ORIGINAL CONTRIBUTION

Neurofibrillary Pathology in Alzheimer Disease With Lewy Bodies

Two Subgroups

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Background: While NFT frequency is reportedly reduced in AD+DLB, we often encounter abundant neocortical NFTs in such cases and decided to investigate this discrepancy.

Objective: To compare neurofibrillary tangle (NFT) frequency in Alzheimer disease with concomitant dementia with Lewy bodies (AD+DLB) with NFT frequency in "pure" AD.

Methods: Neurofibrillary tangle frequency, as well as regional staging of neurofibrillary degeneration modified from Braak, was scored in 160 autopsy cases of primary dementia (80 AD+DLB cases and 80 pure AD cases).

Results: Neurofibrillary tangle and modified Braak scores were lower in AD+DLB, as reported previously. Yet, neocortical NFT scores assumed markedly different patterns in the 2 groups ($P = .001$). In pure AD, NFT scores of "frequent" were predominant: more cases exhibited frequent than moderate or sparse NFTs. In AD+DLB, the distribution of NFT scores was bimodal: NFTs were either frequent or few to absent. Neocortical NFT scores in the AD+DLB group tended to parallel the severity of other types of tau cytopathology (neuropil threads and tau-positive plaque neurites).

Conclusions: Cases of AD+DLB may be divided into 2 subgroups based on the extent of neocortical neurofibrillary pathology. These findings could have implications for disease pathogenesis and treatment.

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While the clinical and neuropathological overlap between Alzheimer disease (AD) and Parkinson disease has long been recognized,[1-7] the terminology used for cases exhibiting AD with concomitant Lewy bodies has varied widely among investigators. In the past, we have referred to such cases as AD with Parkinson disease changes. Other commonly used terms include *Lewy body variant of AD,*[3] *diffuse Lewy body disease*,[1,4] and *senile dementia of the Lewy body type*.[7] More recently, the Consortium on Dementia with Lewy Bodies (DLB) recommended that the term *DLB* be used for cases with subcortical or cortical Lewy bodies and a clinical history of dementia. This generic term was chosen "because it acknowledges the presence of [Lewy bodies] without specifying their relative importance in symptom formation with respect to other degenerative or vascular pathology that is simultaneously present."[8] Therefore, in the present study, we will use the term *AD+DLB* to refer to cases exhibiting the neuropathology of dementia with Lewy bodies in the presence of concomitant AD changes.

In 1993, Hansen et al[9] were the first to report that the brains of patients with the Lewy body variant of AD frequently exhibit no or only few neurofibrillary tangles (NFTs) in the neocortex. Other investigators have subsequently confirmed this finding.[10-12] While we, too, have observed AD+DLB cases with only sparse neocortical NFTs, we have also encountered many AD+DLB cases with abundant neocortical NFTs. To explore this discrepancy, we evaluated the frequency and distribution of neocortical NFTs in a series of 80 AD+DLB cases and compared our findings with those in 80 "pure" AD cases.

RESULTS

All 160 cases exhibited neocortical neuritic plaques in sufficient numbers to meet CERAD criteria for the diagnosis of definite AD; the AD and AD+DLB groups did not differ with regard to semiquantitative neuritic plaque scores assessed on silver-stained sections (data not shown). Nigral

METHODS AND MATERIALS

CASE MATERIAL

We reviewed a series of 160 autopsy cases of primary dementia: 80 consecutive AD+DLB cases accessioned in our laboratory and 80 pure AD cases matched as closely as possible for age, sex, and duration of dementia (**Table 1**). While some of the 160 patients were enrolled in the Emory Alzheimer's Disease Center, Atlanta, Ga, and clinically followed up, the majority of the cases came to us from elsewhere in the community. For many of these community cases, only limited clinical data were available.

The criteria of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) were used for the diagnosis of AD.[6] The criteria of the Consortium on DLB were used for the classification of DLB cases as "brain stem predominant," "limbic/transitional," or "neocortical."[8]

HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL TECHNIQUES

Formalin-fixed, paraffin-embedded sections of hippocampus and entorhinal, frontal, temporal, and parietal cortex were stained using the modified Bielschowsky silver method. Adjacent sections of frontal and temporal cortex were also immunolabeled with anti-tau (rabbit polyclonal, Accurate Chemical and Scientific, Westbury, NY), as previously described.[13] Briefly, sections were incubated at 37°C with 5% milk block followed by anti-tau (1:100), linked with peroxidase using an avidin-biotin complex method (ABC Elite Kit, Vector Laboratories, Burlingame, Calif), and developed with 3,3′-diaminobenzidine.

