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RESEARCH ARTICLE

Empiric Refinement of the Pathologic Assessment of Lewy-Related Pathology in the Dementia Patient

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Abstract

Lewy-related pathology (LRP) is a common pathologic finding at autopsy in dementia patients. Recently criteria for categorizing types of LRP in dementia patients were published, though these criteria have yet to be systematically applied to large dementia samples. We examined a large (n = 208) referral-based autopsy sample for LRP, and applied the published criteria for LRP categorization to these cases. We found almost half (49%) of LRP positive cases from this sample were not classifiable. However, modifying the published criteria by reducing the number of regions requiring examination, allowing more variability in LRP severity scores within specific brain regions, and adding an amygdala predominant category permitted classification of 97% of LRP positive cases from the referral-based sample. Application of the modified criteria to an unrelated community-based autopsy sample (n = 226) allowed classification of 96% of LRP positive cases. Modest modifications in the published criteria permit a significantly greater number of dementia cases with LRP to be classified. In addition, this modification allows for more limited sampling of brain regions for classification of LRP. We propose that these modified criteria for the categorization of LRP be utilized in patients with a history of dementia.

INTRODUCTION

Lewy-related pathology (LRP), including both classic Lewy bodies and abnormal alpha-synuclein (SNCA) deposition in Lewy inclusions and neurites, is a common pathologic change observed in dementia patients. LRP is linked to Dementia with Lewy Bodies (DLB), a clinico-pathologic entity that is thought to be the second most common substrate for dementia in the elderly (15). There are at least two subtypes of DLB: the much more common form that coexists with pathologic changes of Alzheimer's disease (AD), and the less common form that appears to be the sole pathologic correlate of cognitive impairment and is thought by some to overlap with patients who have Parkinson's disease (PD) plus dementia (16, 17). Needless to say, clinical diagnosis and pathologic categorization of these entities are challenges that have motivated now three consensus consortia, the most recent of which published their recommendations in 2005 (15–17). A component of the 2005 DLB consensus recommendations proposed a new scheme for the assessment of LRP using SNCA immunohistochemistry (IHC). This scheme included classification of cases into three "types" based on anatomic distribution and severity (brainstem predominant, limbic (transitional), diffuse neocortical). To date, this proposed classification scheme has not been systematically applied to an autopsy sample of dementia patients.

In this study, we applied the published LRP classification scheme to a large referral-based autopsy sample from a consortium of five AD research centers. The results suggested that the proposed scheme would fail to categorize a large percentage (49%) of dementia subjects with LRP. However, with modifications including reduction in the number of sections examined, greater latitude in LRP severity scoring within each category, and the addition of an amygdala-predominant LRP classification, we found we could classify almost all LRP positive dementia cases. These findings were confirmed when the modified criteria were applied to a separate community-based autopsy case series with dementia.

METHODS

Samples and case selection

All autopsies were from patients enrolled in the Lewy Body-Associated Dementias Research Study (LADRS) or the University of Washington AD Patient Registry (ADPR). The LADRS sample is a referral-based dementia sample obtained from a collaboration of five NIH-funded AD centers. All autopsied cases from these five institutions with sufficient clinical and pathologic information were considered for inclusion. The ADPR is a community-based dementia sample that enrolled individuals from a health maintenance organization in the Puget Sound region. Subjects in the ADPR had demographic characteristics similar to those of the region's population aged 65 and over (1, 10).

Neuropathologic evaluation

All cases underwent assessment for LRP using SNCA IHC with antibody LB509 (1:50 to 1:400, Zymed, San Francisco, CA, USA) (9). Cases with questionable LB509 immunoreactivity were evaluated with a second antibody to nitrated SNCA (syn 303, 1:1000) (6). IHC for SNCA was performed on 10-μm sections that were pretreated (either 88% formic acid for 5 minutes or protease-K for 1 minute), exposed to 3% hydrogen peroxide, blocked in 5% milk, incubated with primary antibody for 1 h at room temperature, and then detected with avidin-biotin complex using diaminobenzidine as chromogen substrate (11). The positive control for each IHC run was a case of DLB. Negative control for each was elimination of primary antibody. Braak staging (2) for neurofibrillary tangle pathology and Consortium to Establish a Registry for AD (CERAD) plaque score (20) were accomplished with modified Bielschowsky-stained sections, tau IHC, or both.

