Contribution of Lewy Body Inclusions to Dementia in Patients With and Without Alzheimer Disease Neuropathological Conditions

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Context: Lewy bodies (LBs) are intraneuronal inclusions in the brain that have been increasingly recognized as neuropathological lesions with relevance not only to Parkinson disease but also to Alzheimer disease. However, the degree to which the density of LBs in the brain contributes to the severity of dementia has not been clear.

Objective: To determine the degree to which LB “burden” contributes to dementia.

Design: Brain specimens were examined from 273 consecutive autopsies of elderly subjects residing in a nursing home. The numbers and densities of LBs were determined in multiple brain regions, and their correlation with a measure of cognition and functional status (Clinical Dementia Rating) during the 6 months preceding death was determined.

Setting and Patients: Postmortem study of nursing home residents.

Results: The severity of dementia correlated significantly and positively with the density of LBs. These correlations were independent of other neuropathological disorders commonly associated with dementia, including Alzheimer disease. The density of LBs correlated significantly with dementia severity whether or not the diagnostic criteria for Alzheimer disease were met and after the contribution of classical Alzheimer disease lesions, neuritic plaques, and neurofibrillary tangles had been accounted for by partial correlation analysis.

Conclusion: Lewy body inclusions appear to contribute significantly to cognitive deficits in the elderly in a manner that is independent of other neuropathological disorders.

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THE RELATIONSHIP between Parkinson disease (PD) and Alzheimer disease (AD) has sparked a great deal of interest because of the overlapping features of these 2 disorders. There appears to be a continuum of deficits ranging from pure movement disorder (PD) to pure dementia (AD), with a large number of cases manifesting combined cognitive and extrapyramidal changes.1,2 Classically, the neuropathological presentation of PD is marked by the presence of the intracellular inclusions called Lewy bodies (LBs), located in the substantia nigra,3 while AD is marked by the hallmark neuritic plaques (NPs) and neurofibrillary tangles (NFTs).4,5 Lewy bodies may or may not coexist in the cortex with NPs and NFTs.6,8 A case of dementia with LBs but no AD changes in the cortex is referred to as “pure” diffuse Lewy body dementia (DLBD).5,9 The co-occurrence of plaques, however, may change the diagnosis to either the common form of DLBD or the Lewy body variant (LBV) of AD.10-12

Whatever neuropathological process LBs represent, their impact on cognition may be complex; at times the presence of LBs may appear to be the sole pathological marker of disease, but at other times they may be found in conjunction with Alzheimer-type neuropathological findings.6,7,13 To what degree do the LBs add to the cognitive “burden” in both instances? Different studies14-18 using different methods of neuropsychological and neuropathological assessments and relatively small sample sizes have reached different conclusions. The lack of clear consensus regarding the relationship between LBs and cognitive deficits may be because of numerous factors, including the small sample sizes investigated and the absence of a broad spectrum of cognitive impairment. Additionally, most studies of the relationship between LB density and cognition have investigated these relationships within the context of specific neuropathological entities, such as PD, DLBD, or LBV, and not across all the disease entities in which LBs could be found.
SUBJECTS AND METHODS

SUBJECTS

The brain tissue specimens used in this study were derived from consecutive autopsies performed on 273 subjects who had been residents of the Jewish Home and Hospital in Manhattan and the Bronx, NY. Autopsies were performed after receiving consent for autopsy from each subject’s legal next-of-kin. The demographic characteristics of the study cohort are the following: (1) 71 men and 202 women; (2) 239 white, 26 black, 1 Asian, 6 Hispanic, and 1 unknown; (3) a mean ± SD postmortem interval of 7.9 ± 8.9 hours (range, 1.2-56.6 hours); (4) a median Clinical Dementia Rating (CDR) score of 3.0 (range, 0-5); and (5) mean ± SD age at death of 86.97 ± 6.95 years (range, 60-107 years).