EVALUATION OF NEUROPATHOLOGICAL CHANGES

Neurofibrillary tangles were scored semiquantitatively by 2 of us (M.G. and S.S.M.) on silver-stained sections from all 5 brain regions using a 4-tiered system (none, sparse, moderate, and frequent) following CERAD guidelines[6]; any interrater discrepancies were discussed and resolved. Scores representing a modification of the staging method of Braak and Braak[14] were also derived from these data. For example, a case having only sparse NFTs in the hippocampus would be classified as Braak stage I, while a case with frequent NFTs in the hippocampus, entorhinal cortex, and frontal, temporal, and parietal neocortex would be classified as Braak stage VI. Braak scores between these 2 extremes would be assigned using the algorithm shown in **Table 2**.

Neuritic plaques (plaques containing dystrophic neurites) were scored semiquantitatively on silver-stained sections of frontal and temporal cortex using the 4-tiered scale developed by CERAD.[6] Adjacent, tau-immunolabeled sections were evaluated semiquantitatively for neuropil threads (NThs) and senile plaques with tau-positive neurites (SP-tau) using a similar rating system.

Finally, the 76 AD+DLB cases for which adequate materials were available were classified as brain stem predominant, limbic/transitional, or neocortical DLB according to the guidelines published by the Consortium on DLB.[8] We chose to assess Lewy body frequency on hematoxylin-eosin–stained sections rather than ubiquitin-immunolabeled sections, because both Lewy bodies and NFTs are ubiquitin positive and can be difficult to distinguish from one another, particularly in cases that have a high frequency of NFTs.

STATISTICAL ANALYSES

Semiquantitative NFT scores and modified Braak scores were analyzed using the Wilcoxon Two-Sample Test to compare the scores in the AD and the AD+DLB groups. The proportions of cases in categories of NFT and Braak scores in the 2 groups were compared using the χ^2 test. The NFT scores of none and sparse were pooled for the χ^2 analysis, since both of these scores were accompanied by a paucity of tau cytopathology and thus appeared quite similar neuropathologically. A similar strategy has been used previously by Hansen et al.[9] Each Braak stage, however, was treated as a separate category.

The relationship between the Consortium on DLB classification and NFT frequency was assessed using the Kruskal-Wallis test and the χ^2 test to compare semiquantitative NFT scores and modified Braak scores among DLB categories. For all analyses, $P \leq .05$ was considered significant. The lack of standardized clinical data in a large number of patients precluded clinicopathological correlational analyses.

Lewy bodies were observed in all AD+DLB cases; 79 of the 80 cases exhibited cortical Lewy bodies as well (**Figure 1**). We were able to evaluate 76 of our 80 AD+DLB cases according to the classification scheme of the Consortium on DLB. Of these 76 cases, 6 were classified as brain stem predominant, 14 as limbic/transitional, and 56 as neocortical DLB. In the remaining 4 cases, the archival tissues available were not adequate for classification.

As expected, modified Braak scores were shifted downward in the AD+DLB group relative to the AD group ($P<.001$, Wilcoxon Two-Sample Test; **Figure 2**). Ninety percent of the AD cases had Braak scores of V or VI, with the remaining 10% having scores of IV. In the AD+DLB group, 70% had scores of V or VI, and 30% had scores of III or IV. Regional NFT scores were significantly lower in the AD+DLB group than in the pure AD group in the hippocampus ($P = .01$), entorhinal cortex ($P = .03$), temporal cortex ($P = .001$), and parietal cortex ($P = .002$); in frontal cortex, the difference did not reach statistical significance ($P = .08$). In only 3 of the 80 AD+DLB cases were neocortical NFTs absent on all sections examined.