Assessment of SNCA IHC

A section from each of the following regions was assessed for LRP in both the LADRS and ADPR samples: medulla (including dorsal motor nucleus of the vagus nerve, raphe nuclei, lateral tegmentum), substantia nigra, amygdala, transentorhinal cortex, cingulate gyrus, and either the superior or middle frontal gyrus. In addition, sections of the locus coeruleus, superior and middle temporal gyri, and inferior parietal lobule were evaluated in the LADRS cases. As recommended by the 2005 DLB consensus (17), regional severity of LRP was scored as none (0), mild (1), moderate (2), severe (3) or very severe (4). This scoring scheme includes both Lewy body inclusions and Lewy neurites as LRP, and thus either or both could be present for a region to be scored as "1" or greater. LADRS cases were evaluated by two of us (JBL or RH) including 89 cases that were evaluated independently by both investigators. Cases from ADPR were evaluated by one of us (JBL).

Statistics

Inter-rater agreement was assessed using kappa statistics (7). Data for dual-rated cases were randomly selected from one of the two raters for all other analyses. Discordance (classifiable vs. not-classifiable) between the published and proposed methods of LRP classification was assessed using McNemar's test (7). Intercooled Stata 8.2 for Windows (College Station, TX, USA) was used in all analyses.

RESULTS

A total of 417 autopsied LADRS cases were available. Of these, 324 autopsied LADRS cases had a clinical diagnosis of "probable AD", "possible AD" or "dementia, type unknown" using NINCDS-ADRDA criteria, or of DLB using McKeith criteria, made within the respective AD Center Clinical Cores (15, 17, 18). Sufficient tissue sampling for case inclusion was present in 208 LADRS cases, and 125 of these cases had any LRP. A total of 300 autopsied ADPR cases were available. Of these, 260 cases fulfilled DSM III criteria for dementia. Sufficient tissue sampling for case inclusion was present in 226 cases, and 126 of these cases had LRP. Modal (range) Braak neurofibrillary tangle stage (2) and CERAD plaque score (20) for LRP positive cases was 5 (1 to 6) and frequent (absent to frequent), and for LRP negative cases was 5 (0 to 6) and frequent (absent to frequent). Breakdown of case selection within each sample and selected case demographic and neuropathologic characteristics are listed in Table 1.

Application of the published LRP staging scheme from the Third Report of the DLB Consortium (17) to the referral-based LADRS sample allowed classification of only 51% (64/125) of cases (Table 2). The vast majority of these classifiable cases were in the diffuse neocortical category (61/64, 95%), while only three cases (5%) were classifiable as limbic, and none as brainstem predominant. Reasons for cases being unclassifiable included absence of brainstem LRP in one of three regions (medulla, locus ceruleus, substantia nigra) when other regions were positive (not permitted for any classification using the published criteria), presence of LRP in only the amygdala (without brainstem or other limbic LRP), and amygdala LRP score that was too high for the limbic classification (published criteria do not permit a score of "4" in the amygdala for the limbic classification).

To improve the number of classifiable cases, we first modified the published criteria by reducing the number of regions analyzed, because many unclassifiable cases had inconsistent findings across regions that did not allow classification within the published criteria. We selected brain regions to optimize for the fewest number needed while maintaining assessment of all regions relevant to LRP. Reducing the number of regions to five (medulla, substantia nigra, amygdala, cingulate gyrus, frontal cortex) allowed classification of 62% of LRP positive LADRS cases. We found that inclusion of additional neocortical regions did not improve categorization of cases. Our second set of modifications included adding an "amygdala predominant" category (to capture unclassifiable cases with limited LRP outside of the amygdala), expanding the range of severity of LRP within regions (to capture cases with LRP severity scores that were too high or too low for inclusion within a category), and allowing cases that fulfilled criteria for two categories to be assigned to the more anatomically rostral category (eg, a case fulfilling limbic and neocortical categories would be classified as neocortical). We decided to keep separate amygdala predominant and limbic/transitional categories because previous work from our group and others suggests that LRP in these two categories may be significantly different (11, 22). Future studies will determine the clinical significance of these two LRP categories.

		LADRS	ADPR
Autopsies (n)	Total available	417	300
	Dementia diagnosis*	324	260
	& with sufficient tissue sampling	208	226
	& with LRP in any region	125	126
	& with LRP in amygdala, SN, or medulla	125	126
Age†	At onset (mean ± SD)	68 ± 9	76 ± 6
	At death (mean ± SD)	78 ± 8	84 ± 6
Male : female (n)†		68:57	52:74
CERAD neuritic plaque score (n)†	None	4	3
	Sparse	2	9
	Intermediate	16	20
	Frequent	103	94
Braak stage (n)†	0	0	0
	I or II	10	23
	III or IV	38	36
	V or VI	77	67

Table 1. Cases evaluated from the referral-based LADRS sample and the community-based ADPR sample. Abbreviations: LADRS = Lewy Body-Associated Dementia Research Study; ADPR = Alzheimer's Disease Patient Registry; LRP = Lewy-related pathology; SN = substantia nigra; CERAD = Consortium to Establish a Registry for AD; AD = Alzheimer's disease; DLB = dementia with Lewy Bodies.

