</td>
<td>6 (3/3)</td>
</tr>
<tr>
<td>All cases combined</td>
<td>83.5 (9.5)</td>
<td>45 (27/18)</td>
</tr>
</tbody>
</table>

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**Figure 1.** Representative examples of microvascular lesions assessed in the present study: multiple microinfarcts in the frontal cortex (a; arrows), white matter gliosis (b), focal cortical gliosis (c), and severe DWMD and PVD (d). Sections were stained with Globus silver impregnation (a through c) and Luxol van-Gieson stain (d). Scale bar: 1000 mm (a), 250 μm (b), and 125 μm (c).
poral cortex (area 20), frontal cortex (area 9), and parietal cortex (area 40) bilaterally were cut into 20-μm-thick sections and stained with the Gollub silver impregnation technique (Figure 1). To assess DWMD and PVD, coronal slices at the level of the anterior border of the corpus callosum were embedded in paraffin, cut into 20-μm-thick sections, and stained with Luxol-van Gieson stain (Figure 1).

Microinfarcts and focal cortical gliosis were assessed semiquantitatively in 10 sections per area with the following score: 0 (absence of such lesions), 1 (<3 lesions per slide), 2 (3 to 5 lesions per slide), or 3 (>5 lesions per slide). Semiquantitative assessment of white matter gliosis was made in the same number of sections with the following rating scale: 0 = absent, 1 = mild, 2 = moderate, and 3 = severe. For each lesion, a total score was obtained by adding the scores of each area. The severity of DWMD and PVD in each hemisphere was estimated in Luxol-van Gieson–stained sections with the semiquantitative scale described above. Scores for each hemisphere were added to obtain a total score. Braak NFT and Aβ-protein deposition staging and semiquantitative assessment of microvascular pathology were performed by 2 independent investigators (E.K. and C.B.) who were blinded to the clinical findings, with a high interrater reliability (κ = 0.88 to 0.95).

The 45 cases included in this study represent consecutive autopsies from the institutions mentioned above who met inclusion criteria (CDR within 3 months of death) and none of the exclusion criteria (history of stroke or other central nervous system disorders, Braak NFT stage > II, macroinfarcts or lacunes on macroscopic examination). Permission for autopsy was obtained in all cases from family members, and the study was approved by the ethics committee.

After normalization of the neuropathological variables, maximal likelihood ordered logistic regression with proportional odds was used to evaluate the association between CDR scores (the dependent variable) and neuropathological parameters (Aβ-protein deposition staging 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 the neuropathological variables. Because the Aβ-protein deposition staging is an ordinal scale, it was entered into the models as a dummy variable (3 levels). The number of cases, a maximum of 5 independent variables were entered into each multivariate model. Maximum likelihood ordered logistic regression can be used to measure the relationship between an ordinal outcome variable (CDR) and several independent variables. This method can also evaluate the amount of variability of the outcome variable (ie, the CDR score) that can be explained by the independent variables (ie, age, Aβ-protein phase, microvascular pathology). Thus, it provides an estimate of the strength of the relationship. Statistical analyses were performed using Stata software package, release 6.

**Results**

Mild Aβ-protein deposition was present in most cases (stage A, 46.7%; stage B, 28.9%). Despite the presence of minimal NFT pathology confined to the entorhinal cortex, > 24% of cases displayed substantial Aβ-protein deposition within the medial temporal lobe (stage C, 17.8%; stage D, 6.7%). Although age and Aβ-protein deposition staging were significantly related to clinical outcome (P < 0.05), they predicted only 5.4% and 8.0% of the CDR variability, respectively. Among the different types of microvascular pathology, only cortical microinfarcts, DWMD, and PVD were significantly associated with CDR score (Figure 2). In a univariate model, the microinfarct score explained 36.1% of the variability in CDR (P < 0.001); PVD score, 10.6% (P < 0.001); and DWMD, 4.6% (P < 0.05).

**Discussion**

Strengths of the present study include the detailed analysis of the different types of microvascular lesions in cortical areas bilaterally, controlling for the most important confounding variables (ie, substantial NFT pathology corresponding to limbic and neocortical Braak stages and macroinfarcts), and use of multivariate models for statistical analysis that can estimate the predictive value of each neuropathological measure, taking into account the strength of their interaction. In the present series, cortical microinfarcts explain ~ 20% of the clinical variability after controlling for age and Aβ-protein deposition stage. This value is comparable to that previously reported for NFT Braak staging in a large autopsy series, indicating that the assessment of cortical microinfarcts may represent a
predictor of cognitive decline in the absence of substantial NFT pathology as strong as NFT staging in elderly individuals without vascular pathology.\textsuperscript{4,13}

DWMD and PVD were also related to cognition in the present series. Several MRI studies addressed the correlation of white matter lesions and cognitive dysfunction in the elderly with conflicting results.\textsuperscript{24–27} Two recent studies comparing MRI with postmortem data demonstrated a poor correlation between the presence of whiter matter hyperintensities and demyelination.\textsuperscript{28,29} In fact, white matter lesions depicted on MRI correspond to variable combinations of myelin and axonal loss, as well as scattered microinfarcts, astroglisisis, and dilatation of periventricular spaces.\textsuperscript{28} It is thus not surprising that the clinical validity of white matter lesions is difficult to establish in the absence of neuropathological data.\textsuperscript{27} Our autopsy series provides important evidence of an association between cognitive decline and both DWMD and PVD in the elderly.

The present data also allow evaluation of the strength of the relationship between each type of microvascular pathology and cognition. The semiquantitative assessment of cortical microinfarcts in frontal, parietal, and temporal association areas and hippocampus represents the most promising neuropathological variable in terms of clinicopathological correlations. Our results also show that PVD accounts for double the clinical variability compared with DWMD. Interestingly, although prior neuroimaging studies have suggested that both diffuse white matter and periventricular lesions correlate with cognitive deterioration,\textsuperscript{30,31} more recent data indicate that periventricular white matter lesions may be the strongest determinant\textsuperscript{12–34} of cognitive performance. In contrast, focal cortical and white matter glissos seem to have no clinical validity.

These new findings may also be relevant to current efforts of neuropathological standardization in the field of vascular and mixed dementia. The absence of widely accepted neuropathological criteria for vascular and mixed dementia mostly reflects the difficulty in evaluating the relative clinical impact of macrovascular and microvascular lesions and AD-type changes in mixed conditions. The present observations define distinct patterns of clinical validity within the heterogeneous group of microvascular lesions and provide an estimate of their cognitive consequences. This information is of particular value for the design of future clinicopathological studies that need to be conducted in prospectively documented elderly cohorts to address the combined effect of clinically significant microvascular changes, macrovascular pathology, and AD lesions in brain aging.

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References


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