While the majority of cases in both the pure AD (96%) and the AD+DLB (87%) groups exhibited frequent NFTs in limbic regions (hippocampus or entorhinal cortex), neocortical NFT scores showed different patterns in the 2 groups (χ^2 test; frontal cortex: $P = .004$, temporal cortex: $P = .006$, parietal cortex: $P = .004$). In the pure AD group, more cases exhibited frequent than either moderate or sparse NFTs in each region of neocortex; the numbers of cases with ratings of moderate or sparse were similar for this group (Figure 1 and

Table 1. Demographic Information*

	"Pure AD"	AD + DLB
No. of cases	80	80
Age at death, y		
Mean	76.2	75.5
Range	50-90	46-93
Duration of dementia, y		
Mean	6.4	6.6
Range	1-17	1-18
Sex, No.		
Men	39	41
Women	41	39

*AD indicates Alzheimer disease; DLB, dementia with Lewy bodies.

Table 2. Algorithm Used to Derive Modified Braak Stages From Semiquantitative Neurofibrillary Tangle (NFT) Scores*

	CERAD NFT Score		
Modified Braak Stage	Entorhinal Cortex	Hippocampus	Sum of Neocortical Scores (F + P + T)
I	1	0	0
II	1 or 3	1	0
III	3 or 5	3 or 5	0
IV	3 or 5	3 or 5	1-3
V	3 or 5	3 or 5	4-14
VI	5	5	15

*0, 1, 3, and 5 (none, sparse, moderate, and frequent) are the semiquantitative ratings used by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) for regional ratings of NFT frequency.[6] F + P + T indicates frontal plus parietal plus temporal.

