Inverse Relationship Between Cerebrovascular Lesions and Severity of Lewy Body Pathology in Patients With Lewy Body Diseases

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Abstract
Cerebrovascular pathology is a major cause of stroke and mortality. Studies on prevalence of cerebrovascular pathologies in dementia with Lewy bodies (DLBs) and Parkinson disease (PD) patients are scarce and contradictory. We aimed to determine the prevalence and severity of cerebrovascular pathologies in DLB and PD and to analyze their relationship to LB pathology. The prevalence and severity of atherosclerosis in the circle of Willis, cerebral amyloid angiopathy, cerebral infarcts, hemorrhages, small-vessel disease, white matter lesions, including the Consortium to Establish a Registry for Alzheimer Disease (CERAD) protocol as well as Braak neurofibrillary tangle stages for AD pathology were analyzed in autopsy-verified DLB (n = 13), PD (n = 102), and control subjects (n = 53). In all patient groups, the extent of LB pathology was inversely correlated to the severity of most vascular pathologies (atherosclerosis, infarcts, and white matter lesions; all p < 0.05). By contrast, cerebral amyloid angiopathy, CERAD, and Braak neurofibrillary tangle stages were positively correlated with LB pathology (p < 0.05). Whereas the overall prevalence and severity of small-vessel disease, infarcts, hemorrhages, and white matter lesions were comparable among both disease groups, the extents of atherosclerosis, cerebral amyloid angiopathy, CERAD, and Braak neurofibrillary tangle stages were significantly higher in DLB than in those of PD patients (p < 0.05). Microinfarcts were statistically more prevalent in each patient group than in controls, whereas gross infarcts predominated in controls (p < 0.05 each). In conclusion, DLB and PD patients with advanced LB pathology were less likely to show severe cerebrovascular disease or history of stroke.

Key Words: Cerebrovascular disease, Dementia with Lewy bodies, Lewy body disease, Parkinson disease.

INTRODUCTION
Cerebrovascular pathologies have been recognized as a major cause of stroke and mortality in the elderly (1) and may coexist with other age-related diseases, such as Lewy body (LB) disease (2, 3) and Alzheimer disease (AD) (4–7). Lewy body diseases include the diagnoses of dementia with LBs (DLB) and Parkinson disease (PD). Neuropathologically, the presence of LBs and Lewy neurites in the central and peripheral nervous systems is a hallmark feature of DLB and PD (2, 8).

Disturbances of cerebral blood flow are frequent in DLB and PD (2, 9, 10), and they might influence clinical courses of the diseases. Such perturbations may result from vascular lesions, a concept that merits closer attention. Most previous studies, however, have only investigated the prevalence of stroke in PD patients compared with controls and have obtained contradictory results. For example, some reports indicate higher frequencies of stroke in PD patients (11–15); others observed less prevalence of stroke (16–19); and others failed to note any difference compared with controls (20–23). The few existing autopsy-controlled studies compared the prevalence of vascular lesions between DLB or PD patients and that of controls and did not specifically address the relationship between LB pathology and cerebrovascular disease (3, 20, 23).

The aim of this study was to examine the prevalence and severity of different cerebrovascular pathologies in DLB and PD and to assess the relationship between cerebrovascular and Lewy pathologies. Our findings reveal an inverse relationship between the major cerebrovascular pathologies and the severity of LB pathology.