*Probable or possible AD, DLB or dementia of type unknown (LADRS) or DSM-III R criteria for any dementia (ADPR).
†Age, gender, plaque score and Braak stage refer to those autopsies that had any LRP.

Application of the above modifications of the published criteria allowed classification of 97% (121/125) of LRP positive LADRS cases (Tables 2 and 3): brainstem predominant (5/125, 4%); amygdala predominant (23/125, 18%); limbic/transitional (26/125, 21%); and neocortical (67/125, 54%). The remaining four unclassifiable cases were categorized as "mixed". All LADRS cases originally categorized by the published criteria as neocortical or limbic remained in the same categories with the modified criteria. Direct comparison of the published and the proposed modified criteria for categorization of LRP in these two autopsy samples found a significantly greater number of LADRS cases to be classifiable using the modified criteria (Table 4) (comparison of discordance using McNemar's test had $P < 0.00005$ for both ADPR and LADRS data, see also Table 3).

Application of our proposed modified scheme to the community-based autopsy sample (ADPR) allowed classification

Lewy body type pathology	Nine regions assessed* n (%)	Five regions assessed† n (%)
Brainstem-predominant	0 (0%)	1 (1%)
Limbic (transitional)	3 (2%)	10 (8%)
Diffuse neocortical	61 (49%)	67 (54%)
Unclassifiable	61 (49%)	47 (38%)

Table 2. LRP categorization in LRP positive LADRS cases using all nine regions recommended by the published criteria* or using a subset of five regions. Abbreviations: LADRS = Lewy Body-Associated Dementia Research Study; LRP = Lewy-related pathology; SN = substantia nigra.

*Reference (17).
†medulla, SN, amygdala, cingulate gyrus, frontal cortex.

Table 3. Proposed modified criteria for categorization of Lewy-related pathology (LRP) in patients with dementia. Results from two autopsy series. Abbreviations: LRP = Lewy-related pathology; SN = substantia nigra; LADRS = Lewy Body-Associated Dementia Research Study; ADPR = Alzheimer's Disease Patient Registry; AD = Alzheimer's disease.

Predominant region	LRP severity scoring with proposed criteria*				Results	
	SN or medulla†	Amygdala	Cingulate gyrus	Frontal cortex	LADRS n (%)	ADPR n (%)
Brainstem	1+ in either	0–2	0–1	0	5 (4%)	20 (16%)
Amygdala	0–1 in both	1+	0–1	0	23 (18%)	24 (19%)
Limbic	1+ in either	2+	1–3	0–1	26 (21%)	22 (18%)
Neocortical	1+ in either	2+	2+	2+	67 (54%)	55 (44%)
Mixed	Cases not classifiable by modified criteria				4 (3%)	5 (4%)

*Severity of LRP was scored according to published consensus criteria as none (0), mild (1), moderate (2), severe (3) or very severe (4) (17).
†For medulla, the highest score in dorsal motor nucleus of the vagus nerve, raphe nuclei or lateral tegmentum was considered representative and 0 means no LRP in all three subregions of medulla.

Table 4. Comparison of published (17) and proposed criteria for the classification of LRP in dementia patients from the LADRS sample. Abbreviations: LRP = Lewy-related pathology; LADRS = Lewy Body-Associated Dementia Research Study.

		Proposed	
		LADRS (n = 125)	
		Classified	Not classified
Published	Classified	64	0
	Not classified	57	4
	P (McNemar's)	<0.00005	

of 96% of cases (Table 2): brainstem predominant (20/126, 16%); amygdala predominant (24/126, 19%); limbic (22/126, 18%); neocortical (55/126, 44%). Five cases (5/126, 4%) were not classifiable and were categorized as "mixed".

All LRP positive cases from both samples had LRP in at least one of three regions: amygdala, substantia nigra or medulla. In the LADRS sample, isolated LRP, which is LRP present in only one anatomic region, was observed in the amygdala in 15 cases and in the medulla in 1 case. In the ADPR sample, isolated LRP was observed in the amygdala in 17 cases, the medulla in seven cases, and the substantia nigra in three cases.