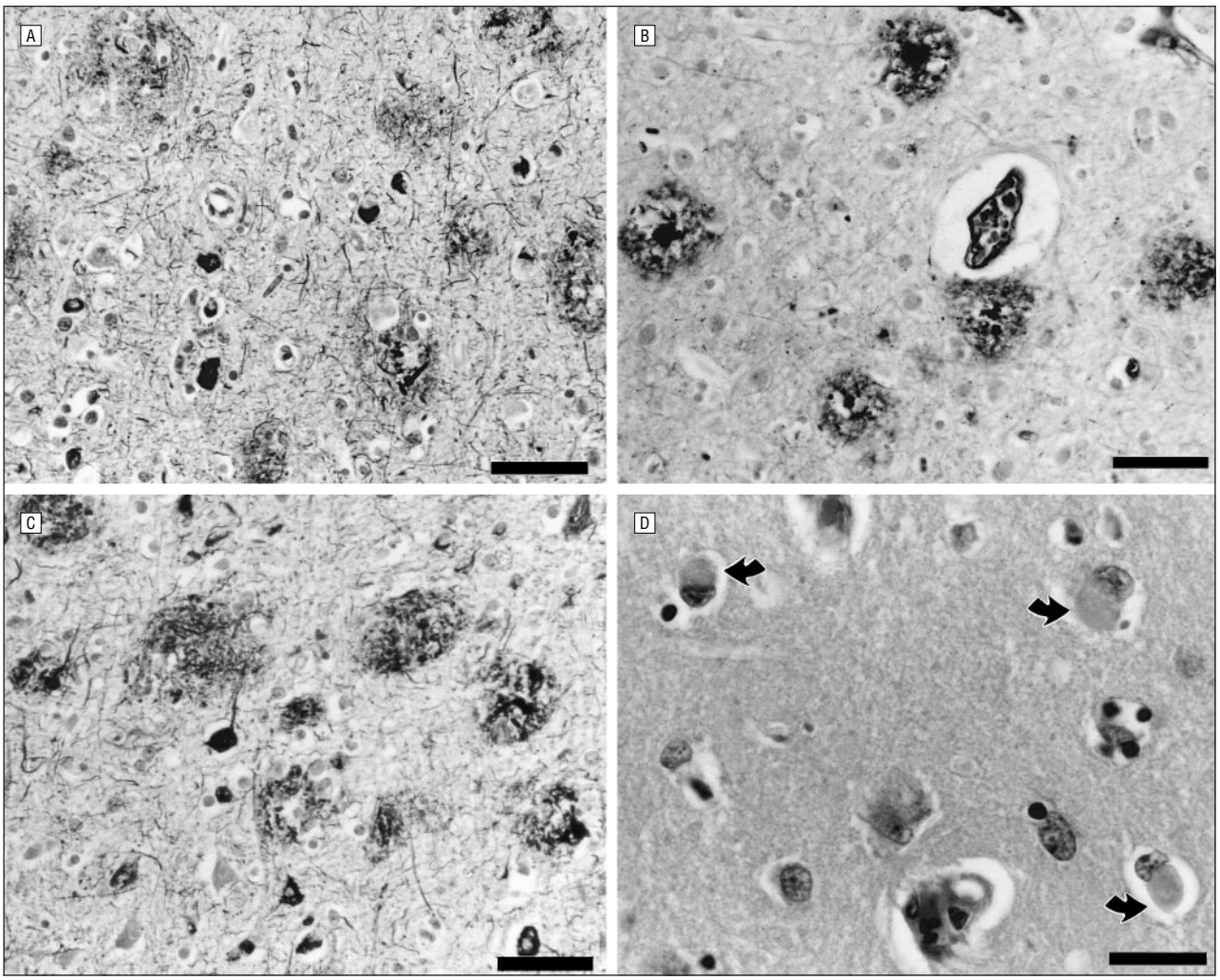

Figure 1. *In Alzheimer disease (AD), the majority of cases exhibited frequent neocortical neurofibrillary tangles (NFTs) (A). In contrast, cases of AD plus dementia with Lewy bodies (AD+DLB) generally fell into 2 groups: those with sparse or no NFTs (B) and those with frequent NFTs (C). Cortical Lewy bodies (arrows), present in 79 of the 80 AD+DLB cases, were often prominent, as in the "frequent NFT" case shown in D. Scale bars: A through C, 50 µm; D, 25 µm.*

Figure 3). In the AD+DLB group, however, the distribution of NFT scores was bimodal: the majority of cases had either frequent or few to no NFTs in the 3 regions of neocortex (Figure 1 and **Figure 4**). Relatively few AD+DLB cases had an NFT rating of moderate.

We next examined the relationship between neocortical NFT scores and senile plaques with SP-tau and NThs. For this and all subsequent analyses, *neocortical NFT score* represents the median of the 3 regional neocortical scores for each individual. In the AD+DLB group,

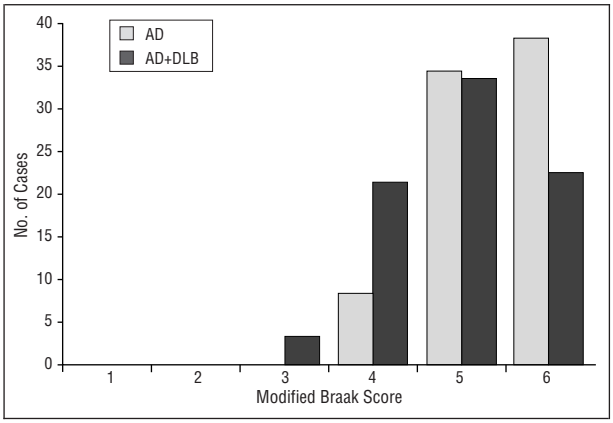

Figure 2. *Modified Braak scores were shifted downward in the Alzheimer disease (AD) plus dementia with Lewy bodies (AD+DLB) group compared with the AD group (P<.001).*

low NFT scores were most often accompanied by low SP-tau and NTh scores; conversely, cases with high NFT scores frequently had high SP-tau and NTh scores (**Figure 5**). In the pure AD group, cases with frequent neocortical NFTs tended to have high SP-tau and NTh scores, but cases with sparse to moderate NFTs had widely variable SP-tau and NTh scores (data not shown).

There was a tendency for lower cortical Lewy body scores to be associated with higher neocortical NFT scores; 5 (83%) of the 6 brain stem predominant cases and 10 (71%) of the 14 limbic/transitional cases had median neocortical NFT scores of 5 (ie, frequent), while only 23 (41%) of 56 neocortical cases had frequent neocortical NFTs. This difference did not reach statistical significance, however ($P = .10$).