MATERIALS AND METHODS
Subjects
We investigated 115 autopsy brains (13 DLB and 102 PD; age range, 55–89 years). Most PD cases, but only 2 DLB
cases, were obtained from the movement disorder unit of the MST Hospital Group in Enschede, The Netherlands. Clinical diagnoses of all persons had been made during life following established criteria for the clinical diagnosis of DLB or PD (2, 24). Informed consent for autopsy was obtained for all patients. We also investigated an age- and sex-matched control population (n = 53). These controls represented consecutive randomly selected autopsy series. Clinical data of the controls were compiled retrospectively from medical records, and neuropathologic investigations of the brains were carried out for the presence of PD and AD pathologies, cerebrovascular lesions, and other common degenerative diseases. Inclusion criteria were the absence of PD pathology as well as other neurological diseases, such as neuropsychiatric, neurodegenerative, infectious/inflammatory, or brain tumors, but not cerebrovascular disease. According to their medical records, none of the controls was demented, although specific dementia rating scales were not recorded. The study was approved by local ethics committees (Enschede, The Netherlands, and Frankfurt, Germany). The demographic and pathological characteristics of controls, patient groups, and neuropathologic subgroups are shown in the Table.

**Neuropathologic Analysis**

All autopsies fulfilled published criteria for macroscopic and microscopic diagnoses of DLB or PD (2, 8). Brains were fixed in 4% formaldehyde. Extensive analysis of brain pathology was performed on serial 10-μm paraffin sections from different brain regions to facilitate DLB consensus criteria (2), the Consortium to Establish a Registry for AD (CERAD) protocol (25), PD staging (26), and Braak neurofibrillary tangle (NFT) staging (27).

**Evaluation of LB Pathology**

Using conventional hematoxylin and eosin and α-synuclein staining, LB pathology was assessed on histological sections from the middle frontal gyrus, inferior parietal lobule, superior and middle temporal gyri, transcortentorial cortex, and anterior cingulate region as outlined in the DLB consensus criteria (2). Assessment of LB pathology followed the same consensus guidelines: brainstem (LB score, 0–2), limbic (LB score, 3–6), and neocortical (LB score, 7–10). Additional sections from brainstem (dorsal motor nucleus of the vagus nerve, locus caeruleus, raphe nuclei, tegmental pedunculopontine nucleus, and substantia nigra), basal nuclei of Meynert, hypothalamic tuberomammillary nucleus, hippocampus, and anterior medial temporal lobe with amygdala and entorhinal cortex were also evaluated to facilitate staging of PD-related pathology according to Braak et al (26).

**Evaluation of AD-Related Pathology**

To verify the degree of AD-related pathology, the presence of cortical neuritic plaques was assessed according to CERAD guidelines in the middle frontal gyrus, inferior parietal lobule, and superior and middle temporal gyri in Bielschowsky preparations (25). Braak NFT staging was performed using the Gallyas silver method in the following sections: entorhinal and transcortentorial cortices with adjacent temporal neocortex (at the mid-uncal level), hippocampus with adjacent parahippocampal temporal neocortex (at the level of the lateral geniculate body), and occipital cortex (Brodmann Area 17).

**Assessment of Atherosclerosis in Basal Surface Arteries of the Circle of Willis**

Atherosclerosis (AS) was examined macroscopically in basal surface arteries of the circle of Willis. The severity of AS was assessed as described previously: 0, absent (no macroscopically detectable AS plaques); 1, mild (AS plaques in at least 1 artery without compromising the lumen); 2, moderate (<50% lumen obstruction of at least 1 artery); and 3, severe (>50% lumen obstruction of at least 1 artery) (7, 28). To confirm the AS diagnosis, blocks from the most severely affected arteries were embedded in paraffin, and tissue sections were stained with elastica van Gieson.

**Assessment of Small-Vessel Disease**

The presence of small-vessel disease (SVD), that is, arteriosclerotic, arteriolosclerotic, lipohyalinotic (fibrinoid necrosis), and microaneurysmal formation in small parenchymal brain vessels, was documented using hematoxylin and eosin– and elastica van Gieson–stained sections. For this purpose, the following 5 cortical brain regions (including adjacent subcortical white matter) were used: middle frontal gyrus, inferior parietal lobule, superior and middle temporal gyri, and occipital gyri.