Eighty-nine LADRS cases were randomly selected and evaluated by both study neuropathologists (RH, JBL), each blinded to the other's results. In 87 (98%) of these cases, the raters agreed on the presence or absence of LRP. In the remaining two cases, both pathologists found no evidence of LRP, but one rater assessed the cases as incomplete because of insufficient sampling in one region. There were no cases in which one rater found LRP and the other rater did not. Inter-rater agreement for the severity of LRP averaged 77% across all nine brain regions, resulting in an average kappa statistic of 0.66. Inter-rater agreement for classification of LRP predominance improved from 58% using the published criterion (17) to 83% using the proposed criteria (kappa statistic of 0.47 and 0.78, respectively).

DISCUSSION

Debate continues over the clinical and pathophysiologic significance of LRP, particularly in patients with coexistent AD (19). However, this debate requires practical and reproducible definitions of LRP to allow reasonable comparisons among studies. Refinement of the classification or staging of LRP increases precision of case characterization that is the underpinning of clinical, pathologic, genetic and pharmacologic studies. It is for these reasons that the new consensus criteria for DLB (17) should be applauded as an insightful and significant advance.

We applied the published criteria (17) to a large referral-based autopsy sample of dementia (LADRS sample). Our results revealed that 49% of cases with dementia and LRP were not classifiable using the published scheme. In contrast, our modified classification criteria categorized 97% of patients with dementia and LRP, a highly significant difference when compared with the published method ($P \leq 0.00005$). The high rate of classification using the modified criteria was confirmed in another, entirely separate, community-based sample. The modified criteria did not result in the re-classification of any case that had been classifiable using the published criteria. Thus, our modifications only improved on the classification of previously unclassifiable cases. Finally, the modified criteria improved the inter-rater reliability for LRP classification.

The improved classification of LRP positive dementia cases with the proposed criteria was due to several reasons. First, as demonstrated in the LADRS sample, restricting the number of regions sampled and scored for LRP reduced the number of unclassifiable cases by 23% (61 to 47 unclassifiable cases, Table 2). The criteria were then altered to allow for a wider variation of LRP severity score within non-neocortical regions. We found many cases would not fulfill criteria for the non-neocortical categories because of LRP severity scores that were more variable than allowed by the published criteria. Finally, we added a new category of "amygdala predominant" LRP. Cases with LRP relatively restricted to the amygdala are frequently observed in dementia samples (8, 11, 12, 14, 21, 22), and were approximately 20% of the LRP positive cases in both samples.

Braak *et al* have suggested that LRP can be staged in PD and suggest the initial pathology likely occurs in the medulla and anterior olfactory structures (3, 5). However, this staging scheme may not be applicable to dementia samples (3). Braak's staging scheme would suggest that all LRP positive cases should have LRP in the medulla prior to the development of this pathology in other more rostral regions. This was clearly not the case in our sample, and others have found similar results in other dementia samples (8, 11, 12, 14, 21, 22). Distinct anatomic patterns of LRP may occur in PD vs. other dementing disorders such as AD (5). Of note, Marui *et al* (13) also suggested a LRP staging scheme that included a stage I similar to our amygdala predominant category. Unfortunately, they did not include brainstem sampling or a brainstem predominant category in their study.

Uchikado *et al* (22) have examined AD cases with amygdala predominant LRP. They hypothesized that AD with amygdala predominant LRP may be a distinct form of synucleinopathy because of the relative absence of LRP outside of the amygdala. However, they found few other pathologic or clinical differences when compared with other AD cases (those without LRP or with more anatomically diffuse LRP). Unfortunately, the clinical data for their sample were retrospective and limited to a small subset of cases. Clearly, more data are needed to examine the clinical and pathophysiologic significance of amygdala predominant LRP, as this subgroup was almost 20% of our LRP positive cases in both autopsy samples.

Using two large dementia samples with autopsy, we have shown that (i) screening with SNCA IHC of single sections of medulla, midbrain with substantia nigra, and amygdala captured 100% of autopsies with any LRP as determined in much wider sampling; and (ii) our proposed modifications for the classification of dementia with LRP using a total of five tissue sections led to categorization of the vast majority of cases, a significant improvement over the current published protocol. Thus, besides increasing the number of cases classified, the modifications to the published LRP classification scheme will permit pathologists to screen dementia cases for LRP with only three sections, and to classify the stage of the LRP with five. It is important to stress that the patients evaluated here were clinically demented and that optimal pathologic classification of LRP in movement disorders or non-demented individuals (4, 5) may be different.

The clinical and pathophysiologic significance of LRP in any anatomic pattern in the brain is still unclear. Nevertheless, it is critical to have established protocols that consistently and efficiently categorize LRP in autopsy cases. Indeed, such protocols will serve as an essential foundation from which future studies can determine clinical correlates, biochemical changes and genetic associations with LRP in different regions of brain from patients with dementia.