COMMENT

Since the initial report by Hansen and colleagues[9] in 1993, other investigators have also noted a reduced frequency of NFTs in the brains of patients with AD+DLB[10-12] and a downward shift in Braak scores relative to those of patients with AD.[15] In the present study, we replicate these findings in our 80 AD+DLB cases. Interestingly, although 35 of our 80 AD+DLB cases were plaque predominant, only 3 of these 35 cases failed to show any NFTs in the neocortical regions examined. Thus, absence of NFTs in a standard sampling of various regions of neocortex is relatively rare, even among AD+DLB cases.

While our AD+DLB cases *as a group* had lower NFT frequencies, the reduction in NFT scores in this group stemmed from a paucity of NFTs in only a subset of cases; 35 (44%) of our 80 AD+DLB cases fell into this subset. A second, equally important subset (38 [47.5%] of our 80 AD+DLB cases) exhibited *frequent* neocortical NFTs; these cases were indistinguishable from the pure AD cases with regard to neurofibrillary pathology. While the bimodal pattern of NFT scores seen in the AD+DLB group was not recapitulated in the Braak scores for this group, this discrepancy may be attributable to the modification used to calculate Braak scores from semiquantitative NFT scores, as described earlier.

The proportion of plaque-predominant cases (44%) among our AD+DLB cases was lower than the two thirds

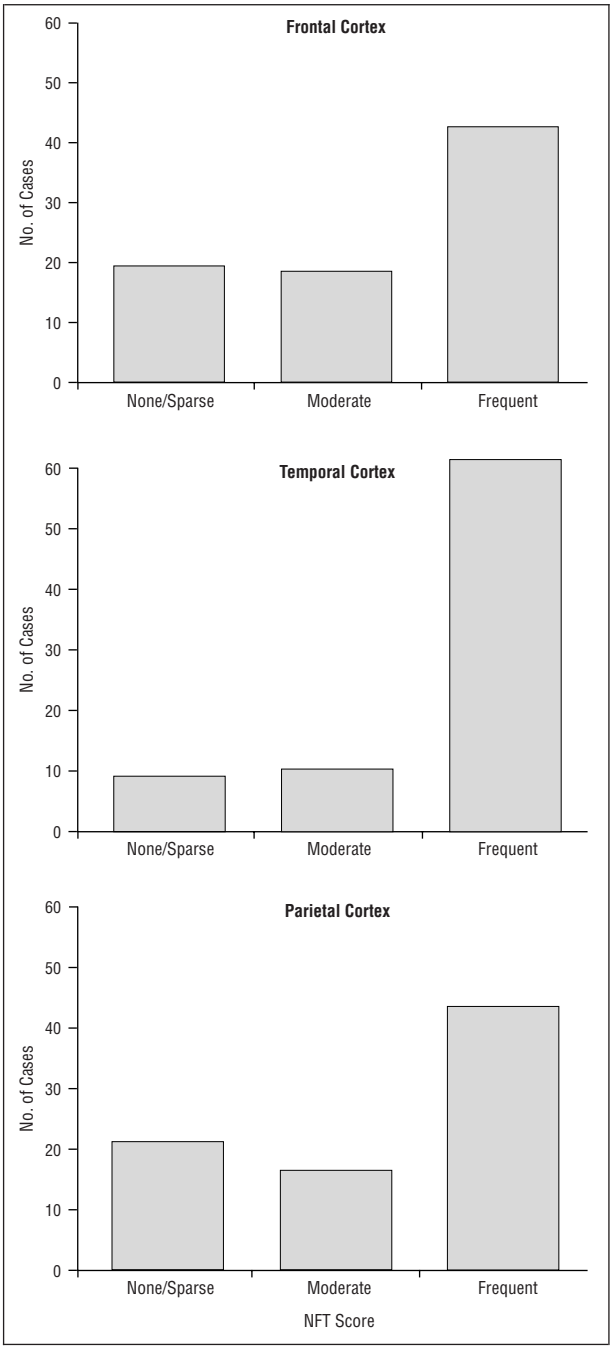

Figure 3. *Neocortical neurofibrillary tangles (NFTs) in "pure Alzheimer disease" (AD). In all 3 regions of neocortex, more AD cases had an NFT rating of frequent than either moderate or sparse. The number of cases with moderate and sparse NFT ratings were similar.*

observed by Hansen et al.[9] This difference may be related to technical factors; Hansen et al[9] used thioflavine S staining to detect NFTs, while we used sensitive silver stains. Our confidence in the validity of our findings is further bolstered by our observation that in both of our AD+DLB subgroups, neocortical NFT scores paralleled scores for other tau-related pathology, ie, SP-tau and NThs.

