Prevalence of mixed neuropathologies in autopsied older adults and associations with clinical disease progression

Willa Domino Brenowitz

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Reading Committee:

Walter A. Kukull, Chair

Stephen E. Hawes

Rebecca A. Hubbard

Christopher D. Keene

W. T. Longstreth Jr

Program Authorized to Offer Degree:

Epidemiology

University of Washington

Abstract

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Willa Domino Brenowitz

Chair of the Supervisory Committee:
Professor Walter A. Kukull
Epidemiology

Introduction: Many older adults have multiple brain pathologies (aka mixed neuropathologies) at autopsy; however, the clinical importance of mixed neuropathologies is not well established. The objectives of this dissertation were 1.) to examine the prevalence of Alzheimer's disease neuropathologic change (ADNC), Lewy body disease (LBD), vascular brain injury (VBI), and their co-occurrence and 2.) to assess whether mixed neuropathologies are associated with clinical disease progression.

Methods: In Chapter 2, we examined the prevalence and co-occurrence of ADNC, LBD, and VBI using data on 2,742 autopsied clinical research volunteers who were evaluated at U.S. Alzheimer's Disease Centers and whose data was in the National Alzheimer's Coordinating Center (NACC) database. Because results may differ by study population, we compared findings to 499 autopsied participants from a population-based cohort study, the Adult Changes in Thought (ACT) study. Secondly, in autopsied NACC participants we examined associations of mixed neuropathologies with progression in overall clinical impairment (Chapter 3) and impairment in 4 specific cognitive domains (Chapter 4). The Clinical Dementia Rating Sum of

Boxes measured overall clinical impairment and we calculated domain scores for memory, attention, language, and executive function from standardized neuropsychological test scores. We used linear mixed effects models with adjustment for covariates and inverse probability weights to account for potential autopsy selection bias. We tested whether associations between clinical progression and ADNC were modified by co-occurring LBD or VBI.

Results: LBD or VBI were common in ADNC participants in NACC (58.6%) and ACT (68.2%). Limbic LBD (in NACC) and amygdala only LBD (in ACT) were associated with high ADNC. In NACC, cortical LBD was associated with intermediate ADNC. The relationship between VBI and ADNC was inconsistent. Annual clinical progression was slightly faster for ADNC+LBD compared to ADNC only (1.9 points; 95% confidence interval [CI]: 1.7, 2.0 vs. 1.7; 95% CI: 1.6, 1.8) and slightly slower for ADNC+VBI (1.5, 95% CI: 1.3, 1.6). ADNC interacted with LBD ($p=0.002$) and VBI ($p=0.003$), such that the rate of progression was slower in those with dual neuropathologies than if each neuropathology contributed independently to progression. In secondary models, this result was found in those with high but not intermediate ADNC. Participants with ADNC+LBD generally had worse trajectories of cognitive domains compared to ADNC only, particularly for attention and executive function.

Conclusions: Many participants with ADNC had co-occurring LBD and VBI, although only LBD were associated with ADNC. Prevention and treatment of dementia may require methods to detect mixed neuropathologies and multifaceted disease-modification strategies. Among older adults with ADNC, the effect of additional pathologies on clinical progression may be greater in those with intermediate levels of ADNC than in those with severe ADNC.

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Chapter 1. Introduction

This dissertation addresses a gap in knowledge regarding the clinical importance of multiple co-occurring brain pathologies capable of causing dementia, known as mixed neuropathologies. Dementia is typically caused by brain damage that results in a loss of cognitive functioning and the ability to perform daily activities of living.[1] Alzheimer's disease (AD) is the most common cause of dementia (accounting for 60-80% of cases). However, vascular brain injury (VBI) and Lewy body disease (LBD) are also common causes of dementia and may occur concomitantly with AD neuropathologic change (ADNC).[1]

Some common approaches to studying dementia have limitations. Using broad clinical outcomes may be too simplistic.[2] Etiologic subtypes of dementia are associated with distinct brain abnormalities (neuropathologies) and patterns of symptoms.[1] Alternatively, focusing on specific diseases, such as AD (based on clinical criteria or neuropathologic diagnosis), likely ignores other relevant brain co-morbidities.[3] Gold standard of etiologic diagnosis is based on neuropathologies, which currently can only be studied at autopsy, although biomarkers can detect ADNC.[4] Demented older adults often have mixed neuropathologies at autopsy.[5–16] Several studies have found that mixed neuropathologies are more common with increasing age,[9,12,14,15] which may be due to increased burden of co-morbidities and less resilience to neuronal loss.[17]

AD dementia is the 6th leading cause of death, and it is the only top 10 cause of death without an effective preventive or treatment method.[1] Meanwhile, the fastest growing sector of the U.S. population is the oldest old (aged 90+); dementia prevalence and its costs are expected to increase drastically in the future.[18] Although mixed neuropathologies are common in autopsy

samples;[5–10] prevalence estimates are varied, likely due to differing study designs and classification criteria. Additionally, much remains unknown about mixed neuropathologies--most importantly, whether mixed neuropathologies represent a separate pathogenesis and clinical progression from relatively "pure" pathologies, as suggested,[17] or instead are an artifact of aging or late-stage relatively severe dementia. ADNC, VBI (in particular microinfarcts), and LBD are associated with poor performance in multiple cognitive domains.[19] ADNC and neocortical LBD have also been associated with more rapid cognitive decline prior to death.[20,21] These prior studies modeled pathologies as additive (or independent) effects, although it is possible that pathologies interact to predict more severe clinical disease.

Conceptual model of mixed neuropathologies

Not all individuals with brain pathology actually become demented. In fact, most elderly brains have mild to moderate pathologic burden at autopsy.[22–24] However, odds of dementia are higher for those with mixed neuropathology compared to those without.[9] The threshold for clinical expression of disease could be moderated by the brain's capacity to compensate for pathologic burden and/or the location of the pathology. Presence of one pathology may reduce an individual's ability to compensate for additional pathologies.[9,25,26] This effect may be due to overall higher pathologic burden or, alternatively, interactions between pathologies may enhance cognitive dysfunction.

Focus on mixed neuropathologies traditionally centered on concomitant vascular pathology and ADNC.[17,27–31] Vascular risk factors are associated with AD dementia; and cerebrovascular disease may even contribute to development of ADNC.[32] However, other studies suggest ADNC

and VBI are independent.[23,33] LBD and ADNC also commonly coexist, with or without concomitant vascular pathology,[34–37] and combinations of non-AD-type pathologies are possible.[15] Neurodegenerative diseases, characterized by accumulation of abnormal proteins, may interact synergistically, such that accumulation of one protein may enhance accumulation of others.[38–44] In particular, basic science research suggests that amyloid (a hallmark of ADNC) and α-synuclein (the key protein in Lewy bodies) may interact. However, research is limited on whether specific vascular pathologies, such as VBI, or LBD interact with ADNC to predict severity of clinical impairment and progression.

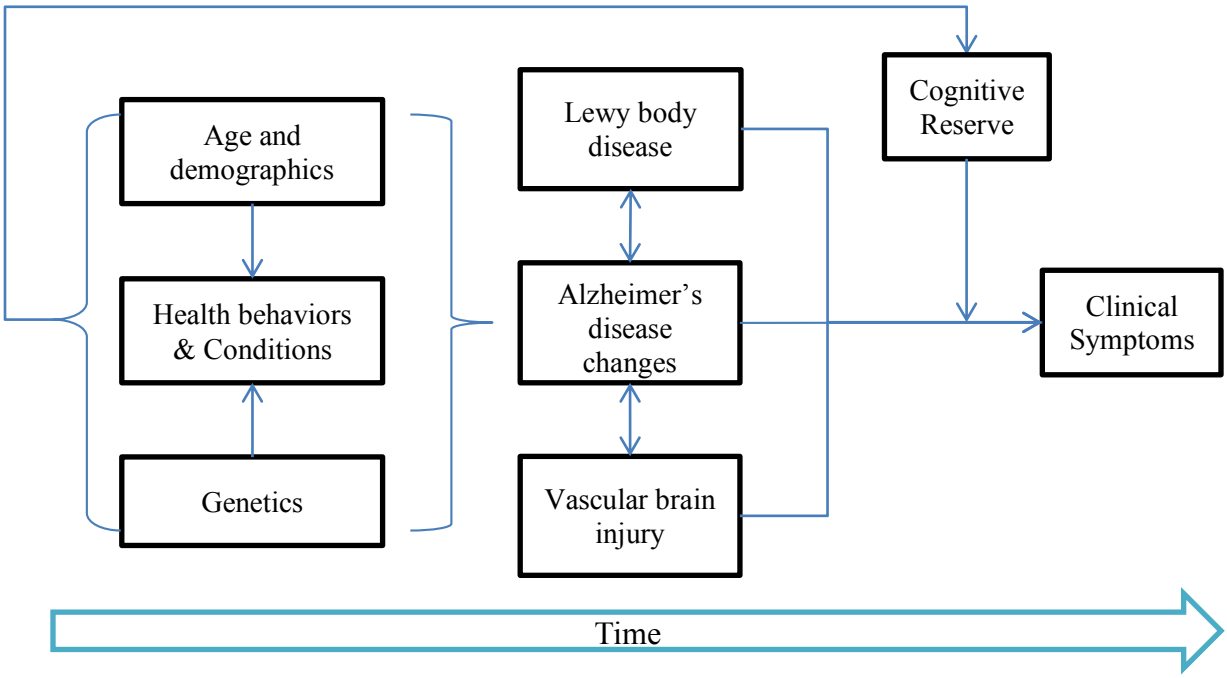

Figure 1.1 Conceptual model

In a simplified example, Figure 1.1 illustrates how ADNC, LBD, and VBI may affect clinical symptoms. This conceptual framework is adapted from Richards & Deary's model for cognitive reserve[45] and the pathophysiological cascade model for AD.[4] Precipitating factors such as age, health status, and genetics may influence development of neuropathologies. Clinical symptoms, such as cognitive decline, could be produced by interactions between coexisting

neuropathologies (which could develop simultaneously or serially). Alternatively, pathologies may develop independently of each other. Cognitive reserve, e.g. cognitive resilience to pathologic burden such as through higher education or social engagement,[46–49] may modify the relationships between pathologies and clinical symptoms.

The goal of this dissertation is to more precisely characterize and understand more completely the clinical impact of mixed neuropathologies. A comprehensive view of pathologic causes of dementia may help improve the development of effective treatment and preventive strategies. If mixed neuropathologies are indeed associated with more severe cognitive impairment it will help demonstrate a need to better account for and understand mixed neuropathologies in future research. This result would have important implications for treatment and prevention strategies, suggesting that treatments of individual disease (e.g. ADNC) alone may not effectively reduce risk of dementia and that methods that reduce risk of multiple forms of dementia will have a greater impact on the population. Alternatively, finding no associations may suggest that one primary pathology is responsible for dementia onset and that mixed neuropathologies may only occur at later stages or provide little additional effect.

This dissertation focuses on mixed neuropathologies defined by co-occurring ADNC, VBI (defined by gross and microscopic infarcts), and LBD. In Chapter 2 we describe the prevalence of pathologies found at autopsy, test whether LBD or VBI occur more frequently in those with ADNC, and compare characteristics between those with mixed and single ADNC. We used data on autopsied clinical research volunteers evaluated at U.S. Alzheimer's Disease Centers (ADCs) and whose data was in the National Alzheimer's Coordinating Center (NACC) database. Since

clinical research volunteers may differ from community-based samples of older adults,[50] we also used data on autopsied participants from a population-based cohort, the Adult Changes in Thought (ACT) study. We conducted an in-depth sub-analysis using detailed data abstracted from neuropathology reports on a portion of NACC participants who were evaluated at the Pacific Northwest Dementia and Aging Neuropathology Group (PANDA) ADCs, which includes Oregon Health & Science University and University of Washington ADCs.

In Chapters 3 and 4 we describe results from longitudinal analyses on progression of clinical disease in autopsied NACC participants and sub-analyses in PANDA ADC participants. Detailed clinical assessments in these participants allowed us to evaluate associations between mixed neuropathologies (focusing on ADNC+VBI and ADNC+LBD) and overall clinical impairment (Chapter 3) as well as impairment in specific cognitive domains (Chapter 4). We conducted these analyses mindful that autopsy studies often comprise a select group of participants who may not represent the overall study population.[51,52,50] Selection bias can result when selection into the observed study sample is driven jointly by the exposure (or cause of the exposure) and the outcome (or cause of the outcome).[53,54] Restricting analyses to the autopsy sample in such a case could induce a spurious association between the exposure and outcome.[53–55] We used an analytic tool, inverse-probability weighting (IPW),[56,57] to try to identify and account for potential selection bias. This method attempts to refer results in Chapters 3 and 4 to the broader NACC study population, which may be more generalizable than the autopsy sample. Chapter 5 concludes this dissertation with a summary and discussion of overall research findings and avenues for future research.

Chapter 2. Co-occurrence of Alzheimer's disease neuropathologic change, Lewy body disease, and vascular brain injury in clinic and community-based samples.

ABSTRACT

Introduction: Lewy body disease (LBD) and vascular brain injury (VBI) may be associated with Alzheimer's disease neuropathologic change (ADNC). Prior findings are inconsistent and may differ between study populations.

Methods: We estimated the co-occurrence of LBD, VBI (gross infarcts and cortical microinfarcts), and intermediate to high ADNC (moderate/frequent neuritic plaques & Braak stage III-VI). Using descriptive statistics and hypothesis tests (χ^2 or Fisher's exact tests), we compared the prevalence of LBD subtype (brainstem, limbic, cortical, amygdala only/other) and VBI by level of ADNC (no/low, intermediate, high). We examined demographic and clinical profiles of participants with ADNC only compared to mixed ADNC. Data came from the National Alzheimer's Coordinating Center (NACC) database on clinical research volunteers enrolled at U.S. Alzheimer's Disease Centers (n=2,742) and the population-based Adult Changes in Thought (ACT) study (n=499).

Results: LBD or VBI were common in ADNC participants in NACC (58.6%) and ACT (68.2%). Limbic LBD (NACC) and amygdala only LBD (ACT) were associated with high ADNC (both, $p<0.001$). In NACC, cortical LBD was associated with intermediate ADNC ($p<0.001$). The relationship between VBI and ADNC was inconsistent between samples. Clinical AD dementia was slightly less common in those with mixed ADNC compared to ADNC only.

Conclusions: The majority of autopsied brains with ADNC had coexisting LBD and VBI. Our data support a link between LBD and severity of ADNC but not VBI and ADNC.

INTRODUCTION

Vascular brain injury (VBI) and Lewy body disease (LBD) commonly co-occur with Alzheimer's disease neuropathologic change (ADNC) among older adults.[34,30,58] Pre-mortem cognitive impairment is more likely in those with mixed neuropathologies compared to those with single or no neuropathologies at autopsy.[9,59,25,26] Whether the co-occurrence of VBI and LBD in ADNC is due to synergistic interactions or to overlapping independent processes is unclear.

LBD is present in up to 60% of individuals with ADNC.[35] ADNC are defined by amyloid plaques and tau neurofibrillary tangles.[60,61] LBD is typically a hallmark of Parkinson's disease and Dementia with Lewy bodies and is characterized by Lewy bodies (inclusions of α-synuclein).[62] In brains of many people with ADNC, Lewy bodies are limited to the amygdala with little involvement of other regions.[35] But cortical LBD is also associated with amyloid burden in most studies[42,36,63–65] and with neurofibrillary tangles in some studies[36,63] but not others.[42,64,66]

Between 30% and 70% of participants with ADNC also have co-occurring vascular neuropathologies.[32,58] A wide range of vascular lesions can be present; however, VBI with gross and microscopic infarcts is considered the most important vascular contributor to dementia.[67] Prevalence of ADNC with co-occurring VBI increases with age.[12,14] In some studies, specific

types of VBI, in particular cortical infarcts and microinfarcts, are more common in those with ADNC.[68,69] However, other studies have found no relationship between VBI and amyloid burden.[23,33]

Inconsistencies in prior study findings could be due to small sample sizes as well as differences in study design, age distribution of study populations, sample selection, clinical assessments, neuropathologic assessment protocols, and classification criteria. Findings on associations between neuropathologies and dementia may differ between clinic-based convenience samples and community or population-based autopsy samples.[70] One prior study found mixed and vascular pathologies were more common in community-based samples, while high ADNC, LBD, and atypical findings were more common in the clinic-based sample.[50] Data from large databases may more precisely characterize relationships between ADNC, LBD, and VBI in clinic and community-based settings. Such research may provide expected prevalence estimates of brain comorbidity in clinic compared to community-based studies, of relevance in particular to clinical trials and development of disease modification strategies.