NEUROPATHOLOGICAL ASSESSMENT

The neuropathological procedures have been described previously. Representative blocks of the following were examined: superior and midfrontal gyrus, orbital cortex, basal ganglia with basal forebrain, amygdala, hippocampus (rostrocaudal and caudal levels with adjacent parahippocampal and inferior temporal cortex), superior temporal gyrus, parietal cortex (angular gyrus), calcarine cortex, hypothalamus with mammillary bodies, thalamus, midbrain, pons, medulla, cerebellar vermis, and lateral cerebellar hemisphere from the right side of the brain. Sections from paraffin-embedded blocks were stained using hematoxylin-eosin, modified Bielschowsky, modified thioflavlin S, anti-Aβ amyloid, anti-β-amyloid, anti-tau, and anti-ubiquitin (Dako Corp, Carpinteria, Calif). The immunohistochemical method used was an avidin-biotin staining procedure with diaminobenzidine detection. The distribution of cases with respect to primary neuropathological diagnoses is shown in Table 1. The division of cases into those with or without LB neuropathological features was used to determine the contribution of LB and AD neuropathological conditions to dementia severity in some of the analyses performed below.

Every case was evaluated for the extent of neuropathological lesions using the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) neuropathological evaluation. Data on the density ratings for LBs are presented here. The CERAD ratings of NPs and NFTs and direct density measures of cortical NPs were used in some of the correlational analyses described below. Multiple high-power fields (5 fields, ×20) were examined in each slide, and the density of LBs, NPs with and without cores, and NFTs was rated on a 4-point scale (absent, sparse, moderate, or severe), according to the scoring criteria established by CERAD. Additionally, to ensure the validity of the CERAD ratings and to obtain a more quantitative estimate of LB densities, a subset of the entire cohort (145 of 273) was selected randomly for a more quantitative assessment of LB densities. Quantitative data regarding the density of LBs (per square millimeter) in the superior/midfrontal gyrus (Brodmann area 9), orbital frontal cortex (Brodmann area 45/47), superior temporal gyrus (Brodmann area 21/22), and cingulate gyrus (Brodmann area 24/32) were collected. When LBs were unevenly distributed in each slide, LBs in the region with the highest density were counted. Although quantitation of lesions in the densest region ensures that the maximal impact of the lesions is estimated, this procedure can overestimate the overall lesion burden. Lewy bodies were distinguished from globoid NFTs morphologically. Identification of LBs in modified Bielschowsky stained sections was confirmed by ubiquitin immunohistochemical analysis.

ASSESSMENT OF DEMENTIA

The CDR scale was used to document the severity of dementia in all subjects in the study as part of the formal review of the postmortem charts. These reviews were conducted in all cases that underwent autopsy. For each subject, a CDR score was obtained for the 6 months prior to death using a careful review of all information contained within each patient’s chart, including admitting diagnoses, nurse’s notes, social work records, psychiatric and neurological consultation results, medication histories, results of mental status testing, and all other medical records and laboratory studies. For a subset of these subjects (n=93), a more extensive postmortem determination of CDR scores based on multiple evaluations was performed as described previously, and a consensus CDR score was derived. The correlation between the initial CDRs from the postmortem chart review by the first reviewer and the consensus CDR scores of these 93 cases was r=0.96 (P<.001). A second subset of the subjects (n=22), albeit small, had undergone neuropsychological assessments during life and had participated in longitudinal studies of cognitive function with instruments such as an expanded form of the CERAD neuropsychological test battery, the Mini-Mental State Examination (MMSE), and Alzheimer’s Disease Assessment Scale (ADAS). Twenty-two of the subjects studied were assessed antemortem using the MMSE. The correlation between the CDR score assigned for the current study and the MMSE score was r=−0.48 (P=.03). If only those subjects who had undergone the MMSE within 1 year of death were considered (n=14), then the correlation between the consensus CDR and the last MMSE rose to r=−0.73 (P=.003).

To clarify the impact of LBs on cognitive function, we undertook a study of 273 brains from patients referred consecutively from a long-term care facility. These brains underwent autopsy and were well characterized with regard to diagnosis and quantitative measures of specific pathological changes, including LBs. We report the results of the analyses performed to study the relationship of LBs to cognitive deficits without regard to grouping of subjects relative to specific neuropathological diagnostic categories. We considered the contribution of AD-type pathological conditions (eg, NPs and NFTs) to the cognitive deficits of subjects with LB inclusions secondarily, in an effort to determine whether LB-associated cognitive deficits could be explained on the basis of NP and NFT densities.

RESULTS

The purpose of this study was to explore the relationship between the density of LBs in the brain and cogni-
The correlation of LB counts in the cortex alone did not reach statistical significance when the data from subjects with severe dementia (CDR, 5) were eliminated from the analysis. The assignment of CDR scores and the determinations of LB neuropathology were performed totally independent of each other by investigators who were blind to each others' ratings.