Despite differences in NFT scores, the AD+DLB group did not differ significantly from the AD group with regard to neuritic plaque scores on Bielschowsky silver-stained sections. Similar findings have been

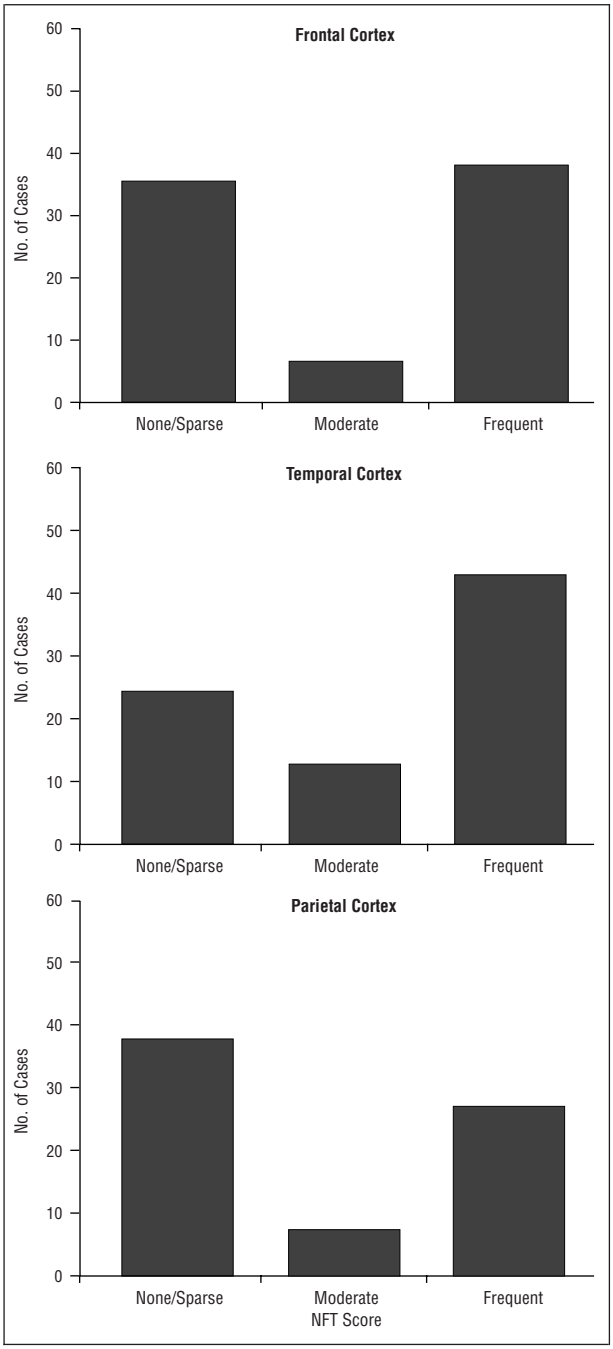

Figure 4. *Neocortical neurofibrillary tangles (NFTs) in Alzheimer disease plus dementia with Lewy bodies. Note the bimodal distribution, with the vast majority of cases having either frequent or sparse to no NFTs. Few cases exhibited moderate NFTs.*