In this study we estimated the prevalence and co-occurrence of ADNC, LBD, and VBI (defined as gross and microscopic infarcts) at autopsy, and examined whether LBD or VBI occurred more frequently in those with ADNC or without. We also compared characteristics of autopsied participants with ADNC only to those with co-occurring LBD or VBI to explore potential predictors of mixed pathology in ADNC. Data came from a large database of clinical research volunteers who were evaluated at U.S. National Institute on Aging (NIA)-funded U.S.

Alzheimer's Disease Centers (ADCs) as well the Adult Changes in Thought (ACT) study, a population-based study in Seattle, WA.

METHODS

Data sources and study populations

U.S. Alzheimer's Disease Centers

The National Alzheimer's Coordinating Center (NACC) maintains data from participants evaluated prospectively by one of 34 past and present NIA-funded ADCs.[71,72] Participants in the Uniform Data Set were evaluated annually at an ADC using a standardized protocol beginning September 2005; neuropathology data based on autopsy results was available for those who had died and consented to autopsy evaluation.[72,73] Individual ADCs recruit and enroll participants according to their own protocols. Some, but not all, ADCs require participants' consent to autopsy prior to enrollment. Participants enroll with any level of cognition, ranging from normal to demented. Written informed consent was obtained from all participants and their study co-participants; institutional review board (IRB) approval was obtained from all individual ADCs. This current study was approved by the University of Washington IRB.

The study sample flow is shown in Figure 2.1. Between September 2005 and September 2015, 32,479 total participants had a clinical evaluation, of whom 6,507 died and 3,835 were autopsied. Because of low prevalence in population-based studies and potential for confounding, 1,063 autopsied participants were excluded with Down's syndrome, prion disease, autosomal dominant genetic diseases, frontotemporal lobar degeneration, and other rare causes of dementia. We also

excluded 30 additional participants missing neuropathologic information on ADNC, LBD, or VBI. The analytic sample for the current study comprised 2,742 autopsied participants.

Additional sub-analyses were conducted on 239 participants in the Oregon Health & Science University (OHSU) and 97 in the University of Washington (UW) ADCs. Both ADCs upload data to NACC and serve as their own data repositories. These two ADCs collaborate in the Pacific Northwest Dementia and Aging Neuropathology Group (PANDA), which also includes the ACT study. Brain tissue collection, histochemical staining, and reporting follow standardized procedures through this agreement. Both ADCs recruit patients seen in clinic for diagnoses, treatment, or clinical trials for enrollment; however, autopsied participants seen at OHSU were recruited from a number of cohort studies focusing on healthy aging in older adults, which are described elsewhere, such as the Oregon Brain Aging Study,[74] the Klamath Exceptional Aging Project,[75] the Intelligent Systems for Assessing Aging Change,[76] and the Oregon Center for Aging and Technology Living Laboratory.[77] Hereafter we refer to OHSU and UW ADCs collectively as the PANDA ADCs.

Adult Changes in Thought study

ACT [U01 AG006781] is a longitudinal community-based prospective cohort study of older adults. In contrast to NACC, participants in ACT were enrolled from a well-defined underlying population of community dwelling older adults receiving care in a health maintenance organization. ACT is described in detail elsewhere.[78] Briefly, a random sample of Group Health Cooperative members aged 65 and older in the Seattle area were invited to participate. Individuals with dementia at baseline were not enrolled. Participants were followed every 2 years

until time of dementia diagnosis, death, or drop-out: 2,581 persons were enrolled in 1994-1996, 811 in 2000-2002, and continuous enrollment started in 2004; 5,074 participants had completed at least one visit between 1994 and May 2015 of which 2,537 had died. Neuropathological assessments were conducted on participants who had died and consented to autopsy. Procedures were standardized with PANDA ADCs as part of the collaborative effort PANDA. Thus although ACT, OHSU ADC, and UW ADC differed in the population of patients studied, the determination of neuropathologic findings was through an identical assessment protocol. The Group Health and University of Washington IRBs approved the ACT study. All participants provided written informed consent, and next of kin consented to autopsy. The University of Washington IRB approved the use of ACT data in the current study. The analytic sample for the current study consisted of 499 autopsied ACT participants (Figure 2.1), having excluded 32 with missing pathologic information on ADNC, LBD, or VBI.

Neuropathological features

With the exception of the common assessment protocol used by PANDA ADCs, each ADC conducted neuropathologic assessments according to its own protocols but following consensus guidelines. Results were uploaded to the NACC database using a standardized form. Additional information on number of microinfarcts as well as number, size, and approximate age of gross infarcts was abstracted from PANDA ADC neuropathology reports to supplement NACC data. In ACT, gross and microscopic lesions were collected using the same standardized assessment protocols used in the PANDA ADCs. Measures were classified similarly across all data sources unless otherwise specified.

ADNC included Consortium to Establish a Registry for Alzheimer's Disease (CERAD) scores of neuritic plaque densities (none, sparse, moderate, frequent)[79] and Braak stages for tau neurofibrillary pathology (none, I-II, III-IV, V-VI).[80] ADNC was categorized semi-quantitatively (no/low, intermediate, and high). No/low ADNC was defined as no/sparse neuritic plaques & any Braak stage OR any neuritic plaques & Braak stage 0-II. Intermediate ADNC was defined as moderate or frequent CERAD plaques & Braak stage III-IV; and high ADNC was defined as moderate or frequent plaques & Braak stage V-VI. Lacking Thal phasing[81] for amyloid plaques this operationalization overlaps but does not correspond exactly to the levels of ADNC as defined by new NIA-Alzheimer's Association criteria.[60]

Vascular pathology encompassed VBI and indicators of vessel disease. In NACC, presence or absence of gross infarcts (small or large artery) and cortical microinfarcts (infarcts in the cortex that were only detected microscopically) were recorded. Acute and old lesions were included. In ACT, OHSU ADC, and UW ADCs microinfarcts were assessed following methods developed in the Honolulu Asia Aging Study and defined as "a focal lesion attributed to ischemia, found only on microscopic examination, and judged to be temporally remote".[82] Microinfarcts were categorized as cortical (present, absent) or subcortical (present, absent). Old or chronic gross infarcts were defined as present or absent. In all data sources, cerebral amyloid angiopathy and atherosclerosis were recorded as none, mild, moderate, or severe.

In NACC, presence of Lewy bodies was assessed according to established guidelines.[83] LBD was defined as presence of Lewy bodies in any brain region examined and categorized as present or absent. LBD subtype was classified as either none, brainstem predominant, limbic

(transitional), cortical (diffuse), or region not specified/other. In ACT, LBD subtype was classified as either none, brainstem predominant, limbic, cortical, or amygdala only.

Hippocampal sclerosis is considered a separate disease entity with potentially multiple etiologic origins.[84] In older NACC form versions (prior to 2014) hippocampal sclerosis was reported present as a primary or contributing neuropathologic diagnosis, while in the newest form version and in ACT presence of hippocampal sclerosis was recorded as unilateral, bilateral, or laterality unknown. Hippocampal sclerosis for this study was classified as present or absent.

Clinical characteristics

Demographic characteristics included age, sex, education, race/ethnicity, and cohort [ACT participants only] or ADC [NACC participants only]. For the purposes of this study, we focused on health histories as of the last clinical visit. In NACC, health history was obtained via co-participant or self-report, medical records and/or judgement of the examining clinician. In ACT, history of co-morbid medical conditions were self-reported. In both studies, *APOE* ε4 allele status (at least one vs. none) was classified for consenting participants who underwent *APOE* genotyping. In NACC ADCs, either a single clinician or consensus group of clinicians made a diagnosis of normal cognition, impaired but not mild cognitive impairment (MCI), MCI, or demented after a review of all evaluation information available. Primary and contributing etiologic diagnoses are assigned for all participants with MCI or dementia, following established guidelines.[72] In ACT, dementia was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition.[85] A complete dementia work-up was only conducted on participants who had a Cognitive Abilities Screening Instrument[86] score 85 or below at their visit

or who reported symptoms suggestive of dementia onset. Dementia due to AD (e.g. clinical AD dementia) was defined in both NACC and ACT as a primary clinical diagnosis of probable or possible AD according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria.[87]

Statistical analyses

We ran analyses for NACC and ACT separately. We calculated the frequency and prevalence (calculated as the number participants with the pathology / total autopsied participants) of ADNC (intermediate to high), LBD, VBI, and other major pathologies in each data set. We estimated the frequency of co-occurrence of intermediate to high ADNC, LBD, and VBI. We used eulerAPE[88] to create Venn/Euler diagrams that accurately illustrate the overlap of each pathology. We examined overlap of pathologies in both samples overall, as well as stratified by age (65-89 vs. 90+). To investigate whether the differences between samples was due to restriction in ACT enrollment, of having to be non-demented and 65 years or older at baseline, we examined co-occurrence of pathologies in NACC excluding those participants who would not have met criteria for ACT.

We next examined the prevalence of LBD subtypes and VBI along the continuum of ADNC (none/low, intermediate, and high). We used Pearson χ^2 test or Fisher's exact test (if any categories included <10 participants) to determine whether the prevalence of LBD subtype and VBI differed by level of ADNC. Because neuropathologic assessments may have differed between some ADCs and ACT we also separately examined prevalence of pathologies among demented in PANDA ADCs, which share neuropathologic protocols with ACT. Finally, using

descriptive statistics we examined clinical and demographic characteristics of those with ADNC and LBD (ADNC+LBD), ADNC and VBI (ADNC+VBI), or ADNC and LBD and VBI (ADNC+LBD+VBI) compared to those with ADNC only, defined as intermediate to high ADNC, no LBD, no VBI. All tests were two-sided, and $\alpha = 0.05$. Analyses were conducted using R (version 3.2.1).[89]

RESULTS

Participant characteristics

Demographic and clinical characteristics of 2,742 autopsied NACC participants and 499 autopsied ACT participants is shown in Table 2.1. NACC participants were evaluated at 31 ADCs; 3 to 252 participants were seen per ADC. Compared to ACT participants, NACC participants were more likely to be demented at last visit and to have at least one *APOE* ε4 allele. They were also less likely to be female, to have low education, and to have a clinical history of stroke. Demographics of non-demented participants were relatively similar between NACC and ACT. NACC participants with dementia were on average younger at death (by almost 10 years) compared to ACT participants.

Prevalence of individual pathologies for NACC and ACT autopsied participants is shown in Table 2.2. NACC autopsied participants with dementia had a higher prevalence of high ADNC and LBD, but a lower prevalence of VBI than did ACT autopsied participants with dementia. Because neuropathologic assessments may have differed between some ADCs and ACT, we also separately examined prevalence of pathologies among demented in PANDA (OHSU and UW) ADCs, which share neuropathologic protocols with ACT. Compared to ACT, LBD was more

prevalent and VBI were less common in all ADCs (Table 2.3). Prevalence of ADNC was lower in OHSU (55%) compared to other ADCs (84%). OHSU participants also had a higher mean age at death (89.1 years [SD: 10.6] vs. 78.1 years [SD 11.9]) and a lower prevalence of dementia (50%) at last visit than other ADCs (82%; see Table 2.4 for demographics in each sub-sample).

Co-occurrence of LBD and VBI in ADNC

The frequency of autopsied participants with ADNC, LBD, VBI, and their combinations is shown in Figure 2.2. Presence of multiple pathologies was common. In NACC, 59% of participants with ADNC had LBD or VBI, and in ACT, 68%. Co-occurrence of ADNC and LBD was more common in NACC than ACT (38% vs. 20% of participants with ADNC), while the co-occurrence of ADNC and VBI was less common in NACC than ACT (30% vs. 60% of participants with ADNC). There were 885 participants from NACC who would have met basic ACT entry criteria of being non-demented and 65 years or older at baseline; 26% of participants with ADNC had co-occurring LBD and 38% had co-occurring VBI. Venn/Euler diagrams stratified by age (65-89 vs. 90+) are shown in Figure 2.3. Among 684 NACC participants and 207 ACT participants older than 90 years at death, the prevalence of LBD in ADNC was similar in both samples (22% in NACC vs 17% in ACT), but VBI in ADNC was still less common in NACC than ACT (45% in NACC vs 61% in ACT).

Prevalence of each LBD subtype is shown across levels of ADNC in Figure 2.4. In NACC, the prevalence of limbic, cortical and other/unknown, LBD significantly differed by ADNC (all, $p<0.001$). Prevalence of brainstem LBD was similar across levels of ADNC ($p=0.2$). The proportion of autopsied with limbic LBD was greater in those with high ADNC compared to low

and intermediate ADNC. Prevalence of other/unknown LBD was also greater in those with high ADNC compared to low and intermediate ADNC. Interestingly cortical LBD were more common in those with intermediate ADNC compared to low or high ADNC. In ACT, sample sizes were small (n=86 with any LBD); however, amygdala only LBD were more common, in a graded fashion, for those with higher ADNC ($p<0.001$). Prevalence of LBD did not significantly differ by level of ADNC for brainstem ($p=0.3$), limbic ($p=1.0$), or cortical LBD ($p=0.6$).

Prevalence of VBI is shown across levels of ADNC in (Figure 2.5). In NACC, VBI were less common in higher levels of ADNC ($p<0.001$). However, in ACT the prevalence of VBI was slightly higher in those with intermediate or high ADNC ($p=0.03$). We also compared specific VBI sub-types between participants from PANDA ADCs and ACT (Figure 2.5) since they all used the same neuropathological assessment protocol. Gross infarcts were less common in intermediate or high ADNC, in a graded fashion, in PANDA ADCs ($p<0.001$) but not in ACT ($p=0.13$); prevalence of cortical microinfarcts did not differ by level of ADNC in ACT ($p=0.13$) or PANDA ADCs ($p=0.22$).

Clinical characteristics of ADNC only vs. mixed ADNC

Clinical characteristics of autopsied participants with ADNC, with and without co-occurring LBD or VBI are shown for NACC (Table 2.5) and ACT (Table 2.6). Compared to ADNC only, male sex, *APOE* ε4 allele, and dementia were all more common in those with mixed ADNC, particularly ADNC with co-occurring LBD, in both NACC and ACT. Prevalence of dementia and college education was higher in NACC than ACT across all ADNC participants. History of stroke was more likely in those with ADNC and VBI compared to other participants with ADNC

only. This trend was more pronounced in NACC than in ACT. Age at death was lower in ADNC+LBD and higher for ADNC+VBI in NACC, but ages were similar in ACT. Participants with mixed ADNC were slightly less likely than ADNC only to have been diagnosed with clinical AD dementia in ACT; in NACC this trend was limited to those with ADNC and co-occurring LBD.

DISCUSSION

We examined mixed neuropathologies in clinic and community-based samples, with particular focus on the relationships between ADNC and LBD or VBI. Overall we found that mixed neuropathologies were common in both populations. Cortical LBD was associated with intermediate ADNC and limbic or other/unknown was associated with high ADNC in NACC. Amygdala LBD was linearly correlated with high ADNC in ACT. The relationship between VBI and ADNC was not consistent between NACC and ACT. Compared to the ACT study, ADC participants were on average younger and a higher proportion had dementia and co-occurring LBD and ADNC at autopsy. Compared to NACC participants, ACT participants were older on average, and a higher proportion of autopsies had VBI. Despite these differences, characteristics of autopsied participants with mixed ADNC neuropathologies were remarkably similar between studies: the majority had dementia but a clinical diagnosis of AD dementia was slightly less common than in those with ADNC only.

Although prior research has found mixed neuropathologies to be common, estimates of the prevalence of mixed neuropathologies are varied.[58] Our findings complement prior findings that prevalence of VBI was higher in community-based sample, and prevalence of high ADNC and

LBD was higher in a clinic-based sample.[50] However, we found that majority of brains had mixed pathologies in both samples. This findings is contrary to the prior study in which mixed pathologies were more common in the community-based sample.[50] This difference may be due to combining data from multiple ADCs that have heterogeneous study populations. Our results suggest ADNC+LBD may be more common in clinic-based, convenience samples, which tend to enroll younger more severely demented individuals with higher prevalence of *APOE* ε4 allele,[90] while ADNC+VBI may be more common in community-based samples, which enroll cognitively normal older adults and often have age restrictions. Applying similar exclusion criteria to NACC, as well as comparing prevalence in the oldest-old, resulted in more similar estimated prevalence of mixed pathologies between ACT and NACC, although the prevalence of VBI was higher in ACT than in NACC.