The apparent influence of cortical LB density on cognitive status at or near the time of death was not the cortices of all other CDR groups, except for subjects with a CDR score of 4, who did not differ significantly (P<.08) from the cohort with a CDR score of 5. Because the LB-Cortex count-sum variable may not have been normally distributed, these analyses were repeated by performing Kruskal-Wallis, Mann-Whitney, and Spearman rank correlation nonparametric procedures. The statistically significant relationships described above were replicated with these nonparametric tests.

The relationship of the density of cortical LBs to CDR is illustrated well when only those cases with cortical LB density counts greater than 0.0 are considered (n=46). The Pearson product moment correlation between cortical LB density and CDR was stronger when only those cases with LBs were considered (LB-CERAD vs CDR: r=0.52, P<.005; Figure 1). No subject who had LB-type pathological features in the neocortex was cognitively intact. The assignment of CDR scores and the determinations of LB neuropathology were performed totally independently of each other by investigators who were blind to each others' ratings.

The relationship of the density of cortical LBs to CDR is illustrated well when only those cases with cortical LB density counts greater than 0.0 are considered (n=46). The Pearson product moment correlation between cortical LB density counts and CDR for these cases was r=0.52, P<.005 (Figure 2). No subject who had LB-type pathological features in the neocortex was cognitively intact. The assignment of CDR scores and the determinations of LB neuropathology were performed totally independently of each other by investigators who were blind to each others' ratings.

The apparent influence of cortical LB density on cognitive status at or near the time of death was not

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**Table 1. Distribution of Primary Neuropathological Diagnosis in the Study Cohort**

<table>
<thead>
<tr>
<th>Neuropathological Diagnosis</th>
<th>No. (%) of Patients</th>
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<tbody>
<tr>
<td>Cohort without LB neuropathological features</td>
<td>227 (100)†</td>
</tr>
<tr>
<td>No significant pathological conditions</td>
<td>27 (12)</td>
</tr>
<tr>
<td>Definite, probable, or possible AD</td>
<td>138 (61)</td>
</tr>
<tr>
<td>Cerebrovascular disease with or without AD</td>
<td>49 (22)</td>
</tr>
<tr>
<td>Other†</td>
<td>13 (6)</td>
</tr>
<tr>
<td>Cohort with LB neuropathological features</td>
<td>46 (100)</td>
</tr>
<tr>
<td>Definite, probable, or possible AD with occasional LBs</td>
<td>12 (26)</td>
</tr>
<tr>
<td>Definite, probable, or possible AD + diffuse LB disease</td>
<td>13 (28)</td>
</tr>
<tr>
<td>Diffuse LB disease</td>
<td>10 (22)</td>
</tr>
<tr>
<td>PD or PD + AD</td>
<td>11 (24)</td>
</tr>
</tbody>
</table>

*LB indicates Lewy body; AD, Alzheimer disease; and PD, Parkinson disease.*
†Percentages do not add to 100% because of rounding.
‡For example, Pick disease, microinfarcts, meningioma, hemorrhage.
altered by the presence or absence of AD-type pathological conditions. First, we performed a partial correlation analysis of CDR scores vs total cortical LB counts controlling and accounting for mean cortical density of NPs and total CERAD NFT density scores. This analysis yielded results nearly identical to those outlined above (LB-CERAD vs CDR: \(r = 0.27\), \(P = .001\), \(n = 273\); LB-Cortex vs CDR: \(r = 0.32\), \(P < .001\), \(n = 145\)). Second, the correlations of CDR with LB counts were significant for subjects who did not receive a CERAD neuropathological diagnosis of AD (LB-CERAD vs CDR: \(r = 0.36\), \(P = .03\), \(n = 93\); LB-Cortex vs CDR: \(r = 0.35\), \(P < .04\), \(n = 40\)) and for subjects who did receive CERAD diagnoses of definite, probable, or possible AD (LB-CERAD vs CDR: \(r = 0.26\), \(P = .009\), \(n = 180\); LB-Cortex vs CDR: \(r = 0.37\), \(P < .001\), \(n = 104\)) even after the influence of NP and NFT densities had been accounted for by partial correlation analysis. These same relationships held true even when only those subjects receiving a CERAD diagnosis of definite AD were considered (LB-CERAD vs CDR: \(r = 0.36\), \(P = .007\), \(n = 101\); LB-Cortex vs CDR: \(r = 0.38\), \(P = .004\), \(n = 57\)).