**Table.** Demographic and pathological features of individuals studied

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls</th>
<th>PD</th>
<th>DLB</th>
<th>Neuropathologic Subgroups</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (men/women)</td>
<td>53 (31/22)</td>
<td>13 (5/8)</td>
<td>102 (60/42)</td>
<td>Brainstem Limbic Neocortical</td>
</tr>
<tr>
<td>Age, mean (SD), years</td>
<td>74.2 (7.9)</td>
<td>75.5 (6.2)</td>
<td>75 (6.8)</td>
<td>14 (10/4) 57 (33/24) 44 (22/22)</td>
</tr>
<tr>
<td>Age at onset, mean (SD), years</td>
<td>69.7 (5.8)</td>
<td>64.3 (10.8)</td>
<td>63.1 (10.8)</td>
<td>72.4 (4.9)* 64.3 (10.7) 63.1 (10.8)</td>
</tr>
<tr>
<td>Disease duration, mean (SD), years</td>
<td>8 (4.5)</td>
<td>10.4 (6.9)</td>
<td>9.5 (4.5)</td>
<td>9.5 (4.6)</td>
</tr>
<tr>
<td>All infarcts present, n (%)</td>
<td>28 (52.8)</td>
<td>5 (38.5)</td>
<td>43 (42.2)</td>
<td>8 (57.1) 24 (42.1) 16 (36.4)</td>
</tr>
<tr>
<td>Size of infarcts, n (%)</td>
<td>3 (10.7)</td>
<td>4 (80)</td>
<td>33 (76.7)</td>
<td>5 (62.5) 18 (75) 14 (87.5)</td>
</tr>
<tr>
<td>Cases with microinfarcts</td>
<td>25 (89.3)</td>
<td>1 (20)</td>
<td>23 (53.5)</td>
<td>6 (75) 13 (54.2) 5 (31.3)</td>
</tr>
<tr>
<td>Cases with WMLs, n (%)</td>
<td>7 (13.2)</td>
<td>4 (30.8)</td>
<td>27 (26.5)</td>
<td>4 (28.6) 14 (24.6) 13 (29.5)</td>
</tr>
</tbody>
</table>

*Significantly older at disease onset than the limbic (p = 0.004) or neocortical groups (p = 0.006). DLB, dementia with Lewy bodies; PD, Parkinson disease patients; WMLs, white matter lesions.
cortex (Brodmann Area 17). Supplementary tissue blocks of deep white matter (WM) from the frontal, parietal, occipital, and temporal regions, and of basal ganglia and thalamus were also evaluated. Severity of SVD was scored semiquantitatively, as previously described: 0, none; 1, mild; 2, moderate; and 3, severe (29).

Assessment of Cerebral Amyloid Angiopathy

Severity of cerebral amyloid angiopathy (CAA) was assessed semiquantitatively on serial anti-\(\alpha\)-\(\beta\)-immunostained 10-\(\mu\)m paraffin sections consisting of leptomeningeal and cortical vessels. The same 5 brain regions studied for the presence of SVD were examined to determine the severity of CAA. Finally, CAA was graded according to the method of Vonsattel et al (30), which involves a 4-point CAA scale as follows: 0, vessels devoid of amyloid; 1, mild; 2, moderate; and 3, severe involvement of vessels (with signs of hemorrhage).

Assessment of Cerebral Infarcts, White Matter Lesions, and Hemorrhages

The presence and severity of infarcts, WM lesions (WMLs), and hemorrhages were determined after full neuropathologic examination at macroscopic and microscopic levels. For gross inspection, both hemispheres were coronally sectioned at 1-cm intervals, and horizontal slices were made through the mesencephalon, pons, medulla oblongata, and the cerebellum. Age, size, location, and number of infarcts were documented. For histological analysis, hematoxylin and eosin–stained sections from the following unilaterally sampled brain regions were investigated: 1) middle frontal gyrus; 2) inferior parietal lobule; 3 and 4) superior and middle temporal gyri; 5) occipital cortex; 6) entorhinal including adjacent transentorhinal cortex; 7) anterior cingulate region; 8) hippocampus; 9) basal nucleus of Meynert; 10) putamen; 11) nucleus caudatus; 12) pallidum; 13) thalamus; 14) hypothalamus; 15–18) deep WM regions from the frontal, parietal, occipital, and temporal regions; 19) mesencephalon; 20) pons; 21) medulla oblongata; and 22) cerebellum. Infarcts in each of these regions were summarized with the rating scales regardless of their number or their qualitative characteristics: 0, absent; 1, present. Individual scores of each region were subsequently summed into a single infarct distribution/severity score for each patient ranging from 0 to 22. In theory, cases could achieve a severity score of more than 22 if they displayed infarcts during gross inspection because supplementary tissue blocks were also excised from each infarct identified on gross examination.