Our study adds to the evidence of positive association between ADNC and LBD.[42,36,63–65] We found limbic and/or amygdala only LBD was more common in those with high ADNC compared to low or intermediate ADNC. In NACC, amygdala only LBD may have been classified as limbic or other/unknown, which may account for the associations with ADNC in those regions. As suggested, the co-occurrence of ADNC and amygdala LBD merely represents a subtype of pathologic AD.[34] Participants with ADNC+LBD were more likely to have the *APOE* ε4 allele than those with ADNC only, similar to other studies.[91,92] This association suggests the development of LBD in ADNC may be due to shared genetic effects. Alternatively, ADNC may enhance or trigger the process of LBD. For instance, in animal models, alpha-synuclein, amyloid, and tau proteins interact to accelerate development of neuropathology and cognitive decline.[93]

Interestingly, cortical LBD were associated with intermediate but not high levels of ADNC, primarily in NACC. This result is consistent with prior evidence that cortical LBD is associated with higher amyloid burden, but not higher Braak stage,[42] and that demented individuals with mixed neuropathologies typically have lower levels of ADNC compared to individuals with ADNC only.[5,94] Discrepancies in prior findings regarding neurofibrillary tangles, in which some studies found positive associations[36,41,63] while others did not[37,42,64,66] may be accounted for by the non-linear association we observed between cortical LBD and level of ADNC.

Although, VBI may increase accumulation of amyloid,[95] the link between ADNC and VBI may also be due to independent effects.[17] Presence of VBI was inversely associated with ADNC in NACC overall as well as in the PANDA ADCs. In ACT, although VBI was somewhat related to level of ADNC, such an association was not consistent when looking at individual VBI sub-types, unlike other studies in which cortical VBI were associated with ADNC.[68,69] Since the PANDA ADCs share neuropathologic assessment with ACT, differences in VBI assessment are unlikely to explain these findings. Perhaps this result is due to selection towards earlier onset dementia in NACC; individuals with ADNC in NACC may be more likely to die prior to development of VBI. In NACC, prevalence of ADNC is lower in those who died after age 80 while prevalence of vascular pathology increases with older age at death.[96] Together, we did not find strong evidence for a positive association between VBI and ADNC, which is consistent with other studies,[23] including a recent study using biomarker data.[33]

We found clinical profiles of ADNC mixed with LBD or VBI differed from ADNC only. Higher proportions of those with ADNC+LBD (with or without VBI) were male and had an *APOE* ε4

allele compared to ADNC only, consistent with similar findings in other studies.[66,91] A high proportion of participants with ADNC+VBI (with or without LBD) were male and had a history of stroke compared to ADNC only, characteristics associated with VBI in general.[17,97] As in prior studies,[98] clinical diagnosis of AD dementia was fairly sensitive (generally 70-90% of those with ADNC), even in those with additional pathologies. Participants with mixed ADNC were slightly less likely to be diagnosed with clinical AD than those with ADNC only. Additional research is needed to determine the clinical relevance of these findings.

Some limitations exist with the use of retrospective autopsy data. Assessment of neuropathologies may differ between centers and methodologies change over time. Newly identified pathologic features, such as TAR DNA-binding protein 43 (TDP-43),[99] were not available for most participants. NACC and ACT studies include predominantly Caucasian and well-educated older adults, which may limit generalizability. There may have been some misclassification of dementia status prior to death; in particular clinic-only assessment may have underestimated the prevalence of dementia in NACC compared to ACT which conducts home-based assessments as well.[70] In this study we focused on findings within the autopsy samples, which may not represent the prevalence of pathologies in overall study populations.[52] Prior findings in ACT suggest that prevalence of neuropathologies among autopsied demented participants is representative of all participants who developed dementia.[51] However, participants with dementia are overrepresented in the ACT autopsy sample,[100] and more so in NACC, so this finding may have biased individual prevalence estimates upwards compared to overall ACT and NACC study populations.

Despite the limitations there are important strengths to this study. We used data from two large autopsy samples with extensive clinical and pathologic information which allowed use to examine the co-occurrence of ADNC, LBD, and VBI from multiple perspectives. To examine potential biases, we made comparisons between autopsied clinical research volunteers included in NACC and autopsied participants in ACT, a population-based study, and we included sub-analyses among ACT, and the PANDA ADCs that shared identical neuropathologic assessment protocols. This enabled us to examine whether differences in our findings were related to the neuropathological assessment protocols, the populations studied, or some other factors.

Assessing the similarities, we find further evidence suggesting that ADNC are common with LBD or VBI, especially in demented patients. Our findings point to an association between ADNC and LBD, although whether these factors are synergistic or related to shared pathogenic processes remains to be determined. Clinical characteristics of participants with ADNC, differed between those with and without co-occurring LBD and VBI. Given that over 50% of participants with ADNC in our study had co-occurring LBD or VBI, effective prevention and treatment of clinical AD may need to target multiple disease processes.

TABLES AND FIGURES

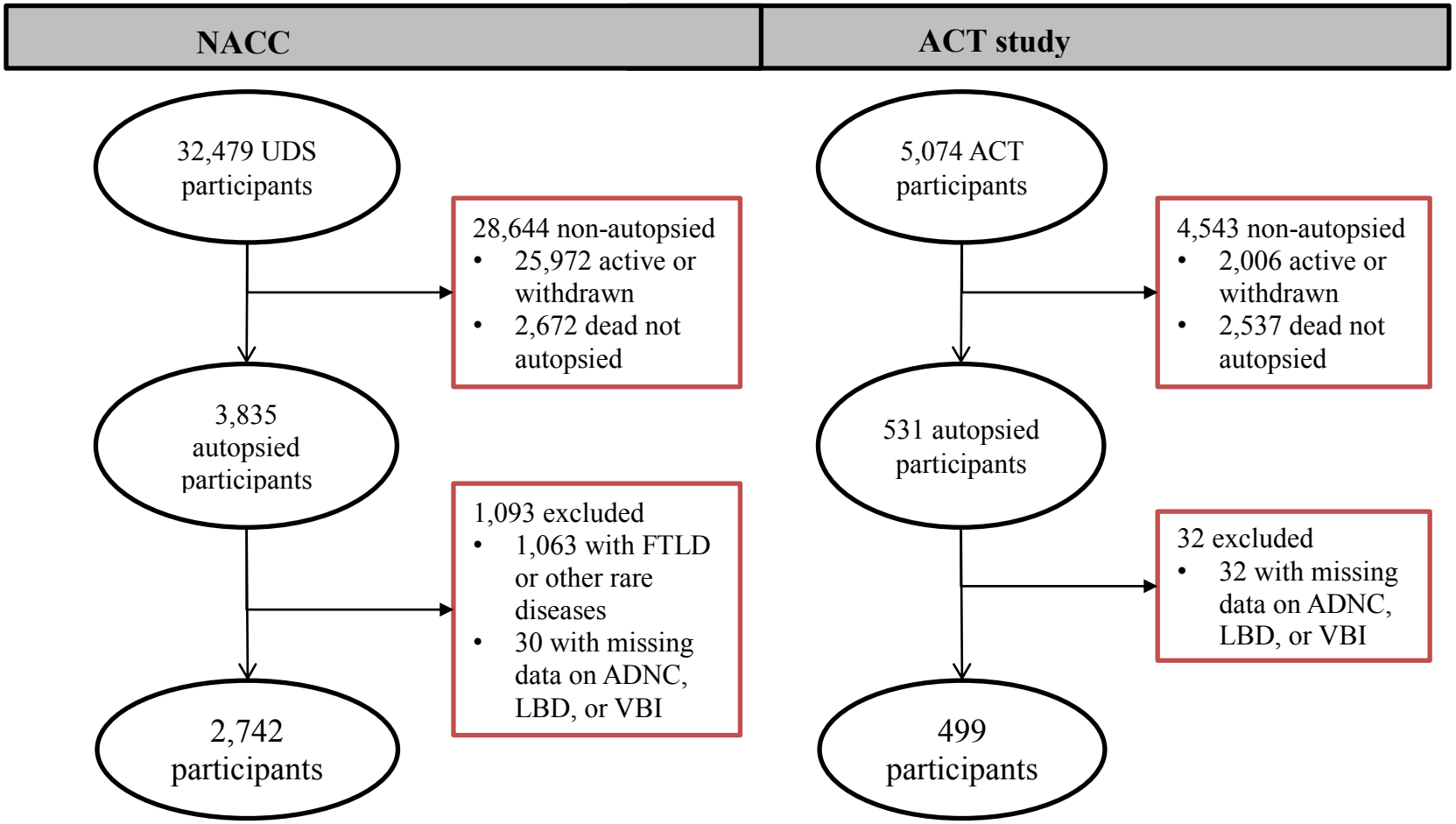

Figure 2.1 Study sample flow chart. ACT; Adult Changes in Thought study; ADNC; Alzheimer's disease neuropathologic change; FTLD, frontotemporal lobar degeneration; LBD, Lewy body disease, NACC, National Alzheimer's Coordinating Center; UDS, Uniform Data Set; VBI, vascular brain injury

Table 2.1 Demographic and clinical characteristics of participants by dementia status

Characteristics*	NACC			ACT study		
	Non-demented	**Demented**	**Total**	**Non-demented**	**Demented**	**Total**
Total autopsies, N	585	2,157	2,742	275	224	499
Age at death, mean (SD)	87.3 (8.8)	80.3 (10.3)	81.8 (10.4)	86.3 (7.0)	89.5 (5.7)	87.7 (6.7)
Female	327 (55.9)	941 (43.6)	1,268 (46.2)	145 (52.7)	120 (53.6)	265 (53.1)
Non-white	24 (4.1)	1,487 (6.9)	171 (6.3)	11 (4.0)	9 (4.0)	20 (4.0)
College graduate	334 (57.1)	1,180 (54.7)	1,514 (55.2)	120 (43.6)	78 (34.8)	198 (39.7)
History of stroke	74 (12.7)	263 (12.3)	337 (12.4)	42 (16.2)	32 (17)	74 (16.6)
APOE ε4 allele	113 (21.5)	1,015 (55.8)	1,128 (48.1)	59 (23.1)	68 (34.9)	127 (28.2)
Clinical AD dementia	NA	1,776 (82.3)	1,776 (42.9)	NA	176 (78.6)	178 (35.7)

ACT, Adult Changes in Thought; AD, Alzheimer's disease; NA, not applicable; NACC, National Alzheimer's Coordinating Center

*N,% unless otherwise specified. Relative frequencies calculated based on complete data. Number of participants missing data in NACC: race=15 (<1%), education=25 (<1%), *APOE* genotype=395 (14.4%), stroke=22 (<1%), and age of onset=27 (1.3%). Number of participants missing data in ACT: *APOE* genotype=49 (9.8%), stroke=52 (10.4%).

Table 2.2 Prevalence of individual pathologies in participants by dementia status

Characteristics*	NACC			ACT study		
	Non-demented	Demented	Total	Non-demented	Demented	Total
Total autopsies, N	585	2,157	2,742	275	224	499
Alzheimer's disease neuropathologic change (ADNC)†	29.6	82.7	71.3	26.2	63.4	42.9
Intermediate ADNC	16.8	13.9	14.5	15.6	13.3	14.6
High ADNC	12.8	68.8	56.9	10.5	50.0	28.3
Lewy body disease (LBD)	16.9	40.8	35.7	13.8	21.4	17.2
Cortical LBD	3.8	18.2	15.1	4.0	8.9	6.2
Vascular brain injury‡	40.7	30.7	32.9	40.7	68.3	53.1
Cortical microinfarcts	20.2	16.2	17.1	29.8	45.5	36.9
Gross infarcts	31.0	22.5	24.3	23.9	46.0	33.9
Other Pathologies						
Severe atherosclerosis	14.5	13.4	13.6	5.6	9.6	7.4
Severe CAA	5.6	15.1	13.1	1.8	2.2	2.0
Hippocampal sclerosis	3.1	13.2	11.0	5.6	12.5	8.7

ACT, Adult Changes in Thought; CAA, cerebral amyloid angiopathy; NACC, National Alzheimer's Coordinating Center

*Percent autopsied unless otherwise noted, calculated based on complete data. Number of participants missing data in NACC: gross infarcts=3 (<1%), atherosclerosis=23 (<1%), CAA=62 (2.3%), hippocampal sclerosis=450 (16.4%). Number of participants missing data in ACT: gross infarcts=3 (<1%), atherosclerosis=14 (2.8%), CAA=2 (<1%), hippocampal sclerosis=14 (2.8%).

†ADNC defined as moderate/frequent CERAD neuritic plaques & Braak stages of neurofibrillary degeneration III-IV (intermediate ADNC) or Braak stages V-VI (high ADNC).

‡VBI defined as any gross infarcts or cortical microinfarcts.

Table 2.3 Prevalence of individual pathologies among demented participants in OHSU and UW ADCs compared to other to ACT and other NACC ADCs

Neuropathologies*	ACT study	NACC-ADCs			
		OHSU	UW	Other ADCs	Total
Total autopsies, N	224	116	84	1,956	2,157
Alzheimer's disease neuropathologic change (ADNC)†	63.4	55.2	85.7	84.0	82.7
Intermediate ADNC	13.3	13.8	8.3	14.1	13.9
High ADNC	50.0	41.4	77.4	70.0	68.8
Lewy body disease (LBD)	21.4	33.6	53.6	40.8	40.8
Cortical LBD	8.9	17.2	26.2	18.0	18.2
Vascular Brain Injury‡	40.7	39.7	34.5	29.3	30.7
Cortical microinfarcts	45.5	20.7	28.6	15.2	16.2
Gross infarcts	46	40.5	19.0	21.6	22.5
Subcortical microinfarcts	39.9	50.0	20.2	NA	NA
Other Pathologies					
Severe atherosclerosis	9.6	14.8	1.2	13.8	13.4
Severe CAA	2.2	4.3	7.1	16.1	15.1
Hippocampal sclerosis	12.5	9.4	6.4	13.7	13.2

ADC, Alzheimer's Disease Center; CAA, cerebral amyloid angiopathy; OHSU, Oregon Health & Science University; NA, not applicable; NACC, National Alzheimer's Coordinating Center; UW, University of Washington

*Percent autopsied unless otherwise specified, calculated for complete data. Number of participants missing data in ACT: subcortical microinfarcts=1 (<1%), atherosclerosis=6 (2.7%), hippocampal sclerosis=8 (3.6%). Number of participants missing data in OHSU: atherosclerosis=1 (<1%), hippocampal sclerosis=10 (8.6%). Number of participants missing data in UW: hippocampal sclerosis=6 (7.1%). Number of participants missing data in other ADCs: gross infarcts=1 (<1%), atherosclerosis=16 (<1%), CAA=51 (2.6%), hippocampal sclerosis=337 (17.2%). Number of participants missing data in NACC overall: gross infarcts=1 (<1%), atherosclerosis=17 (<1%), CAA=51 (2.4%), hippocampal sclerosis=355 (16.5%).

†ADNC defined as moderate/frequent CERAD neuritic plaques & Braak stages of neurofibrillary degeneration III-IV (intermediate ADNC) or Braak stages V-VI (high ADNC).

‡Vascular brain injury defined as any gross infarcts or cortical microinfarcts.

Table 2.4 Participant characteristics in OHSU and UW ADCs compared to other ADCs

Characteristics*	**NACC –ADCs**			
	OHSU	**UW**	**Other ADCs**	**Total**
Total autopsies, N	239	97	2,399	2,742
Age at death, mean (SD)	90.4 (9.3)	80.2 (11.1)	81.0 (10.0)	81.8 (10.4)
Female	148 (61.9)	33 (34)	1,080 (45.0)	1,268 (46.2)
Non-white	4. (1.7)	4 (4.2)	162 (6.8)	171 (6.3)
College graduate	116 (48.5)	66 (68)	1,329 (55.4)	1,514 (55.2)
APOE ε4 allele	60 (26.1)	55 (57.9)	1,011 (50.2)	337 (12.4)
Demented	116 (48.5)	84 (86.6)	1,956 (81.5)	2,157 (78.7)

ADC, Alzheimer's Disease Center, OHSU, Oregon Health & Science University; UW, University of Washington

*N,% unless otherwise specified. Relative frequencies calculated based on complete data. Number of participants missing data in OHSU: education=1 (<1%), *APOE* genotype=9 (3.8%). Number of participants missing data in UW: race=1 (<1%), education=1 (1.0%), *APOE* genotype=2 (2.1%), Number of participants missing data in other ADCs: race=14 (<1%), education=23 (1.0%), *APOE* genotype=384 (16.0%).