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REFERENCES

1. Barnhart RL, van Belle G, Edland SD, Kukull W, Borson S, Raskind M *et al* (1995) Geographically overlapping Alzheimer's disease registries: comparisons and implications. *J Geriatr Psychiatry Neurol* **8**:203–208.
2. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* Berl **82**:239–259.
3. Braak H, Del Tredici K, Bratzke H, Hamm-Clement J, Sandmann-Keil D, Rub U (2002) Staging of the intracerebral inclusion body pathology associated with idiopathic Parkinson's disease (preclinical and clinical stages). *J Neurol* **249**(Suppl. 3):III/1–5.
4. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* **24**:197–211.
5. Braak H, Bohl JR, Muller CM, Rub U, de Vos RA, Del Tredici K (2006) Stanley Fahn Lecture 2005: the staging procedure for the inclusion body pathology associated with sporadic Parkinson's disease reconsidered. *Mov Disord* **21**:2042–2051.
6. Duda JE, Giasson BI, Mabon ME, Lee VM, Trojanowski JQ (2002) Novel antibodies to synuclein show abundant striatal pathology in Lewy body diseases. *Ann Neurol* **52**:205–210.
7. Fleiss JL, Levin B, Paik MC (2003) *Statistical Methods for Rates and Proportions*, 3rd edn. Wiley & Sons: Hoboken.
8. Hamilton RL (2000) Lewy bodies in Alzheimer's disease: a neuropathological review of 145 cases using alpha-synuclein immunohistochemistry. *Brain Pathol* **10**:378–384.
9. Jakes R, Crowther RA, Lee VM, Trojanowski JQ, Iwatsubo T, Goedert M (1999) Epitope mapping of LB509, a monoclonal antibody directed against human alpha-synuclein. *Neurosci Lett* **269**:13–16.
10. Larson EB, Kukull WA, Teri L, McCormick W, Pfanschmidt M, van Belle G, Sumi M (1990) University of Washington Alzheimer's Disease Patient Registry (ADPR): 1987–1988. *Aging (Milano)* **2**:404–408.
11. Leverenz JB, Fishel MA, Peskind ER, Montine TJ, Nochlin D, Steinbart E *et al* (2006) Lewy body pathology in familial Alzheimer disease: evidence for disease- and mutation-specific pathologic phenotype. *Arch Neurol* **63**:370–376.
12. Lippa CF, Fujiwara H, Mann DM, Giasson B, Baba M, Schmidt ML *et al* (1998) Lewy bodies contain altered alpha-synuclein in brains of many familial Alzheimer's disease patients with mutations in presenilin and amyloid precursor protein genes. *Am J Pathol* **153**:1365–1370.
13. Marui W, Iseki E, Nakai T, Miura S, Kato M, Ueda K, Kosaka K (2002) Progression and staging of Lewy pathology in brains from patients with dementia with Lewy bodies. *J Neurol Sci* **195**: 153–159.
14. Marui W, Iseki E, Kato M, Akatsu H, Kosaka K (2004) Pathological entity of dementia with Lewy bodies and its differentiation from Alzheimer's disease. *Acta Neuropathol* Berl **108**:121–128.
15. McKeith IG, Galasko D, Kosaka K, Perry EK, Dickson DW, Hansen LA *et al* (1996) Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. *Neurology* **47**:1113–1124.
16. McKeith IG, Perry EK, Perry RH (1999) Report of the second dementia with Lewy body international workshop: diagnosis and treatment. Consortium on Dementia with Lewy Bodies. *Neurology* **53**:902–905.
17. McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H *et al* (2005) Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology* **65**:1863–1872.
18. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology* **34**:939–944.
19. Merdes AR, Hansen LA, Jeste DV, Galasko D, Hofstetter CR, Ho GJ *et al* (2003) Influence of Alzheimer pathology on clinical diagnostic accuracy in dementia with Lewy bodies. *Neurology* **60**:1586–1590.
20. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM *et al* (1991) The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* **41**:479–486.
21. Parkkinen L, Soininen H, Alafuzoff I (2003) Regional distribution of alpha-synuclein pathology in unimpaired aging and Alzheimer disease. *J Neuropathol Exp Neurol* **62**:363–367.
22. Uchikado H, Lin WL, DeLucia MW, Dickson DW (2006) Alzheimer disease with amygdala Lewy bodies: a distinct form of alpha-synucleinopathy. *J Neuropathol Exp Neurol* **65**:685–697.