Although for purposes of protecting against statistical errors associated with multiple comparisons, the principal analyses were based on summary variables derived from LB density scores for multiple regions, the findings reported above held true for exploratory correlational analyses of LB scores in specific brain regions and CDR scores. Spearman rank correlations calculated for the CERAD ratings of LB density in the 11 brain regions examined and CDR scores ranged between \(r = 0.13\) and \(r = 0.19\), with \(P < .04\) (Table 2). The only 2 brain regions where CDR scores and LB density ratings did not correlate significantly were the inferior parietal lobule and the occipital visual cortex (\(P > .80\)). When the entire cohort was subdivided into subjects with and without a diagnosis of AD, many of the regional correlations between CDR scores and LB density scores remained significant (Table 2). The most conspicuous and consistent regional correlation in the various diagnostic subgroupings was between the density of LBs in the nucleus basalis of Meynert and the CDR, with the \(r\) values ranging between 0.17 and 0.21 (\(P < .04\)) depending on the inclusion or exclusion of subjects with AD diagnoses.

The correlations between the density of LBs in the cortex and CDR outlined above remained significant even when subjects with neuropathological diagnoses of definite PD, possible PD, and DLBD were excluded from the analysis (LB-Cortex vs CDR: \(r = 0.19\), \(P = .003\), \(n = 124\)). Similarly, cortical LB densities correlated significantly with the CDR scores when only those subjects meeting neuropathological diagnoses of definite PD, possible PD, DLBD, and AD with DLBD were included in the analysis (LB-Cortex vs CDR: \(r = 0.51\), \(P = .007\), \(n = 27\)). Although the relationship between cortical LB scores and CDR was stronger in subjects who met the neuropathological criteria for LB diseases, the presence of cortical LBs contributed to the severity of dementia even in those subjects who did not meet the neuropathological criteria for LB-related diseases (LBs in brainstem and diencephalic nuclei and more than 5 LBs per medium-high-power field \([\times 20]\) in 4 neocortical regions).

### Table 2. Regional Correlations of CERAD Lewy Body Density Ratings and CDR Scores for Subjects Within Different Neuropathological Diagnostic Groupings

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Entire Cohort ((N = 273))</th>
<th>Definite, Probable, or Possible AD ((n = 100))</th>
<th>Not AD ((n = 93))</th>
<th>Definite AD ((n = 101))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midfrontal gyrus</td>
<td>0.14*</td>
<td>0.14†</td>
<td>0.11</td>
<td>0.25†</td>
</tr>
<tr>
<td>Superior/midfrontal gyrus</td>
<td>0.13</td>
<td>0.13</td>
<td>0.01</td>
<td>0.25†</td>
</tr>
<tr>
<td>Inferior parietal lobule</td>
<td>0.01</td>
<td>-0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entorhinal cortex</td>
<td>0.14†</td>
<td>0.16†</td>
<td>0.09</td>
<td>0.25†</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.15†</td>
<td>0.16†</td>
<td>0.01</td>
<td>0.13</td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.13†</td>
<td>0.16†</td>
<td>0.09</td>
<td>0.18</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>0.19†</td>
<td>0.12</td>
<td>0.29†</td>
<td>0.10</td>
</tr>
<tr>
<td>Nucleus basalis of Meynert</td>
<td>0.17†</td>
<td>0.16†</td>
<td>0.20</td>
<td>0.21†</td>
</tr>
<tr>
<td>Locus ceruleus</td>
<td>0.14†</td>
<td>0.05</td>
<td>0.29†</td>
<td>0.07</td>
</tr>
<tr>
<td>Dorsal vagus nucleus</td>
<td>0.19†</td>
<td>0.11</td>
<td>0.31†</td>
<td>0.22†</td>
</tr>
</tbody>
</table>

*Regional correlations were calculated using the Spearman rank correlation. CERAD indicates Consortium to Establish a Registry for Alzheimer’s Disease; CDR, Clinical Dementia Rating; AD, Alzheimer disease; and ellipses, no correlation.

†\(P < .05\).