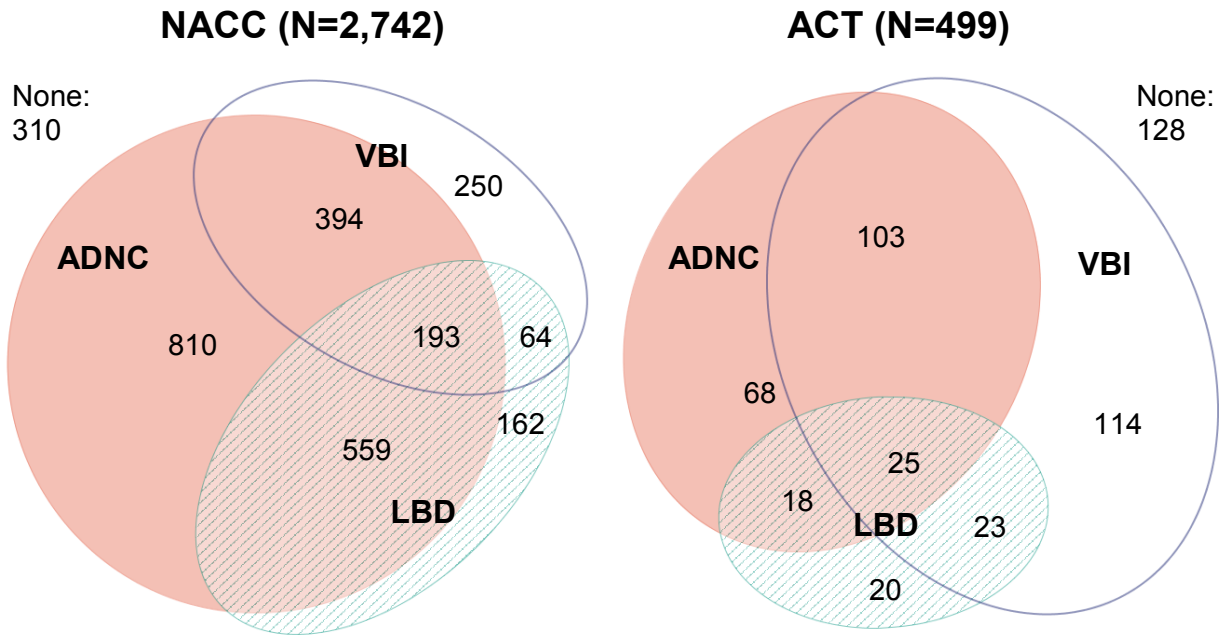

Figure 2.2 Co-occurrence of Alzheimer's disease neuropathologic change (ADNC), Lewy body disease (LBD), and vascular brain injury (VBI). ACT, Adult Changes in Thought study; NACC, National Alzheimer's Coordinating Center. ADNC = moderate/frequent neuritic plaques & Braak stage III-VI; LBD = Lewy bodies in any brain region examined; VBI = gross infarcts and cortical microinfarcts.

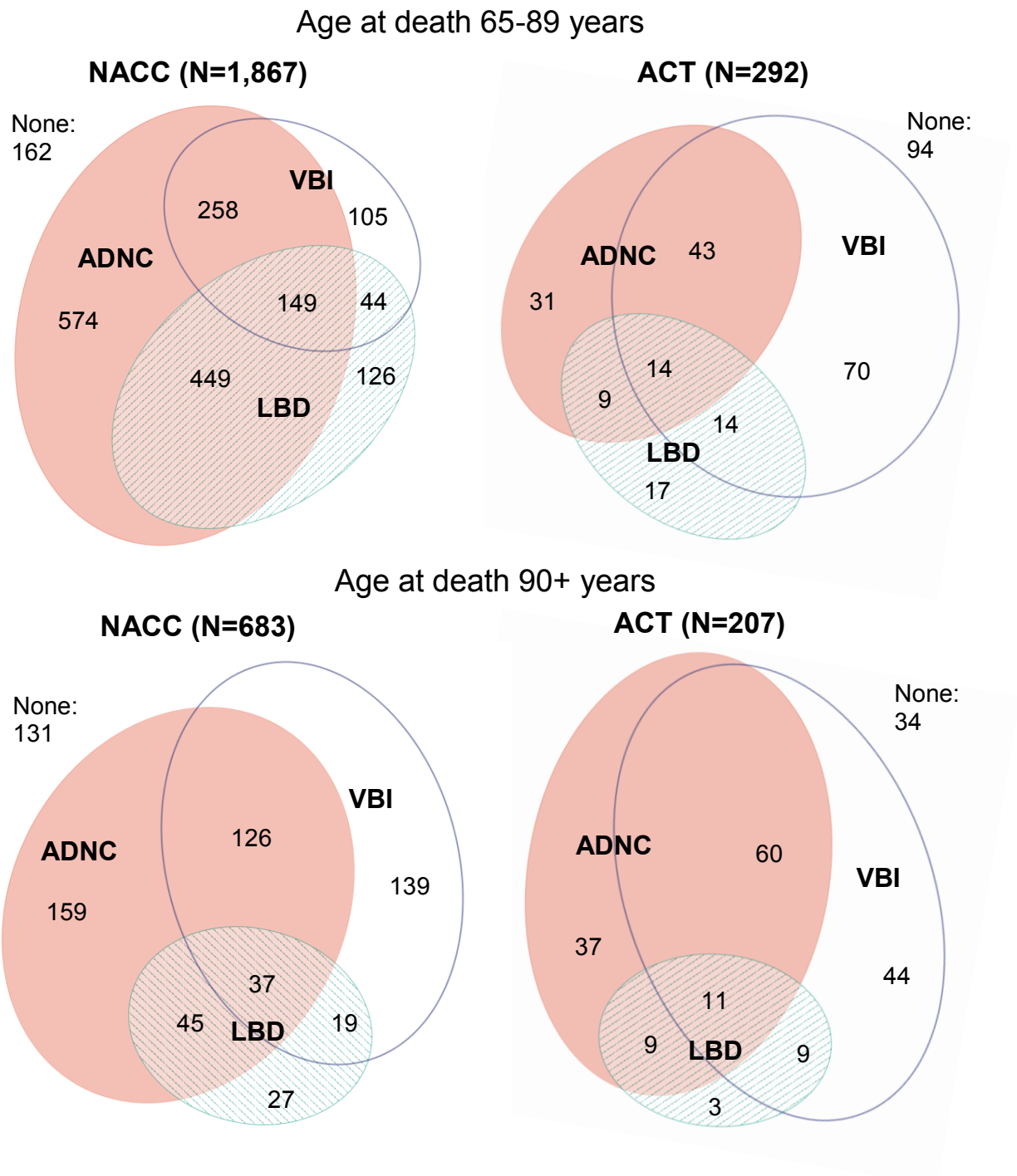

Figure 2.3 Co-occurrence of Alzheimer's disease neuropathologic change (ADNC), Lewy body disease (LBD), and vascular brain injury (VBI) stratified by age. ACT, Adult Changes in Thought study; NACC, National Alzheimer's Coordinating Center. ADNC = moderate/frequent neuritic plaques & Braak stage III-VI; LBD = Lewy bodies in any brain region examined; VBI = gross infarcts and cortical microinfarcts. Note: 191 NACC participants with age of death less than 65 years were excluded.

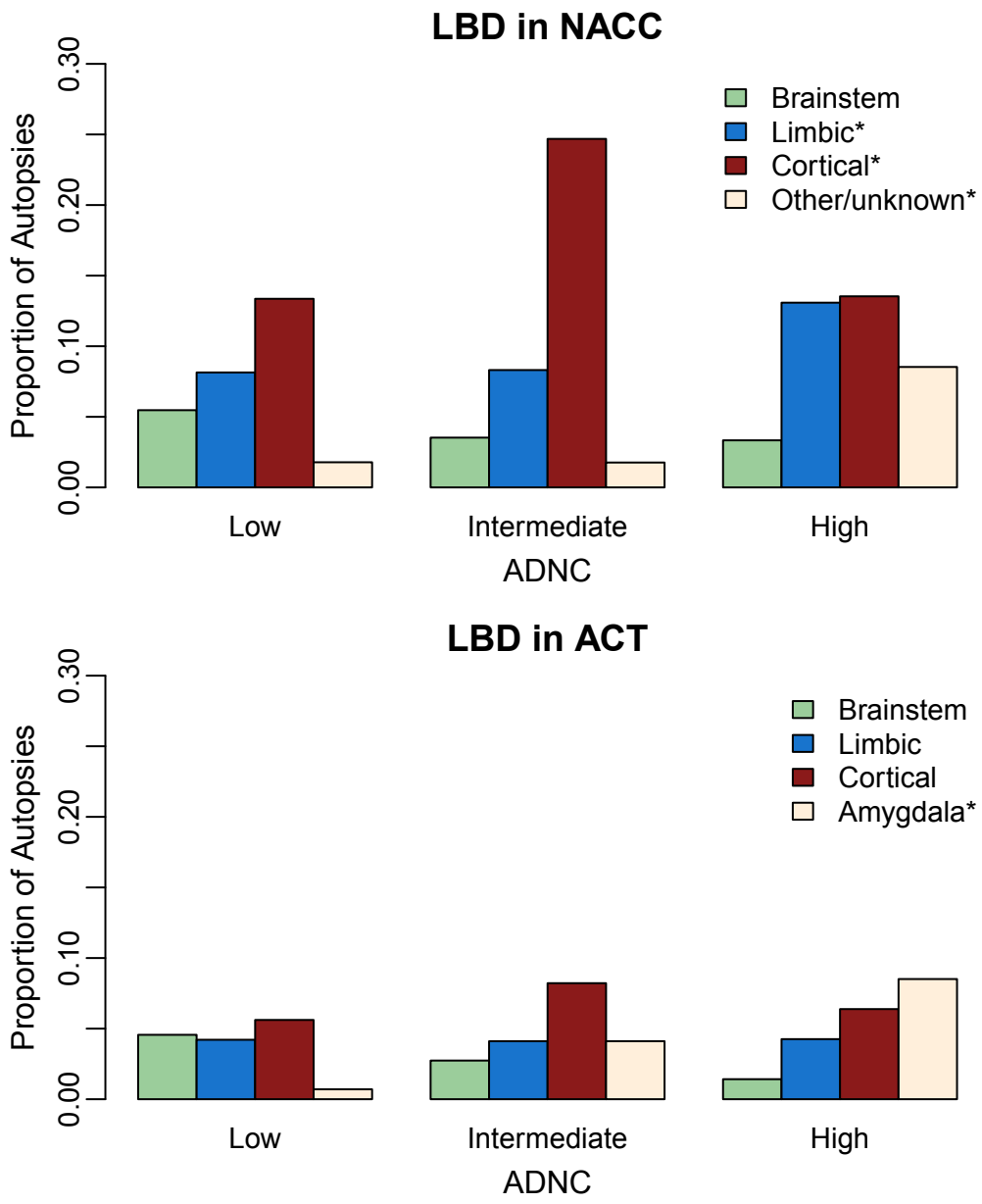

Figure 2.4 Prevalence of Lewy body disease (LBD) subtypes in participants with low, intermediate, and high Alzheimer's disease neuropathologic change (ADNC). ACT, Adult Changes in Thought study; NACC, National Alzheimer's Coordinating Center. Low ADNC= no/sparse CERAD neuritic plaques & any Braak stage OR any neuritic plaques & Braak stage 0-II; intermediate ADNC= moderate/frequent neuritic plaques & Braak stage III-IV; high ADNC = moderate/frequent neuritic plaques & Braak stages V-VI. *$p<0.001$ for difference in prevalence of LBD subtype by level of ADNC based on χ^2 (NACC) or Fisher's exact test (ACT).

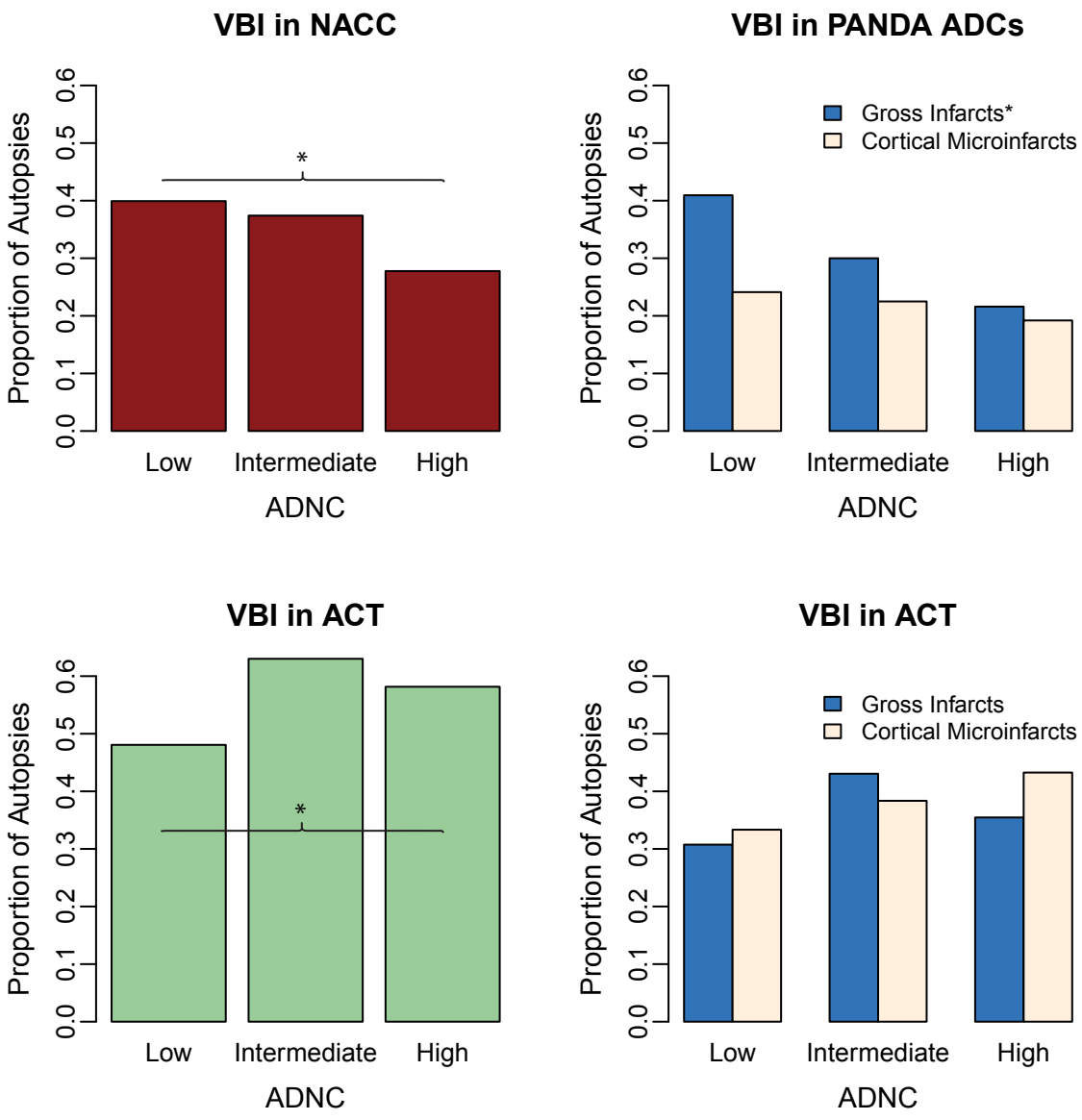

Figure 2.5 Prevalence of vascular brain injury (VBI) in participants with low, intermediate, and high Alzheimer's disease neuropathologic change (ADNC). ACT, Adult Changes in Thought study; NACC, National Alzheimer's Coordinating Center; PANDA ADCs, Pacific Northwest Dementia and Aging Neuropathology Group Alzheimer's Disease Centers (Oregon Health & Science University and University of Washington). Low ADNC = no/sparse CERAD neuritic plaques & any Braak stage OR any neuritic plaques & Braak stage 0-II; Intermediate ADNC = moderate/frequent neuritic plaques & Braak stage III-IV; High ADNC = moderate/frequent neuritic plaques & Braak stages V-VI. *$p<0.05$ for difference in prevalence of VBI by level of ADNC, based on χ^2 (NACC) or fisher's exact test (ACT).

Table 2.5 Demographic and clinical characteristics of NACC participants with Alzheimer's disease neuropathologic change (ADNC) with and without co-occurring Lewy body disease (LBD) or vascular brain injury (VBI)*

Characteristics†	ADNC only	ADNC+LBD	ADNC+VBI	ADNC+LBD +VBI
Total autopsies, N	810	559	394	193
Age at death, mean (SD)	80.2 (10.6)	77.4 (9.5)	84.4 (8.4)	82.9 (10.9)
Female	403 (49.8)	228 (40.8)	184 (46.7)	76 (39.4)
Non-white	37 (4.6)	35 (6.3)	35 (9)	24 (12.5)
College graduate	466 (57.5)	314 (56.2)	203 (51.5)	103 (53.4)
History of stroke	54 (6.8)	30 (5.4)	102 (26.3)	28 (14.6)
APOE ε4 allele	378 (54.9)	307 (64.9)	185 (54.9)	105 (62.5)
Demented	726 (89.6)	538 (96.2)	337 (85.5)	182 (94.3)
Clinical AD dementia	658 (90.6)	421 (78.3)	306 (90.8)	159 (87.4)

NACC, National Alzheimer's Coordinating Center

*ADNC = moderate/frequent neuritic plaques & Braak stage III-VI; LBD = Lewy bodies in any brain region examined; VBI = gross infarcts and cortical microinfarcts.