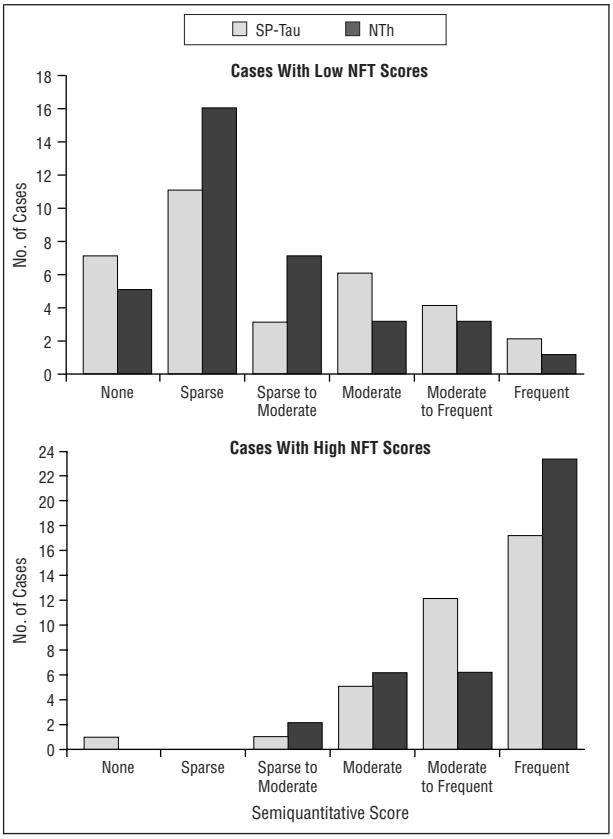

Figure 5. *Tau-related neuropathology. In the Alzheimer disease plus dementia with Lewy bodies (AD+DLB) group, low neurofibrillary tangle (NFT) scores (sparse or no neocortical NFTs) were most often accompanied by low tau-positive plaque neurite (SP-tau) and neuropil thread (Nth) scores. Conversely, cases with high NFT scores (frequent neocortical NFTs) typically had high SP-tau and NTh scores.*

reported by Hulette et al.[10] Nagy et al,[12] however, reported that "for any given level of cognitive deficit, the densities of either all plaques or neuritic plaques alone in the neocortex are significantly lower in cases of AD mixed with other CNS pathology [including AD+DLB cases] than in cases of AD with no other CNS pathology." It is possible that, had we performed actual plaque counts rather than scoring plaques semiquantitatively, we might have observed a similar difference, since a plaque score of frequent encompasses a wide range of actual plaque densities.

The criteria for the diagnosis of AD[6,16,17] and DLB[8,18] continue to be debated. A major issue is the question of whether neocortical NFTs should be included as a diagnostic criterion for AD.[19,20] If so, how many NFTs must be present for a case to qualify as AD? If, for example, only sparse neocortical NFTs are required for the diagnosis, then only 3 of our 80 AD+DLB cases would fail to meet the criteria for AD. If moderate or frequent neocortical NFTs are required, then a substantial proportion (44%) of our AD+DLB cases would be excluded. Do we know enough about the pathobiology of AD to arbitrarily eliminate such cases from the spectrum of AD?

If AD+DLB cases with low NFT counts are not classified as AD, then one needs to consider diagnostic criteria for DLB. Should cortical Lewy body cases with significant numbers of neuritic plaques be lumped with cases lacking plaques, the approach taken by the Consortium on DLB[8]? Hansen and Samuel[19] argue for the separation of pure "diffuse Lewy body disease" cases from Lewy body cases exhibiting any type(s) of plaques, even those with diffuse plaques only, since recent evidence suggests that diffuse plaques represent an early stage of AD.[21] As neuropathological diagnostic criteria for AD and DLB continue to be scrutinized, our finding that AD+DLB cases fall into 2 subgroups warrants consideration.

Even more importantly, and transcending nosological issues, individuals within these 2 pathologically defined subgroups of AD+DLB may respond differently in therapeutic trials. Clinical studies of cholinesterase inhibitors have revealed considerable heterogeneity in the responses of patients with probable AD to cholinergic therapy.[22,23] Preliminary studies of patients who were treated with cholinesterase inhibitors and have subsequently died and come to autopsy suggest that patients with DLB are more likely to be responders than patients with pure AD.[24-26] Interestingly, several investigators have reported that neocortical choline acetyltransferase levels are lower in patients with AD+DLB than in patients with pure AD.[27,28] Perry et al[27] further subdivided their patients with DLB into 2 groups based on the presence or absence of visual hallucinations and found that the hallucinating group had lower neocortical choline acetyltransferase levels than did the nonhallucinating group. Whether these groups respond differently to cholinesterase inhibitors is not known. Additional cliniconeuropathological correlation studies with attention to subgroups such as the clinical subgroups described by Perry et al[27] and the neuropathological subgroups reported in the present study are warranted.

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