‡\(P < .01\).

This study of 273 brains demonstrates that a significant relationship exists between the presence and density of LB inclusions and degree of dementia. The correlation between the density of LBs and the global measure of dementia (CDR) was significant in multiple brain regions, and it was independent of clinical or pathological diagnosis. The burden of LBs appears to be so significant that cognitive function is impaired at virtually the lowest sum of cortical LB density. Speculation that this effect may be an epiphenomenon of AD or “coincidental” is countered by the persistence of the correlation of LB density and CDR even when subjects with neuropathological diagnoses that included AD were eliminated from the analyses. The independence of the contribution of LBs to cognitive deficits from AD pathological conditions was further demonstrated by the correlational analyses that factored out the contributions of AD-related neuropathological lesions (NPs and NFTs) to the measure of dementia. These results demonstrated significant correlations between LB density and CDR in all brains containing LBs, regardless of neuropathological diagnosis.

Although the density of LBs was correlated with the severity of dementia with a high degree of statistical confidence, LB density accounted for only 3% to 29% of the total variance in CDR, depending on whether the entire cohort or only those subjects with LB inclusions were considered. Multiple neuropathological and neurochemical lesions contribute to dementia in elderly populations, including NPs, NFTs, neuronal loss, decreases in indices of synaptic markers, and deficits in multiple neurochemical systems. Data reported by Samuel et al.
and Perry et al.\textsuperscript{11,32} on the neuropathological and neurochemical consequences of LBs in AD are suggestive of an additive model for multiple lesion effects on cognition. What is particularly noteworthy regarding the results presented here is that a relatively independent contribution of LBs to dementia severity can be identified despite the multiplicity of other lesions that also contribute to dementia.

Just as different neuropathological lesions can all contribute to dementia severity, lesions in different neuroanatomical foci can also contribute to cognitive deficits. The results presented in Table 2 demonstrate that dementia can result from LBs in different brain regions. In subjects with AD-like neuropathological features, LB lesions in the neocortex are the best correlates of dementia severity. On the other hand, in the non-AD group, which included subjects with PD and diffuse LB disease, the density of LBs in subcortical structures, such as the substantia nigra and the locus ceruleus, was most highly correlated with dementia severity.

The mechanism(s) through which LBs may be associated with cognitive decline is unknown. It has been suggested that the LBs represent a cytoprotective response to specific insults, agents, and cellular stressors\textsuperscript{20} that may be the ultimate cause(s) of the dementia. Recent studies\textsuperscript{8,33} have identified α-synuclein to be a core component of LBs and a potential contributor to their formation. The identification of mutations in the α-synuclein gene\textsuperscript{14-16} in familial PD strongly supports the hypothesis that LBs may be among the neuropathological manifestations of the abnormal processing or aggregation of α-synuclein. Irrespective of the disease processes that lead to the accumulation of LB inclusions, it is clear that their presence connotes a neuropathological process that is associated with dementia. Lewy bodies appear to reflect a neuropathological process that involves many critical brain regions that have all been implicated as structures subserving memory and cognitive function.\textsuperscript{37,38} The fact that LB density ratings in the nucleus basalis of Meynert were most significantly and consistently (Table 2) correlated with severity of dementia underscores the relevance of major cholinergic deficits in association with the presence of LBs.\textsuperscript{31,32}

In summary, LB density is significantly correlated with degree of dementia. This is true for the cerebral cortex as well as specific subcortical regions. The relationship between LBs and cognitive impairment is not diminished by partialing out the neuropathological hallmarks of AD. Lewy bodies signify a process that has a direct and distinct impact on cognition.

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REFERENCES


Error in Text. In the Observation by Saéz et al titled “Autosomal Dominant Nocturnal Frontal Lobe Epilepsy in a Spanish Family With a Ser252Phe Mutation in the CHRNA4 Gene,” published in the August issue of the Archives (1999;56:1004-1009), 2 errors occurred in the text. In the abstract on page 1004, the last sentence in the “Results” section should have read as follows: “Neither of the 2 polymorphisms found in a series of families with epilepsy were found in our sample.” Also, on page 1008, the second sentence of the third paragraph in the “Comment” section should have read as follows: “Two polymorphisms at positions 555 and 594 (consisting in both cases of a C→T transition in exon 5), described previously in a series of different families with epilepsy, were not found.”