†N,% unless otherwise specified. Relative frequencies presented for complete data. Number of participants missing data: race=11 (<1%), education=19 (1.0%), stroke=20 (1.0%), and *APOE* genotype=289 (14.8%).

Table 2.6 Demographic and clinical characteristics of ACT study participants with Alzheimer's disease neuropathologic change (ADNC) with and without co-occurring Lewy body disease (LBD) or vascular brain injury (VBI)*

Characteristics†	ADNC only	ADNC+LBD	ADNC+VBI	ADNC+LBD +VBI
Total autopsies, N	68	18	103	25
Age at death, mean (SD)	89.1 (6.7)	88.9 (5.8)	90.6 (5.9)	87.8 (6.3)
Female	43 (63.2)	7 (38.9)	60 (58.3)	13 (52.0)
Non-white	5 (7.4)	1 (5.6)	7 (6.8)	1 (4.0)
College graduate	26 (38.2)	9 (50.0)	31 (30.1)	11 (44.0)
History of stroke	2 (3.4)	1 (6.7)	25 (26.9)	1 (4.5)
APOE ε4 allele	20 (33.3)	6 (37.5)	33 (34.4)	12 (60.0)
Demented	35 (51.5)	14 (77.8)	76 (73.8)	17 (68.0)
Clinical AD dementia	33 (94.3)	10 (71.4)	61 (80.2)	14 (82.3)

ACT, Adult Changes in Thought

*ADNC = moderate/frequent neuritic plaques & Braak stage III-VI; LBD = Lewy bodies in any brain region examined; VBI = gross infarcts and cortical microinfarcts.

†N,% unless otherwise specified. Relative frequencies presented for complete data. Number of participants missing data: stroke=25 (11.7%), and *APOE* genotype=22 (10.3%).

Chapter 3. Mixed neuropathologies and progression of scores on Clinical Dementia Rating Scale Sum of Boxes

ABSTRACT

Introduction: How co-occurring neuropathologies may affect clinical disease progression is unclear. We estimated rates of clinical progression in a longitudinal data set and tested whether progression associated with Alzheimer's disease neuropathologic change (ADNC) was modified by Lewy body disease (LBD) or vascular brain injury (VBI).

Methods: Data came from the National Alzheimer's Coordinating Center on 2,046 autopsied participants with a clinical evaluation at a U.S. NIA-funded Alzheimer's Disease Center within 2 years of death. Linear mixed effects models evaluated longitudinal trends in the Clinical Dementia Rating Scale Sum of Boxes (CDR-SB). Models included adjustment for age, sex, race, education, and last visit-death interval, as well as inverse probability of autopsy weights to adjust for potential bias due to autopsy selection.

Results: The annual change in CDR-SB (higher score=worse impairment) for those with ADNC only (1.7 points; 95% confidence interval [CI]: 1.6, 1.8) was slightly slower compared to those with ADNC+LBD (1.9; 95% CI: 1.7, 2.0) but slightly faster than those with ADNC+VBI (1.5; 95% CI: 1.3, 1.6). Interestingly, ADNC interacted with LBD (p=0.002) and VBI (p=0.003), such that the rate of progression was significantly slower in those with dual neuropathologies than if each neuropathology contributed independently to progression. In secondary models, significant negative interactions were present only in those with high but not intermediate levels of ADNC.

Conclusions: Considering interactions is important in characterizing clinical progression associated with neuropathologies. The impact of co-occurring pathologies on progression may also depend on severity of ADNC.

INTRODUCTION

In community-based autopsy studies up to 75% of older adults have multiple brain pathologies (aka mixed neuropathologies).[9,25,59] The most commonly observed pathologies are Alzheimer's disease neuropathologic change (ADNC-defined by β-amyloid plaques and tau neurofibrillary tangles), vascular brain injury (VBI), such as infarcts, and Lewy body disease (LBD-defined by inclusions of α-synuclein). Coexisting neuropathologies may interact synergistically or act independently to influence the dementia syndrome. Current evidence for such interactions is inconsistent.

Lewy body development may be enhanced by ADNC.[34,44] Lewy bodies, particularly those found in the cortex, and ADNC are each associated with cognition[19,100–103] and increased rates of cognitive decline.[20,21] In two studies cognitive decline was faster in individuals with ADNC and co-occurring LBD compared to ADNC only.[104,105] One community-based study reported no significant interactions.[37] Few other studies have reported testing whether concomitant LBD modified the association between ADNC and cognition, or vice versa.

In the presence of VBI, a lower level of ADNC may be necessary to produce cognitive symptoms.[5,27,6,28,30] Although one study reported a synergistic interaction in which presence of VBI in those with ADNC was associated with more severely reduced memory scores,[106] other

studies suggest that ADNC and VBI do not interact.[31,107,33] ADNC in more severe stages may overwhelm the effects of VBI.[108] Co-occurrence of vascular lesions has not been associated with cognitive decline beyond that of high ADNC[32,109] and cerebrovascular disease was more strongly associated with decline in those with lower ADNC.[16]

To our knowledge no prior studies have extensively examined whether LBD or VBI interact with ADNC clinical progression. Such research could help clarify the role of mixed neuropathologies in clinical progression, however testing interactions requires a large sample size. We used National Alzheimer's Coordinating Center (NACC) data on autopsied participants who were clinically evaluated at a U.S. National Institute on Aging-funded Alzheimer's Disease Center (ADC). We evaluated whether autopsied older adults with ADNC with co-occurring LBD or VBI had faster overall clinical progression compared to those with single and low neuropathologies. We tested whether LBD or VBI modified the association between ADNC and progression. If ADNC and LBD or VBI contributed independently to progression, we expected the cumulative association of mixed pathologies with clinical progression to be the sum of the estimates for each pathology, namely additive effects. Significant interactions between ADNC and LBD or ADNC and VBI would indicate that the association with clinical progression was greater (synergistic) or less (antagonistic) than the sum of individual estimates for each pathology.

METHODS

Data sources and study populations

NACC maintains the Uniform Data Set (UDS) on participants who had been prospectively evaluated and autopsied by one of 34 past and present ADCs since September 2005. Participants enrolled with any level of cognition and were examined annually in-person using a standard protocol, described in detail elsewhere.[71,72] Neuropathologic data was collected following a standardized protocol also on participants who had died and consented to autopsy. All participants provided written informed consent and institutional review board (IRB) approval was obtained from all individual ADCs.

As of September 2015, the UDS had 32,479 participants with at least one clinical visit; 6,507 were known to have died of whom 3,835 had been autopsied. 1,063 participants were excluded with Down's syndrome, prion disease, autosomal dominant genetic diseases, frontotemporal lobar degeneration, or other rare causes of dementia, which may conflict with neuropathologic assessment of ADNC or confound clinical conditions. Also excluded were participants missing information on age, sex, race/ethnicity, or achieved education (n=93) and/or neuropathologic information on ADNC, LBD, or VBI (n=30). Participants with their last visit over 2 years prior to death were also excluded (n=532). We excluded 71 participants who did not have ADNC, LBD, or VBI but had other pathologic burden such as hippocampal sclerosis, Braak stage V-VI with sparse or no neuritic plaques, frequent neuritic plaques but Braak stage 0-II, other major pathologies, white matter disease, or some combination of these. Given these exclusions, 2,046 participants with at least one clinical visit remained for analyses; individuals with only one visit

(n=1,571) were included in analytic models at baseline but did not contribute to longitudinal estimates. The study sample flow chart is shown in Figure 3.1.

To supplement NACC data, we abstracted additional information on number of vascular pathologies on participants seen at the Oregon Health & Science University (OHSU) (n=211) and University of Washington (UW) (n=82) ADCs. These two centers have a joint agreement as part of the Pacific Northwest Dementia and Aging Neuropathology Group (PANDA) to follow the same neuropathologic assessment protocol. Both ADCs recruit patients seen in clinic for diagnoses, treatment, or clinical trials for enrollment into the UDS; however, autopsied UDS participants seen at OHSU were also recruited from a number of cohort studies focusing on healthy aging in independent older adults which are described elsewhere, such as the Oregon Brain Aging Study.[74] Subsequently, we will use the term PANDA ADCs to refer to OHSU and UW ADCs combined.

Neuropathological features

ADCs follow consensus guidelines but conduct neuropathologic assessments according to their own protocols, which vary between sites. Neuritic plaque density was defined by Consortium to Establish a Registry for Alzheimer's Disease (CERAD) scores of none, sparse, moderate, frequent.[79] Tau neurofibrillary pathology was measured with Braak stage (none, I-II, III-IV, V-VI).[80] ADNC was defined regardless of a participant's cognitive status and was categorized semi-quantitatively as no/low, intermediate, and high. No/low ADNC was defined as no/sparse neuritic plaques & any Braak stage OR any neuritic plaques & Braak stage 0-II. Intermediate ADNC was defined as moderate/frequent CERAD plaques & Braak stage III-IV; and high

ADNC was defined as moderate/frequent plaques & Braak stage V-VI. This classification overlaps with intermediate to high ADNC as defined by the 2012 NIA-Alzheimer's Association criteria;[60] however, Thal phasing[81] for amyloid plaques was not available for most participants. Assessment for Lewy bodies followed recognized guidelines.[83] LBD was defined as presence of Lewy bodies in any brain region examined. LBD subtype was classified as none, brainstem predominant, limbic (transitional), neocortical (diffuse), or region not specified/other. In NACC, presence of any VBI was defined as any gross infarcts (small or large artery) or any cortical microinfarcts (infarcts in the cortex only seen microscopically) regardless of age. In the PANDA ADCs, assessment of microinfarcts followed methods developed in the Honolulu Asia Aging Study.[82] The number (0,1,2,3, or 4 or more) of cortical and subcortical microinfarcts was recorded separately. Cerebral amyloid angiopathy, atherosclerosis, and arteriolosclerosis were recorded as none, mild, moderate, or severe. Participants with subcortical leukoencephalopathy or white matter rarefaction were considered to have white matter disease. Hippocampal sclerosis was categorized as present or absent. Due to differences in NACC forms, hippocampal sclerosis was considered present if a primary or contributing neuropathologic diagnosis was listed in participants autopsied prior to 2014, while for participants autopsied 2014 or later, presence of hippocampal sclerosis was recorded as unilateral, bilateral, or laterality unknown. Participants were defined as having a low level of neuropathology (low NP) if they were without ADNC, LBD, VBI, or other major pathologic burden.

Clinical impairment

Clinical impairment was quantified at each study visit with the Clinical Dementia Rating Scale Sum of Boxes (CDR-SB),[110] a measure of the overall level of cognitive impairment and

functional disability and sums scores from 1 to 3 over six domains. Specifically, the CDR-SB is a composite measure of deficits in memory, orientation, judgment & problem solving, community affairs, home & hobbies, and personal care that is based on clinical judgment and study co-participant report. The CDR has good reliability.[110] The CDR-SB ranges from 0-18, with increasing score for increasing impairment, and is considered a sensitive measure for staging dementia on a continuum from normal to severe dementia in heterogeneous samples.[111]

Other clinical characteristics

Demographic characteristics included in analyses were age, sex, education, race/ethnicity, and ADC. History of comorbidities, such as depression, heart disease, and stroke were evaluated at each visit. *APOE* genotyping was performed on consenting participants. *APOE* ε4 allele status was classified as at least one or none. Clinician judgment of cognitive, motor, and behavioral problems was recorded at each visit. At all ADCs, either a single clinician or consensus group of clinicians made at each visit a diagnosis of normal cognition, impaired but not mild cognitive impairment (MCI), MCI, or dementia.

Statistical analyses

Modeling clinical progression

To model longitudinal trends in clinical impairment and test associations with neuropathologies, we used multivariable regression modeling via linear mixed effects models. The primary outcome was the CDR-SB score (0 to 18) at each visit, modeled as a continuous measure. Following the approach of other studies,[21] we modeled longitudinal trends in CDR-SB over time such that visits work backwards from last visit to the initial clinical visit. Empirical plots

suggested change in CDR-SB over time was approximately linear. Models included random intercepts for both participants and ADC to account for correlation of CDR-SB scores within the same participant as well as within the same ADC. In addition, random slopes were included for participants to allow for heterogeneity in rate of change in CDR-SB over time between participants. Primary predictors were dichotomous variables for ADNC, LBD, VBI, and time. We added interaction terms between time and each primary pathology term and 3-way interactions of ADNC×LBD×time and ADNC×VBI×time. In primary models the CDR-SB (Y_{ijk}) at time ***k*** (k= 1,…m) for participant ***j*** (j=1,…,n) in ADC ***i*** (i=1,…,p) was specified via the following model (covariates not shown for simplicity),

$$\begin{aligned} Y_{ijk} = {} & \beta_0 + \beta_1 Time_{ijk} + \beta_2 ADNC_{ij} + \beta_3 VBI_{ij} + \beta_4 LBD_{ij} + \beta_5 ADNC_{ij} \times Time_{ijk} + \beta_6 VBI_{ij} \times Time_{ijk} \\ & + \beta_7 LBD_{ij} \times Time_{ijk} + \beta_8 ADNC_{ij} \times VBI_{ij} + \beta_9 ADNC_{ij} \times LBD_{ij} + \beta_{10} ADNC_{ij} \times VBI_{ij} \times Time_{ijk} \\ & + \beta_{11} ADNC_{ij} \times LBD_{ij} \times Time_{ijk} + a_{0i} + b_{0ij} + b_{1ij} Time_{ijk} + \varepsilon_{ij} \end{aligned}$$

where β = fixed effects terms, a = ADC level random effects, b = subject level random effects, and ε = random error. The regression parameter estimate (β) for time described the annual mean rate of change in CDR-SB, namely the slope, in those with low NP. The interaction between time×ADNC, for instance, described modifications to the relationship between time and CDR-SB that were attributable to ADNC without co-occurring LBD or VBI. The model estimate for 3-way interaction between ADNC×LBD×time, for instance, described the difference in annual rate of change of CDR-SB associated with having ADNC and LBD (ADNC+LBD) beyond the contribution of ADNC only and LBD only. In model terms, if each pathology contributed independently to CDR-SB, annual rate of change in CDR-SB for an individual with ADNC+LBD would be the summed estimates of time×ADNC + time×ADNC.

Primary models also included adjustment for potential confounders: age at death, sex, race/ethnicity, education, and interval between last visit and death. As a sensitivity analysis, we included additional adjustment for comorbidities and *APOE* ε4 allele, among the subset of participants with *APOE* genotype information. Due to concern that trends in those with severe dementia drove trends, we conducted a sensitivity analysis on participants without dementia. We also examined rates of progression stratified by high and low education (high school or less vs some college or more) because prior studies have found that the association between cognition and markers of pathologic burden are lower in those with higher educational attainment, which may indicate cognitive or brain reserve.[112,113] In secondary models, we examined associations with semi-quantitative measures instead of dichotomous measures for ADNC pathologies and LBD subtype. In PANDA ADC participants, we examined associations with number of cortical and subcortical microinfarcts, modeled as continuous, separately.

Inverse probability weighting

An important consideration in this study is that participants who have died and consented to autopsy may differ in characteristics from the overall study populations. Characteristics of autopsied and non-autopsied participants in NACC are shown in Table 3.1. Findings in autopsy samples may be biased in certain cases when determinants of autopsy are also potential confounders of the exposure-outcome association,[52] such as age, sex, race, and education.[100] In an attempt to account for potential selection bias, we used inverse probability weighting (IPW)[114] to adjust for differences between the overall study sample and the autopsy sample.[54] Weights were created by first modeling autopsy selection using logistic regression; selection into the autopsy sample was the outcome and factors associated with autopsy were the predictors.

Initially, all factors potentially associated with autopsy as well as potential confounders of the relationship between neuropathology and clinical progression were included in models. Backward selection was used to select variables for final models. In NACC, overall factors predictive of autopsy included in the model were: baseline age, sex, race, education, early birth year (pre 1928), presence of 1 or more comorbidities, CDR-SB at last visit, atypical dementia diagnosis (not clinical AD or vascular dementia), presence of motor symptoms, and volunteering for genotype assessment. In the PANDA ADC subset, predictors were: baseline age, sex, race, education, presence of 1 or more comorbidities, CDR-SB at last visit, and volunteering for genotype assessment. Predicted probability of selection into the autopsy sample was calculated for each participant from the selection model and inverse probabilities were incorporated into the main analytic models described above as weights.[52] To stabilize the weights with low autopsy selection probability, we truncated weights at the 95th percentile,[114,115] which corresponded to 20.7 for NACC overall and 11.7 for PANDA ADCs. Predictive model fit was assessed via ROC curves; the area under the curve was 0.85 for both NACC and the PANDA ADCs. We used bias-corrected and accelerated (BCa) bootstrap CIs in our analyses in order to ensure that uncertainty attributable to the estimated weights was reflected in our parameter estimates.[116] Analyses were conducted using R (version 3.2.1).[89] All tests were two-sided with $\alpha = 0.05$.