The following lesions were differentiated: large infarcts (>10 mm in diameter), lacunar infarcts (cystic lesions, <10 mm in diameter), small infarcts (lesions 2–10 mm in diameter without cystic changes), and microinfarcts that were defined as infarcts (<2 mm) not determinable with certainty during gross inspection of the brain (31). Hemorrhages and WMLs were recorded separately and were designated as present or absent regardless of their sizes.

Immunohistochemistry

Serial 10-\(\mu\)m paraffin sections were used for immunohistochemistry, and immunostained sections were assessed semiquantitatively. Lewy bodies and Lewy neurites were visualized using \(\alpha\)-synuclein immunoreactions with hematoxylin counterstain, as previously described (32). To detect CAA, sections were immunostained after formic acid pretreatment with the monoclonal antibody 6F3D (1:50, 24 hours at \(4^\circ\mathrm{C}\); DAKO, The Netherlands). Primary antibodies were detected with a biotinylated secondary antibody and the ABC complex (Vector Laboratories, Burlingame, CA) and then visualized with 3,3'-diaminobenzidine HCl.

Statistical Analysis

Statistical analysis was performed using Pearson \(\chi^2\) test to compare categorical measurements and 1-way analysis of variance to compare continuous measurements between groups. When inference was made between more than 2 groups, \(p\) values were adjusted for multiple comparisons according to Tukey method. In cases of ordered measurements, the Spearman \(\rho\) statistics for correlation and the Cochran-Armitage trend test were applied. Multiple regression models were fitted to the data to estimate the influence of demographic variables as well as vascular and AD-related pathologies on LB scores. Principal components were estimated to reduce the dimensionality of the complex correlation structure of the neuropathologic variables. The level of significance was set to 0.05. All computations were performed with SAS 9.1 (SAS Institute, Cary, NC) and SPSS 17 (SPSS Inc, Chicago, IL).

RESULTS

We compared all vascular-related measurements across LB disease neuropathologic subtypes classified following consensus guidelines (2). As shown in Figure 1 and the Table, 14 patients were assigned LB scores of 0 to 2 (brainstem subtype), 57 had LB scores 3 to 6 (limbic subtype), and 44 had LB scores 7 to 10 (neocortical subtype). Nearly one half of the PD patients (51%) were classified as limbic subtype, whereas most DLB patients belonged to neocortical subtype (61.5%). Alternatively, the underlying pathology in all DLB and PD cases ranged between PD stages 3 and 6 according to

![Figure 1](http://jnen.oxfordjournals.org/). Percentages of patient groups classified according to their Lewy body (LB) subtype (brainstem, limbic, and neocortical). DLB, dementia with LBs; PD, Parkinson disease.
Braak PD staging (26), in which most patients (77%) were assigned to PD stage 4 or 5. There was a significant and positive correlation between PD stages and LB scores (p = 0.0001).

The frequency and severity of cerebrovascular pathology was analyzed in all cases. The prevalence of hemorrhages was low in all groups (controls, 11.3%; DLB, 0%; and PD, 5.9%); therefore, further analysis was not performed.