RESULTS

Participant characteristics

Among 2,046 autopsied NACC participants, the mean age at death was 79.2 years (standard deviation [SD]: 9.9), the average interval between last clinical visit and death was 9.4 months (SD: 6.0), and the median follow-up of participants with 2 or more clinical visits (n=1,387) was

3.3 years (IQR: 2.0-5.1). Co-occurrence of ADNC, LBD, and VBI at autopsy was common (Figure 3.2); 70.4% of participants had ADNC, of which 37.6% had co-occurring LBD and 29.7% had co-occurring VBI. Characteristics of participants grouped by ADNC, LBD, and VBI neuropathologies are described in Table 3.2. Prevalence of *APOE* ε4 allele and cognitive impairment was slightly higher in those with ADNC+LBD (with or without VBI) compared to ADNC only. Participants with LBD, in general, were more likely to be male and died at a younger age, on average, compared to those without LBD. They also had a higher prevalence of motor problems and psychosis compared to those without LBD. Participants with VBI, with or without co-occurring pathologies, had a higher prevalence of other indicators of cerebrovascular disease (e.g. clinical history of stroke, severe vessel disease, and white matter disease) and they died at an older age, on average, compared to those without VBI. Those with mixed ADNC had a slightly lower prevalence of Braak stage V-VI (e.g. high ADNC) compared to those with ADNC only. Over 40% of those considered to have low NP were cognitively impaired at the last visit. There were relatively few participants with LBD+VBI and ADNC+LBD+VBI, so these groupings were not examined separately in analytic models.

Clinical progression in primary models

On average, participants with ADNC+LBD had the fastest rate of progression followed by ADNC only and then ADNC +VBI (Table 3.3). Progression for ADNC+LBD was borderline significantly faster than ADNC only (p=0.06), while progression for ADNC+VBI was significantly slower (p=0.003). However, average trajectories of those with ADNC, regardless of co-occurring pathology were relatively similar (Figure 3.3). Interestingly, significant negative interactions were present between ADNC and LBD (β: -0.47 points; 95% confidence interval

[CI]: -0.76, -0.22; p=0.002) and between ADNC and VBI (β: -0.34; 95% CI: -0.53, -0.10; p=0.003), such that participants with ADNC and co-occurring LBD or VBI had a lower rate of progression than would be expected if each pathology independently (additively) contributed to progression. For example, in primary models the rate of progression for ADNC+LBD was estimated as 1.85 points per year, however, if pathologies were additive we would expect the average annual progression for ADNC+LBD to be higher: 2.77 points per year based on the estimates for ADNC only (1.70 points) + LBD only (1.07 points).

In sensitivity analyses, results did not differ substantially in models with further adjustment for comorbidities and *APOE* ε4 allele or in unweighted models that did not use IPW to adjust for potential selection bias (Table 3.4). Among those without dementia at baseline, rates of progression were slower overall but the rate of progression diverged further between those with ADNC only and those with ADNC+LBD (Table 3.4). A negative interaction between ADNC and LBD was not significant (β: -0.14; 95% CI: -0.41, 0.11; p=0.3), but the negative interaction between ADNC and VBI was significant (β: -0.39; 95% CI -0.61, 0.17; p=0.002). Compared to those with high education; participants with low education had faster progression of low neuropathology, VBI only, and LBD only but slower progression of ADNC with or without VBI or LBD (Table 3.5). Trends otherwise remained similar to primary models in analyses stratified by low and high education.

Microinfarcts and clinical progression in PANDA ADCs

Among 293 PANDA ADC participants the mean age at death was 87.5 years (SD: 10.6), the average interval between last clinical visit and death was 7.7 months (SD: 5.2), and the median

follow-up of participants with 2 or more clinical visits (n=247) was 3.8 years (IQR: 2.1-5.2). The majority of participants had no cortical microinfarcts (75.8%, n=222) and no subcortical microinfarcts (58.7%, n=172). The distribution of cortical microinfarcts was as follows: 13.0% (n=38) had 1, 4.8% (n=14) had 2, 2.4% (n=7) had 3, and 3.8% (n=11) had 4 or more. The distribution of subcortical microinfarcts was: 7.2% (n=21) had 1, 7.5% (n=22) had 2, 3.1% (n=9) had 3, and 23.5% (n=69) had 4 or more. The number of subcortical microinfarcts in those without ADNC was associated with a significantly faster rate of progression compared to those with low neuropathology (β: 0.10; 95% CI: 0.02, 0.18; p=0.002) but the number cortical microinfarcts was not associated with faster progression (β: 0.05; 95%CI: -0.09, 0.26; p=0.6). The rate of progression of those with ADNC and subcortical microinfarcts was lower than would be expected if effects were additive (β: -0.22; 95% CI: -0.34, -0.08; p=0.003).

Semi-quantitative measures and clinical progression

We also examined progression according to LBD subtype in those without ADNC; both limbic and cortical LBD were associated with faster progression compared to those with low NP (Table 3.6). Finally, we examined progression by severity of ADNC (none/low, intermediate, and high) and interactions with VBI and cortical LBD (Table 3.7). In this analysis we focused on cortical LBD, since subtype may differ by level of ADNC (Chapter 2) and cortical LBD was most strongly associated with progression (Table 3.6). Cortical LBD as well as intermediate and high ADNC only were associated with faster progression than those with low neuropathology (all, p=0.002). Rates of progression were fastest for those with intermediate ADNC and cortical LBD (p=0.04 compared to high ADNC only) followed by those with high ADNC with or without cortical LBD (p=0.2 for high ADNC+cortical LBD vs. high ADNC only). Progression for those

with intermediate or high ADNC and co-occurring VBI were still slightly slower than for intermediate or high ADNC only (all, p=0.002). Trajectories of those with intermediate ADNC+LBD resulted in a similar level of impairment as those with high ADNC at the last visit (Figure 3.4). We found a suggestive synergistic or positive interaction between intermediate ADNC and cortical LBD, which was not significant (p=0.37), with individual estimates shown in Table 3.8. The interaction between intermediate ADNC and VBI was not significant (p=0.4). Trends in those with high ADNC were similar to primary models: negative interactions were significant (p=0.003 for high ADNC×cortical LBD and p=0.03 for high ADNC×VBI).

DISCUSSION

This study reports on novel findings regarding interactions of ADNC and LBD or VBI in the association with clinical progression. Compared to ADNC only, progression in those with ADNC+LBD was faster while progression in those with ADNC+VBI was slower. Interestingly, in primary models trajectories of those with ADNC pathology were relatively similar regardless of co-occurring pathology; and we found significant negative interactions between both ADNC and LBD and ADNC and VBI. In a subset of those without dementia, however, there was no significant interaction of ADNC and LBD and trajectories diverged more from those with ADNC only. In secondary analyses, we found clinical progression to be fastest among participants with co-occurring cortical LBD and intermediate levels of ADNC. Meanwhile, trajectories of progression were similar among those with high ADNC regardless of co-occurring pathology. Primary findings were similar in those with low or high education level, despite small differences in rates of progression, suggesting that the relationships between mixed pathologies and progression were not modified by cognitive reserve. Together these findings suggest that

considering interactions is important in characterizing progression among individuals with multiple pathologies. Concomitant pathologies, in particular LBD, may have a larger effect on progression in those with intermediate compared to high ADNC.

Our primary findings of negative interactions between ADNC and LBD or VBI were unexpected and counter to the hypothesis that pathologies interact synergistically to affect dementia. Few prior studies have explicitly tested for interactions; however, another study using NACC data also found a significant negative interaction such that the association between cerebrovascular disease and dementia was weaker in those with higher Braak stage.[16] Considering our results in secondary models and that NACC participants tended to have severe ADNC, our findings may suggest that ADNC is the primary determinant of clinical progression in those with more severe ADNC, where it could clinically overwhelm co-occurring neuropathologies. This hypothesis is consistent with prior research that co-occurring vascular lesions were not associated with cognitive decline beyond that of ADNC in individuals with high ADNC.[32,108,109] In primary models where we used dichotomous measures for ADNC results were likely skewed towards participants with dementia and high ADNC, due their high prevalence in NACC. These sample characteristics may have led to a ceiling effect in the rates of progression observable.

On the other hand, our secondary findings also suggest there may be synergistic interactions between intermediate ADNC and cortical LBD on the rate of clinical progression. These data complement prior animal studies finding that ADNC and LBD interact to produce more rapid cognitive decline.[93] Only one prior community-based study tested for interactions between ADNC and LBD in associations with dementia or cognitive test scores,[37] although they found no

significant interaction, this could be because of a smaller sample size or because of different sample characteristics (e.g. lower prevalence of dementia). Additionally, our study differs in that we examined whether interactions depended on level of ADNC. Two smaller studies found cognitive decline to be faster in those with ADNC+LBD compared to ADNC only but did not test for interactions.[104,105] Rates of progression were highest for those with ADNC+LBD through all models, consistent with studies showing that dementia is more likely among those with mixed pathologies.[25,31] Previously, in Chapter 2 we found that cortical LBD was associated with intermediate ADNC in NACC, while limbic or amygdala only LBD was associated with high ADNC. LBD in ADNC may represent one or more subtypes of AD, with a distinct pathogenesis and clinical course compared to those with ADNC only.[104,117] More rapid progression in this current study may be due to differences in other symptoms; for instance, motor and behavioral symptoms are also more common in those with LBD than in ADNC.[66]

We did not find evidence for synergistic interactions between ADNC and VBI; in fact those with ADNC+VBI had slower rates of progression than those with ADNC only. This observation may be because participants with ADNC+VBI were less likely to have high ADNC, which could result in residual confounding even after accounting for differences using semi-quantitative measures. Alternatively, participants with ADNC+VBI may have a slower disease course. They were, on average, older at death than those with ADNC only, and in NACC, older age is inversely associated with symptoms among those with ADNC.[118] Several prior studies found no interactions between ADNC and VBI using pathologic[31,107] or imaging data.[33] Additionally, including participants with just one VBI may have diluted the association between VBI and clinical progression. Information on number of infarcts was not available for most participants in

NACC. Our findings in PANDA ADC participants highlight that clinical impairment due to VBI may be detectable only in those with multiple VBI. Interestingly, in this study only subcortical microinfarcts were associated with progression.

This study has several important limitations. Neuropathologic assessments are conducted at autopsy and may not reflect burden of pathology when clinical progression was measured. Neuropathologic assessments could differ between ADCs and over time; we did not have information on TDP-43[99] or Thal phase,[81] so some pathologies may have been misclassified. Statistical interactions and effects may differ from biological interactions,[119] and biological interactions between pathologies may not be captured in clinical-pathologic studies that are based on regression models correlating burden after death to cognition during life. The CDR-SB is based on overall clinical assessment and measures of specific symptoms may be more sensitive to detecting differences between each type of pathology. NACC participants are predominantly Caucasian and well-educated older adults; future studies in diverse populations may better assess the impact of cognitive reserve on mixed neuropathologies.

This study also has important strengths. We used a large data set of standardized clinical evaluations that could provide power to detect statistical interactions. We modeled progression allowing for differences between participants with mixed pathologies and those with individual pathologies. We investigated associations in a subset of participants with the same neuropathologic assessment protocols and explored relationships between clinical progression and semi-quantitative measures of pathologic burden. Finally, we adjusted for potential confounders and attempted to account for potential selection bias due to autopsy sampling.

Overall we found evidence that ADNC+LBD is associated with faster rate of decline than ADNC only, but that this effect is most evident in those with moderate levels of ADNC. Trajectories of those with ADNC+VBI were slower than those with ADNC only, although cumulative VBI may be associated with faster clinical progression compared to those with low neuropathology. Future research with prospective biomarkers is needed to elucidate further the temporal effects of these pathologies in cases with mixed pathologies. Our results demonstrate that mixed neuropathologies are relevant to clinical disease progression but that relationships are complex and may depend upon the stage of ADNC. Modeling individual pathologies as independent factors may overestimate differences in the rate of progression between those with single and mixed ADNC, particularly those with high ADNC. These results suggest differentiating individuals with mixed vs. single ADNC may be challenging in practice.

TABLES AND FIGURES

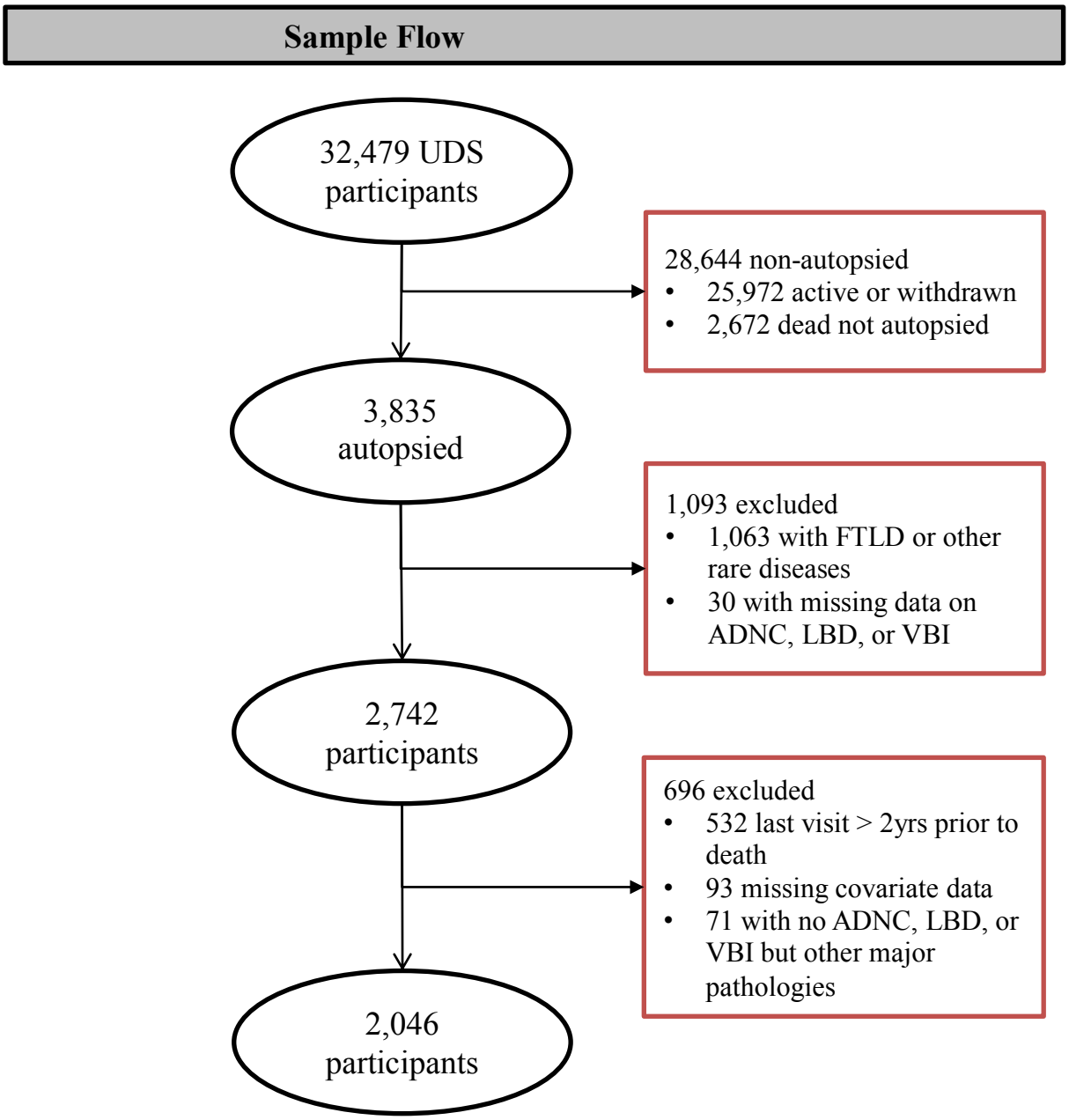

Figure 3.1 Study sample flow chart. ADNC; Alzheimer's disease neuropathologic change; FTLD, frontotemporal lobar degeneration; LBD, Lewy body disease, NACC, National Alzheimer's Coordinating Center; UDS, Uniform Data Set; VBI, vascular brain injury.

Table 3.1 Comparison of autopsied participants to non-autopsied

Characteristics*	Alive/Unknown	Dead, non-autopsied	Autopsied
Total, N	25,972	2,672	3,835
Age at baseline, mean (SD)	71.2 (10.1)	77.5 (9.6)	76.4 (11.4)
Age at last visit, mean (SD)	73.8 (10.6)	79.5 (9.9)	78.7 (11.8)
Birth year <1928 (25th percentile)	5,728 (22.1)	1,394 (52.2)	1,916 (50.0)
Female	15,462 (59.5)	1,275 (47.7)	1,744 (45.5)
Non-white race	5,310 (20.8)	509 (19.4)	236 (6.2)
College graduate	13,768 (53.0)	1,139 (42.6)	2,106 (54.9)
History of heart disease	5,442 (21.1)	973 (36.8)	1,288 (33.7)
History of hypertension, hypercholesterolemia or both	19,764 (76.5)	2,104 (79.0)	2,788 (73.2)
History of stroke	1,596 (6.2)	340 (12.8)	427 (11.2)
Depression	10,972 (42.6)	1,257 (47.5)	2,008 (53.1)
APOE ε4 allele	7,614 (39.9)	718 (41.1)	1,440 (44.2)
Demented at last visit	9,503 (36.6)	1,853 (69.3)	3,057 (79.7)
Rare/atypical dementia type	4,121 (15.9)	605 (22.6)	1,066 (27.8)
Motor problems	5,803 (22.4)	1,313 (49.7)	2,258 (59.7)
Genetics data available	3,967 (15.3)	463 (17.3)	1,252 (32.6)

*N,(%) unless otherwise specified. Participants missing data: race=535 (1.6%), education=230 (<1%), heart disease=224 (<1%), hypertension=176 (<1%), stroke=141 (<1%), depression=318 (1%), motor problems=170 (<1%)

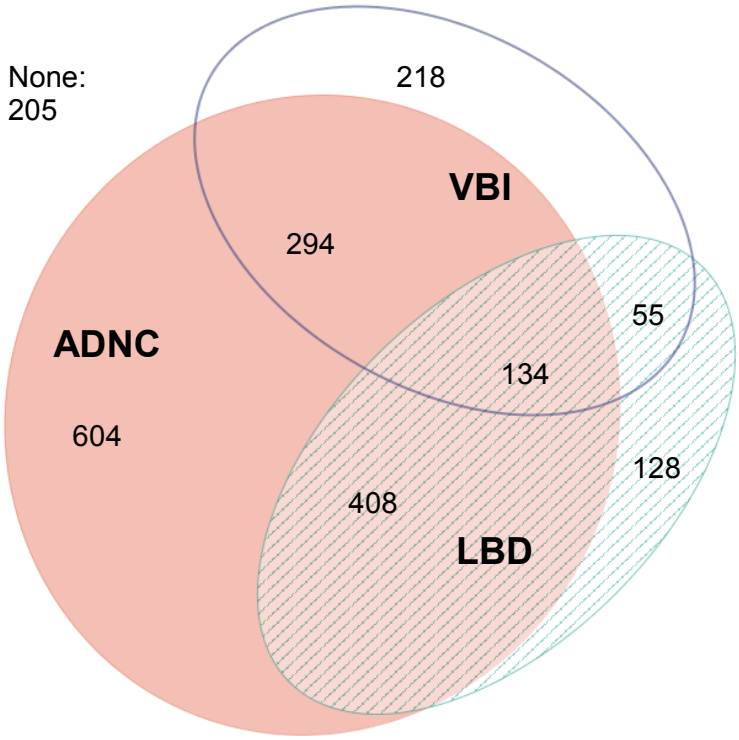

Figure 3.2 Co-occurrence of Alzheimer's disease neuropathologic change (ADNC), Lewy body disease (LBD), and vascular brain injury (VBI). ADNC = moderate/frequent neuritic plaques & Braak stage III-VI; LBD = Lewy bodies in any brain region examined; VBI = gross infarcts and cortical microinfarcts. We used eulerAPE[88] to create Venn/Euler diagrams that accurately illustrate the overlap of each pathology.

Table 3.2 Study participant characteristics stratified by ADNC, LBD, and VBI neuropathologic groupings*

Characteristics†	Low NP	VBI only	LBD only	VBI+LBD	ADNC only	ADNC+ LBD	ADNC+ VBI	ADNC+ LBD+VBI
Total autopsies, N	205	218	128	55	604	408	294	134
Clinical								
Age at death, mean (SD)	86.4 (10.6)	88.2 (9.2)	78.6 (9.8)	84.8 (8.9)	80.3 (10.6)	77.8 (9.4)	84.8 (8.6)	82.9 (8.6)
Female	112 (54.6)	130 (59.6)	32 (25)	20 (36.4)	281 (46.5)	157 (38.5)	137 (46.6)	54 (40.3)
Non-white	13 (6.3)	12 (5.5)	4 (3.1)	4 (7.3)	27 (4.5)	20 (4.9)	26 (8.8)	17 (12.7)
College graduate	114 (55.6)	109 (50)	81 (63.3)	30 (54.5)	349 (57.8)	233 (57.1)	153 (52.0)	71 (53.0)
History of stroke	17 (8.3)	75 (34.4)	3 (2.3)	9 (16.7)	41 (6.9)	19 (4.7)	81 (27.9)	19 (14.3)
APOE ε4 allele	34 (18.1)	40 (20.4)	32 (33)	16 (34)	284 (54.2)	216 (62.6)	136 (54.8)	73 (62.4)
Cognitively impaired	93 (45.4)	147 (67.4)	108 (84.4)	46 (83.6)	583 (96.5)	403 (98.8)	279 (94.9)	132 (98.5)
Motor problems	52 (25.4)	91 (41.7)	91 (71.1)	35 (63.6)	364 (60.3)	305 (74.8)	180 (61.2)	100 (74.6)
Psychosis	11 (5.4)	20 (9.2)	50 (39.1)	14 (25.9)	143 (23.9)	165 (40.6)	71 (24.2)	50 (37.6)
Pathological								
Braak stage V-VI	NA	13 (5.9)	7 (5.5)	1 (1.8)	493 (81.6)	308 (75.5)	208 (70.7)	98 (73.1)
Cortical LBD	NA	NA	59 (46.1)	23 (41.8)	NA	184 (45.1)	NA	45 (33.6)
Severe atherosclerosis	17 (8.4)	63 (29)	11 (8.6)	8 (14.5)	68 (11.4)	36 (8.9)	46 (15.8)	22 (16.4)
Severe arteriolosclerosis	3 (1.7)	54 (25.8)	5 (4.7)	13 (24.5)	43 (8.8)	31 (9.2)	54 (20.8)	37 (30.6)
Severe CAA	8 (4.0)	7 (3.3)	4 (3.2)	5 (9.1)	101 (17.1)	52 (13.0)	54 (19.1)	25 (20)
White matter disease	NA	37 (17.1)	10 (7.9)	10 (18.2)	72 (12.0)	48 (12.0)	48 (16.6)	19 (14.6)
Hippocampal sclerosis	NA	17 (7.9)	8 (6.3)	3 (5.5)	47 (7.9)	29 (7.1)	26 (8.9)	21 (16.2)

ADNC, Alzheimer's disease neuropathologic change; CAA, cerebral amyloid angiopathy; LBD, Lewy body disease; NA, not applicable; NP, neuropathology; VBI, vascular brain injury

*ADNC = moderate/frequent neuritic plaques & Braak stage III-VI; LBD = Lewy bodies in any brain region examined; low NP = no ADNC, no VBI, no LBD, and no other major pathologies; VBI = gross infarcts and cortical microinfarcts.

†N,% unless otherwise specified. Participants missing data: stroke=15 (<1%), APOE genotype=284 (13.9%), psychosis=12 (<1%), atherosclerosis=15 (<1%), arteriolosclerosis=296 (14.5%), CAA=53 (2.6%), white matter disease=31 (1.5%), hippocampal sclerosis=309 (16.4%).

Table 3.3 Estimated annual change in CDR-SB for single and mixed neuropathologies

Neuropathologies*	Annual change in CDR-SB (95%CI)†
Low neuropathology	0.45 (0.35, 0.57)
VBI only	0.54 (0.42, 0.65)
LBD only	1.07 (0.90, 1.30)
ADNC only	1.70 (1.60, 1.81)
ADNC+VBI	1.45 (1.32, 1.60)
ADNC+LBD	1.85 (1.71, 2.00)

ADNC, Alzheimer's disease neuropathologic change; CDR-SB, Clinical Dementia Rating Sum of Boxes; LBD, Lewy body disease; VBI, vascular brain injury

*Based on models with adjustment for age at baseline, sex, non-white race, years of education, and last visit-death interval and weighted by inverse probability of autopsy. Note: A positive value corresponds to increasing impairment over time.

†ADNC = moderate/frequent neuritic plaques & Braak stage III-VI; LBD = Lewy bodies in any brain region examined; low NP = no ADNC, no VBI, no LBD, and no other major pathologies; VBI = gross infarcts and cortical microinfarcts.

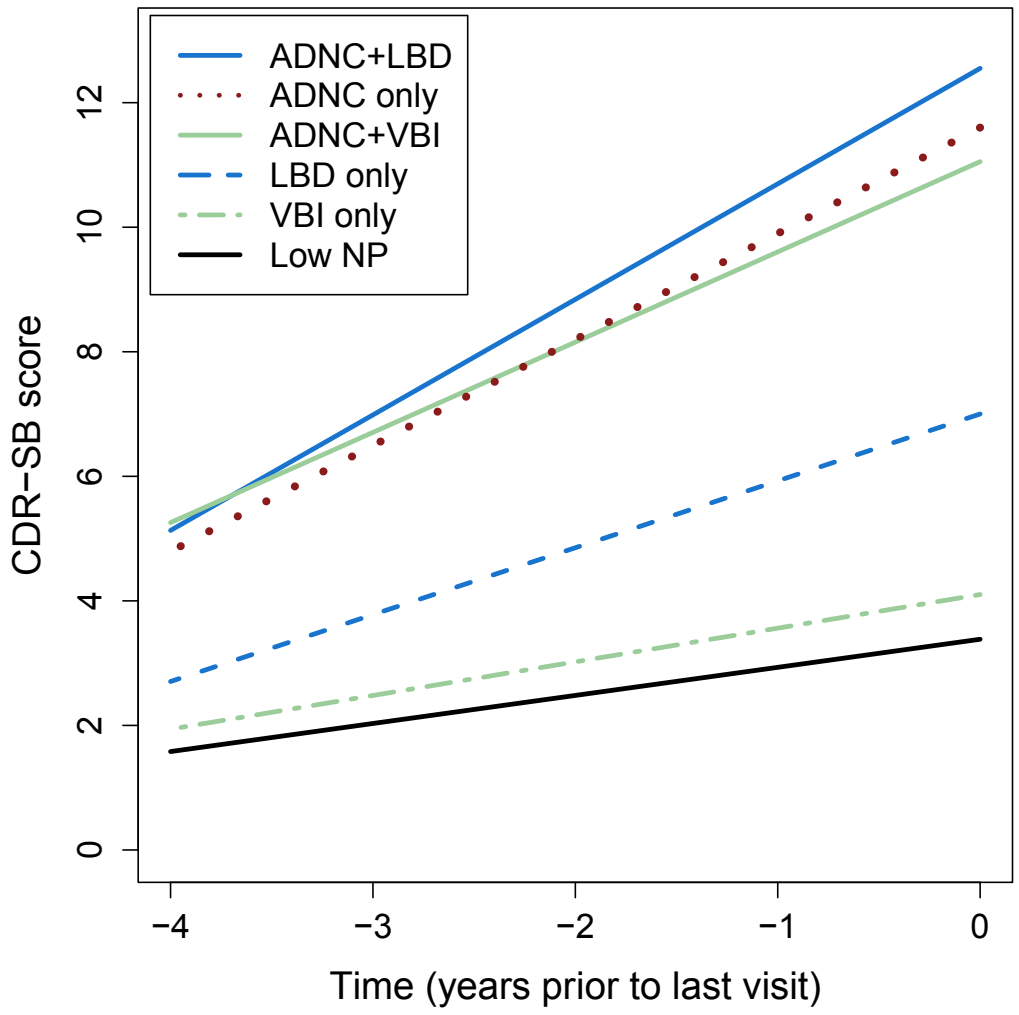

Figure 3.3 Model based population mean trajectories of clinical impairment (CDR-SB) prior to death. ADNC, Alzheimer's disease neuropathologic change = moderate/frequent neuritic plaques & Braak III-VI; CDR-SB, Clinical Dementia Rating Sum of Boxes; LBD, Lewy body disease = Lewy bodies in any brain region examined; low NP, low neuropathology = no ADNC, no VBI, no LBD, and no other major pathologies; VBI, vascular brain injury = any gross infarcts or cortical microscopic infarcts. Progression was faster for those with LDB only (p=0.002) and ADNC only (p=0.002) but not VBI only (p=0.2) compared to those with low NP. Compared to ADNC only progression for ADNC+LBD was borderline faster (p=0.06) while progression was slower for ADNC+VBI (p=0.003). Significant negative interactions were present between ADNC and LBD (p=0.002) and between ADNC and VBI (p=0.003), such that participants with ADNC and co-occurring LBD or VBI had a lower rate of progression than would be expected if each pathology independently contributed to progression.

Table 3.4 Estimated annual change in CDR-SB for single and mixed neuropathologies in sensitivity analyses: unweighted models, additional covariate adjustment, and non-demented subsample

Neuropathologies*	Unweighted Model (n=2,046)†	Additional covariates (n=1,762) ‡	Non-demented (n=940)§
		Annual change in CDR-SB (95%CI)	
Low neuropathology	0.53 (0.42, 0.67)	0.48 (0.05, 0.90)	0.25 (0.19, 0.32)
VBI only	0.61 (0.47, 0.74)	0.63 (0.20, 1.03)	0.34 (0.25, 0.43)
LBD only	1.28 (1.04, 1.53)	0.94 (0.50, 1.41)	0.58 (0.43, 0.78)
ADNC only	1.78 (1.67, 1.88)	1.72 (1.30, 2.09)	1.30 (1.16, 1.45)
ADNC+VBI	1.53 (1.39, 1.68)	1.48 (1.01, 1.87)	1.00 (0.84, 1.19)
ADNC+LBD	1.96 (1.82, 2.11)	1.89 (1.46, 2.29)	1.49 (1.28, 1.70)

ADNC, Alzheimer's disease neuropathologic change; CDR-SB, Clinical Dementia Rating Sum of Boxes; LBD, Lewy body disease; VBI, vascular brain injury

*ADNC = moderate/frequent neuritic plaques & Braak stage III-VI; LBD = Lewy bodies in any brain region examined; low NP = no ADNC, no VBI, no LBD, and no other major pathologies; VBI = gross infarcts and cortical microinfarcts.

†Based on model that did not weight for autopsy selection but include adjustment for age at baseline, sex, non-white race, years of education, and interval between last visit and death.

‡Based on model with adjustment for age at baseline, sex, non-white race, years of education, and interval between last visit and death, comorbidities, and *APOE* ε4 allele and weighted by inverse probability of autopsy selection.

§Based on model with adjustment for age at baseline, sex, non-white race, years of education, and interval between last visit and death and weighted by inverse probability of autopsy selection among participants with CDR global score 0 or 0.5.

Table 3.5 Estimated annual change in CDR-SB for single and mixed neuropathologies stratified by low and high education

Neuropathologies*	High school education or less (n=540)	Some college or more (n=1,506)
	Annual change in CDR-SB (95%CI)	
Low neuropathology	0.71 (0.46, 1.05)	0.37 (0.27, 0.49)
VBI only	0.65 (0.42, 0.96)	0.50 (0.39, 0.62)
LBD only	1.18 (0.79, 1.65)	1.04 (0.84, 1.28)
ADNC only	1.42 (1.20, 1.63)	1.78 (1.65, 1.90)
ADNC+VBI	1.33 (1.08, 1.61)	1.50 (1.33, 1.67)
ADNC+LBD	1.52 (1.24, 1.82)	1.94 (1.80, 2.11)

ADNC, Alzheimer's disease neuropathologic change; CDR-SB, Clinical Dementia Rating Sum of Boxes; LBD, Lewy body disease; VBI, vascular brain injury

*ADNC = moderate/frequent neuritic plaques & Braak stage III-VI; LBD = Lewy bodies in any brain region examined; low NP = no ADNC, no VBI, no LBD, and no other major pathologies; VBI = gross infarcts and cortical microinfarcts.

†Based on models with adjustment for age at baseline, sex, non-white race, years of education, and interval between last visit and death and weighted by inverse probability of autopsy selection. Note: A positive value corresponds to increasing impairment over time.