There was an inverse and significant correlation between LB scores and AS (p = 0.01), between LB scores and SVD (p = 0.002), and between LB scores and cerebral infarcts (p = 0.038) (Figs. 2A–C). The severity of these vascular pathologies declined with increasing LB scores irrespective of clinical disease entity, that is, an overall increase of vascular lesions was more common in patients with brainstem predominant, followed by limbic and neocortical subtypes (all trend tests, p < 0.05; Fig. 3). By contrast, severity of CAA was positively correlated with LB scores (p = 0.002; Fig. 2D) in which advanced CAA pathology was predominantly present in patients with the neocortical subtype compared with patients with other subtypes (p = 0.01). Likewise, there was an inverse and significant correlation between Braak PD stages and the presence of cerebral infarcts (p = 0.009) and a positive correlation between Braak PD stages and CAA (p = 0.0001). However, AS and SVD scores were not correlated with Braak PD stages, although a trend toward an inverse correlation was apparent. The lack of significance could be attributed to ceiling and floor effects because most patients (77%) were categorized as PD stage 4 or 5.

The AS scores were significantly less severe in the PD group compared with those of the DLB group or controls.
 Conversely, the prevalence and severity of cerebral infarcts and SVD revealed no significant differences among patient groups and controls (p > 0.05 each). Infarcts included both cortical and subcortical lesions, the latter consisting mainly of lacunar (cystic) lesions in the basal ganglia. The prevalence and severity of infarcts in the basal ganglia were not statistically different among patients and controls (p = 0.08). There was no gender difference in prevalence of infarcts (women, 37.3%; men, 35.8%). Infarct severity was significantly associated with severity of AS and SVD (both p < 0.0001) but not with CAA scores (p = 0.9), indicating that CAA is not a significant risk factor for infarction. Microinfarcts were more frequent in all patients than in controls, whereas gross infarcts predominated in controls (p < 0.05 each; Table). Cerebral infarcts were considered the cause of death in 4 controls (7.5%) and in none of the disease groups. In both patient groups, the prevalence of gross infarcts became less with advancing LB pathology (trend test, p = 0.05; Table), whereas microinfarcts were generally more common in patients with severe LB pathology. The presence of WMLs was associated with the severity of SVD (p = 0.009) and AS (p = 0.045), but not with that of CAA (p = 0.06). There was no significant association between the presence of WMLs and LB pathology or AD-related pathologies (all p > 0.05). Controls had less WMLs compared with those of DLB and PD patients (Table), although this was not significant (p = 0.2, p = 0.07, respectively).

There were significant correlations between LB scores and CERAD (p < 0.0001), LB scores and Braak NFT stages (p < 0.001), and LB scores and CAA (p = 0.002). All pathological measurements were higher in DLB cases than in PD cases. Only 1 PD case and 1 DLB case showed severe AD pathology (CERAD C, Braak NFT stages V–VI); 13 PD and 4 DLB cases had intermediate AD pathology (CERAD B–C, Braak NFT stages III–IV). Most PD (n = 88) and DLB cases (n = 8) had mild AD pathology (CERAD 0–A, Braak NFT stages 0–IV; as well as CERAD B–C, Braak NFT stages 0–II); 13 (92.9%) of the patients with brainstem predominant, 52 (91.2%) with limbic, and 31 (70.5%) with neocortical subtypes had mild AD pathology. Most controls were at early Braak NFT stages 0 to III (median, stage I) and CERAD 0–A (median, CERAD 0). There were no correlations between any of the AD-related pathologies and cerebrovascular lesions in DLB/PD patients, LB pathological groups, or controls.

To cluster related variables and thereby simplify the complex picture of the large number of pathological variables, a principal component analysis was carried out in all cases for which 8 neuropathologic variables were assessed. The principal component analysis is a standard procedure that discloses similarities and differences of pathological variables. The principal component analysis yielded 2 principal components.
(PC1 and PC2) that accounted for 28% and 24% of the total variance, respectively. PC1 positively correlated with LB scores and delineated both AD-related lesions (Braak NFT stages, CERAD) and CAA, thereby indicating that CAA belongs to the spectrum of AD-related lesions. Conversely, variables that loaded highly on the PC2 reflected mainly cerebrovascular lesions, including severity of AS, infarcts, SVD, and WMLs, most of which negatively correlated with LB scores (Fig. 4).

**DISCUSSION**

The novel finding in this study was the striking inverse correlation between LB pathology and AS, LB pathology and SVD, as well as LB pathology and cerebral infarcts, such that these cerebrovascular lesions were more common and more prominent in patients with the least LB pathology. This reciprocal relationship between cerebrovascular and LB pathologies was independent of disease entity (DLB, PD). Patients with the least LB pathology (brainstem subtype) showed predominant cerebrovascular pathology perhaps because of their oldest age at death. However, we observed that patients with the most prominent LB pathology (neocortical subtype) displayed significantly less cerebrovascular pathology than those with intermediate LB pathology (limbic subtype), albeit with similar ages at death. This indicates that age alone does not predict the severity of cerebrovascular pathology. Here, it is noteworthy that the overwhelming majority of patients (87.8%) displayed either limbic or neocortical subtypes, thus emphasizing the small number of patients in our study with the brainstem subtype. We concede, however, that this study is limited by a referral bias because most PD patients were recruited from a specialized movement disorder clinic where more “atypical” and severely affected patients are likely to be recruited. In addition, our study is retrospective, and hence there may have been inconsistent symptom ascertainment, in particular, pertaining to controls. Prospective studies are, therefore, needed to substantiate the findings presented here.

The reasons for these findings are difficult to explain and require further investigation. Our results are, however, similar to previously reported patterns in AD of severe comorbid cerebrovascular lesions in AD patients with low or intermediate AD pathology (4–7, 33). In line with these results, our findings could be interpreted to indicate that cerebrovascular lesions lower the threshold for developing clinical PD or DLB symptoms. In the present study, there was no correlation between AD-related pathologies and cerebrovascular lesions in all patients, controls, or LB pathological groups. This assessment, however, might have been biased by a floor effect, that is, the presence of mild AD pathology in all these groups. In this context, it should be mentioned that the issue of the relationship between cerebrovascular lesions and AD-related pathologies is still controversial, with some studies indicating positive associations (29, 34–36), others indicating negative associations (5, 6, 37), and still others failed to detect any association (38, 39).

Much attention has been devoted exclusively to exploring the prevalence of stroke in PD patients versus controls, with inconsistent results (11–23). Our data suggest that these discrepancies may have arisen because earlier studies did not consider the burden of cerebrovascular lesions in relation to the extent of the LB pathology. In this context, it should be mentioned that most previously published clinical studies included neither imaging nor neuropathologic data (11–19, 21, 22). Results of the few available autopsy-controlled studies (20, 23) are in accordance with our observations that the prevalence of cerebral infarcts in PD patients and controls is similar. More important, however, our study complements previous findings by showing that the prevalence of microinfarcts was significantly higher in each patient group compared with controls, whereas that of gross infaracts was significantly increased in controls.

Our results confirm previous reports in which most pathological measures, including LB pathology, CERAD, and Braak NFT stages, were higher in DLB cases than those in PD cases (40, 41). In addition, whereas the overall prevalence and severity of infarcts, hemorrhages, SVD, and WMLs were comparable among DLB and PD groups, the extents of AS and CAA were significantly higher in DLB than in those of PD patients. Taken together, our results confirm the frequent overlap of LB- and AD-related pathologies in DLB cases.

In summary, the principal finding in this study was the striking inverse and significant correlation between the severity of LB pathology and most major cerebrovascular lesions, suggesting that patients with higher LB burden were less likely to show severe cerebrovascular pathology or a history of stroke. Alternatively, DLB or PD patients with severe cerebrovascular lesions may be less inclined to develop severe LB pathology. By contrast, the severity of CAA, CERAD, and Braak NFT stages were positively correlated with LB pathology. The link between LB pathology and cerebrovascular lesions, however, is currently not clear and warrants further examination.

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