Table 3.6 Estimated annual change in CDR-SB for LBD subtypes in those with no/low ADNC (n=572)

Neuropathologies	Annual change in CDR-SB (95% CI)*	P-value†
Low neuropathology	0.42 (0.32, 0.54)	---
Brainstem LBD	0.57 (0.32, 0.89)	0.29
Limbic LBD	0.78 (0.54, 1.06)	0.002
Cortical LBD	1.35 (1.10, 1.68)	0.002
Other/unknown LBD	1.14 (0.52, 2.94)	0.05

ADNC, Alzheimer's disease neuropathologic change; CDR-SB, Clinical Dementia Rating Sum of Boxes; LBD, Lewy body disease

*Based on models with adjustment for age at baseline, sex, non-white race, years of education, and interval between last visit and death as well as inverse probability of autopsy selection weights. Participants with ADNC (moderate/frequent neuritic plaques & Braak stage III-VI) were excluded.

†P-value for comparison of annual change in LBD subtype to annual change in those with low neuropathology (no ADNC, no VBI, no LBD, and no other major pathologies).

Table 3.7 Estimated annual change in CDR-SB according to level of ADNC and co-occurring cortical LBD or VBI

Neuropathologies*	Annual change in CDR-SB (95%CI)†
cLBD	1.44 (1.16, 1.75)
Intermediate ADNC	1.10 (0.91, 1.31)
Intermediate ADNC + cLBD	2.27 (1.83, 2.64)
Intermediate ADNC + VBI	1.01 (0.77, 1.25)
High ADNC	1.82 (1.72, 1.93)
High ADNC + cLBD	2.02 (1.75, 2.29)
High ADNC + VBI	1.62 (1.48, 1.76)

ADNC, Alzheimer's disease neuropathologic change; CDR-SB, Clinical Dementia Rating Sum of Boxes; cLBD, cortical Lewy body disease; VBI, vascular brain injury

*Intermediate ADNC = moderate/frequent neuritic plaques & Braak III-IV; high ADNC= moderate or frequent plaques & Braak V-VI; VBI = any gross infarcts or cortical microscopic infarcts.

†Model included adjustment for age at baseline, sex, non-white race, years of education, and interval between last visit and death as well as inverse probability of autopsy selection weights. Note: A positive value corresponds to increasing impairment over time.

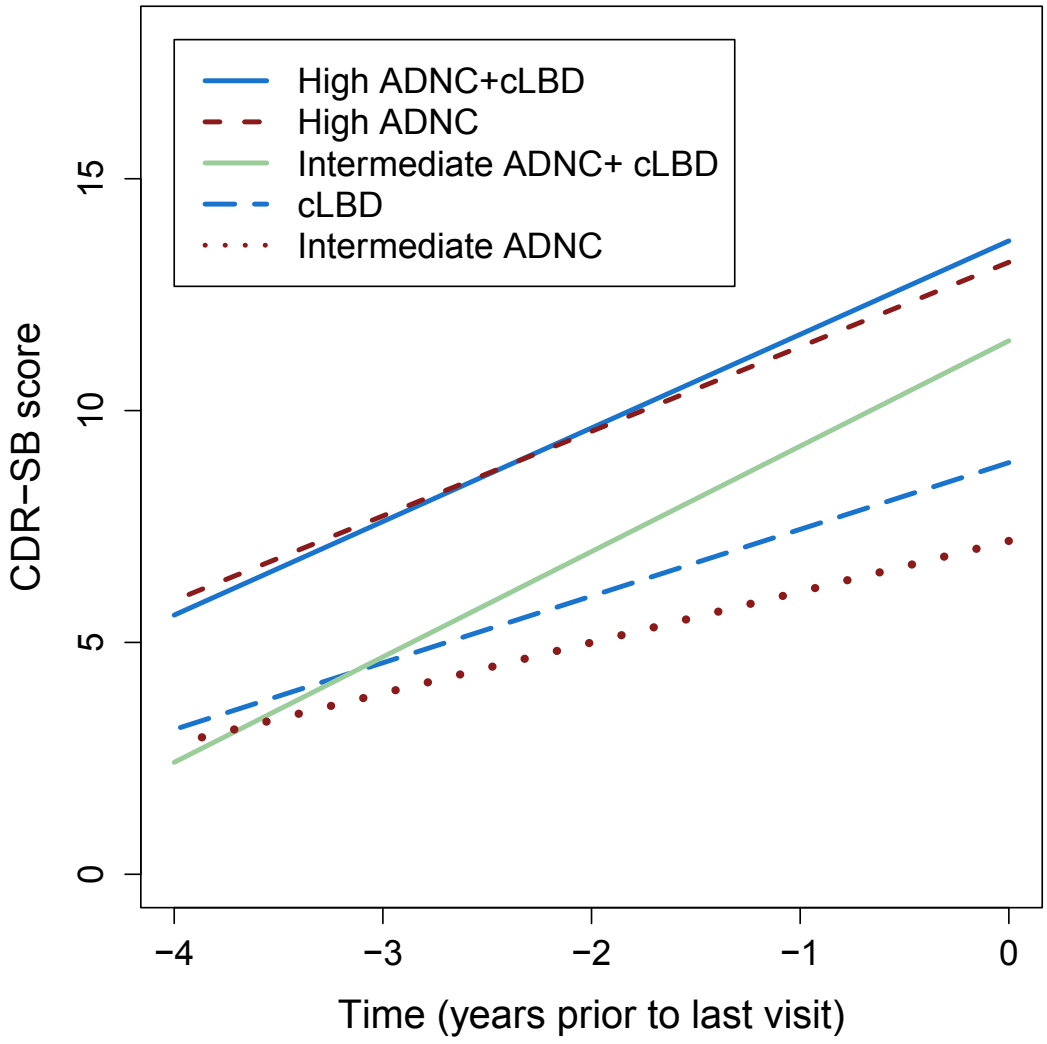

Figure 3.4 Model based population mean trajectories of clinical impairment (CDR-SB) prior to death associated with intermediate and high ADNC with and without co-occurring cortical LBD. ADNC, Alzheimer's disease neuropathologic change; CDR-SB, Clinical Dementia Rating Sum of Boxes; cLBD, cortical Lewy body disease. Intermediate ADNC = moderate/frequent neuritic plaques & Braak III-IV; high ADNC= moderate or frequent plaques & Braak V-VI. Trajectories for ADNC+VBI were similar to ADNC only and are not shown. Compared to high ADNC, progression in intermediate ADNC+cLBD was significantly faster (p=0.04), but progression in high ADNC+cLBD was not significantly different (p=0.2). Significant negative interactions were present between high ADNC and cLBD (p=0.003) such that participants with ADNC and co-occurring LBD or VBI had a lower rate of progression than would be expected if each pathology independently contributed to progression.

Table 3.8 Interactions between intermediate and high ADNC and cortical LBD or VBI

Neuropathologies*	Difference in annual change in CDR-SB (95% CI)†	P-value
Intermediate ADNC×cLBD	0.26 (-0.30, 0.79)	0.37
High ADNC×cLBD	-0.72 (-1.13, -0.28)	0.003
Intermediate ADNC ×VBI	-0.14 (-0.47, 0.18)	0.42
High ADNC×VBI	-0.26 (-0.52, -0.04)	0.03

ADNC, Alzheimer's disease neuropathologic change; CDR-SB, Clinical Dementia Rating Sum of Boxes; cLBD, cortical Lewy body disease; VBI, vascular brain injury

*Intermediate ADNC = moderate/frequent neuritic plaques & Braak III-IV; high ADNC= moderate or frequent plaques & Braak V-VI; VBI = any gross infarcts or cortical microscopic infarcts.

†Number of points difference in annual change of CDR-SB than the combined (summed or additive) estimate for each individual pathology. A negative β = slower annual change than an additive model, a positive β = faster annual change than an additive model

Chapter 4. Mixed neuropathologies and associations with domain specific cognitive decline

ABSTRACT

Introduction: Few studies have tested if decline in specific cognitive domains associated with Alzheimer's disease neuropathologic change (ADNC) is modified by co-occurrence of other pathologies, such as Lewy body disease (LBD) or vascular brain injury (VBI).

Methods: Data came from 1,622 autopsied participants who were evaluated at U.S. Alzheimer's Disease Centers. Standardized Z scores in memory, attention, language, and executive function were derived from neuropsychological test scores assessed at each annual visit. Linear mixed effects models assessed associations between neuropathologies and each domain score with adjustment for confounders and inverse probability weights to account for potential bias in autopsy selection.

Results: Decline in attention (expressed as annual change in standard deviations from average baseline scores of all participants) was significantly faster (p=0.002) for those with ADNC+LBD (β: -0.21; 95% CI: -0.24, -0.18) compared to ADNC only (β: -0.14; 95% CI: -0.16, -0.12). Those with ADNC+LBD generally had worse impairment across follow-up in other domains, especially executive function. Interactions were significant between LBD and ADNC for memory (p=0.04) and VBI and ADNC for language (p=0.02); decline was slower than expected if these neuropathologies acted additively on the rate of decline. In a subset of participants, cortical and subcortical microinfarcts were associated (sometimes borderline) with memory, language, and executive function decline.

Conclusions: ADNC, LBD, and microinfarcts were associated with decline in multiple cognitive domains. ADNC+LBD tended to have worse trajectories across follow-up; this multi-domain effect may increase likelihood of dementia compared to those with ADNC only.

INTRODUCTION

Co-occurrence of Alzheimer's disease neuropathologic change (ADNC), Lewy body disease (LBD), and vascular brain injury (VBI) is prevalent in autopsied older adults.[9,25,58] These common neuropathologies are associated with pre-mortem decline in specific cognitive domains.[21,23] Few studies have compared cognitive decline associated with pathologies between those with multiple co-occurring (aka mixed pathologies) and those with single pathologies.

In prior studies, ADNC, in particular neurofibrillary tangles (measured by Braak stage), has been associated with lower pre-mortem memory, executive function, and language scores.[19,23] Cortical LBD has been associated with increased impairment in multiple domains including memory, perceptual speed, visual spatial abilities, language, and cognitive fluctuations. VBI, such as cortical microinfarcts, has been associated with worse memory, perceptual speed, and visuospatial abilities, while subcortical microvascular brain injury has been associated with executive function and language.[19] In other studies, VBI were not associated with faster decline in specific domains.[21] The few studies that have reported testing interactions between ADNC and LBD or ADNC and VBI have not found significant interactions in their associations with decline in specific domains.[33,37] Prior studies may have been too small to detect significant interactions. In a large multi-center sample, we previously found that LBD and VBI interacted with ADNC in associations with overall clinical progression (Chapter 3).

In this current study, we used data from the National Alzheimer's Coordinating Center (NACC) on participants previously enrolled at U.S. Alzheimer's Disease Centers (ADCs). We evaluated whether autopsied older adults with ADNC and co-occurring LBD or VBI had faster progression of impairment in 4 specific cognitive domains: memory, attention, language, and executive function (a set of cognitive processes necessary for behavior control) compared to those with single or low neuropathologies. We also tested whether the relationships between cognition and LBD or VBI was modified by co-occurrence of ADNC.

METHODS

Data sources and study populations

Data came from NACC's Uniform Data Set (UDS) on participants who had been prospectively evaluated and autopsied at one of 34 past and present ADCs since September 2005. Participants enrolled with any level of cognition and were examined annually in-person using a standard protocol, described in detail elsewhere.[71,72] Neuropathologic data was collected following a standardized protocol also on participants who had died and consented to autopsy. Participants provided written informed consent and each ADC received institutional review board approval.

As of September 2015, the UDS had 32,479 participants with at least one clinical visit; 6,507 were known to have died of whom 3,835 had been autopsied. 1,063 participants were excluded with Down's syndrome, prion disease, autosomal dominant genetic diseases, frontotemporal lobar degeneration, or other rare causes of dementia because these conditions may conflict with neuropathologic assessment of ADNC, confound clinical symptoms, or both. Also excluded were

participants missing information on age, sex, race/ethnicity, or education (n=93), or neuropathologic information on ADNC, LBD or VBI (n=30). Participants with their last visit more than 2 years prior to death were also excluded (n=532). We also excluded 71 participants who did not have ADNC, LBD, or VBI but had other major pathologies such as hippocampal sclerosis, Braak stage V-VI with sparse or no neuritic plaques, frequent neuritic plaques but Braak stage 0-II, other major pathologies, white matter disease, or some combination of these. Because participants in advanced stages of dementia may be unable to complete neuropsychological tests, we excluded 318 participants with severe dementia at baseline, defined as those with Clinical Dementia Rating Sum of Boxes scores (CDR-SB) 16-18.[110,120] An additional 106 participants were excluded due to missing all neuropsychological test scores at all visits. Given these exclusions, 1,622 participants remained for analyses; participants with only one visit (n=1,387) contributed to model estimates at baseline but did not contribute to estimates of longitudinal change. A study sample flow chart is shown in Figure 4.1.

To supplement NACC data, we abstracted additional information on number of vascular pathologies for participants seen at the Oregon Health & Science University (OHSU) (n=201) and University of Washington (UW) (n=71) ADCs. These two centers have a joint agreement to follow the same neuropathologic assessment protocol as part of the Pacific Northwest Dementia and Aging Neuropathology Group (PANDA). Both ADCs recruit patients seen in clinic for diagnoses, treatment, or clinical trials for enrollment; however, participants seen at OHSU were also recruited from a number of cohort studies focusing on healthy aging in independent older adults which are described elsewhere, such as the Oregon Brain Aging Study.[74] In this study, OHSU and UW ADCs will be collectively referred to as PANDA ADCs hereafter.

Neuropathological features

ADCs follow consensus guidelines but conduct neuropathologic assessments according to their own protocols. Neuritic plaque density was defined by Consortium to Establish a Registry for Alzheimer's Disease (CERAD) scores of none, sparse, moderate, or frequent.[79] Tau neurofibrillary pathology was measured with Braak stage (none, I-II, III-IV, V-VI).[80] ADNC was categorized semi-quantitatively (no/low, intermediate, high) regardless of a participant's cognitive status. No/low ADNC was defined as no/sparse neuritic plaques & any Braak stage OR any neuritic plaques & Braak stage 0-II. Intermediate ADNC was defined as moderate/frequent CERAD plaques & Braak stage III-IV. High ADNC was defined as moderate/frequent plaques & Braak stage V-VI. This classification overlaps with intermediate to high ADNC as defined by the 2012 NIA-Alzheimer's Association criteria;[60] however, Thal phasing[81] for amyloid plaques was not available for most participants. Lewy bodies assessment and classification of LBD subtype followed recognized guidelines.[83] LBD was classified by subtype as brainstem predominant, limbic (transitional), cortical (diffuse), or region not specified/other. We further categorized LBD as present in any brain region examined or absent. Presence of any VBI was defined as any gross infarcts or cortical microinfarcts. In NACC, presence of cortical microinfarcts (infarcts in the cortex only seen microscopically), gross infarcts (small or large artery), or both were recorded regardless of age. In the PANDA ADCs, microinfarct assessment followed methods developed in the Honolulu Asia Aging Study.[82] The number of cortical and subcortical microinfarcts was recorded separately as 0, 1 ,2, 3, or 4 or more. Cerebral amyloid angiopathy, atherosclerosis, and arteriolosclerosis were classified as none, mild, moderate, or severe. Participants with subcortical leukoencephalopathy and/or white matter rarefaction were considered to have white matter disease. Hippocampal sclerosis was categorized as present or absent. Due to differences in

NACC forms, hippocampal sclerosis was considered present if a primary or contributing neuropathologic diagnosis was listed for participants autopsied prior to 2014, or if hippocampal sclerosis was recorded as unilateral, bilateral, or laterality unknown for participants autopsied in 2014 or after. Participants were defined as having a low level of neuropathology (low NP) if they were without ADNC, LBD, VBI, or other major pathologic burden.

Cognitive function in specific domains

The UDS neuropsychological test battery comprises 8 tests (12 measures) and is administered at each in-person visit.[121] Cognitive function was quantified in 4 specific domains: memory (episodic memory), attention (and working memory), language, and executive function.[122] Domains were previously identified through factor analysis of neuropsychological tests in the NACC database.[122] These domains were stable longitudinally as well as across groups defined by cognitive status. Scores for each cognitive domain are a composite of individual tests.

The episodic memory tests included the Logical Memory Story A immediate and delayed recall.[123] The attention domain included Digit Span Forwards and Backward tests, which assess attention and working memory.[123] Language tests included animals and vegetables list generation,[124] which test verbal fluency, and the Boston Naming Test, which assesses naming ability.[125] The executive function domain was measured by the Digit Symbol[126] and Trail Making test Part A and Trail Making test Part B,[127] which evaluate processing speed and executive function. To create domain specific scores, each test was converted into z scores and averaged. Z scores were calculated by subtracting individual raw scores from the mean and dividing by the standard deviation of scores at the initial visit (for all autopsied and non-

autopsied participants). Scores for each domain can be interpreted as the units of SDs from the baseline average score. If one or more test was missing the corresponding domain was considered missing for that participant's visit.

Covariates

Demographic characteristics included in analyses were age, sex, education, and race/ethnicity, as well as ADC. History of comorbidities, such as depression, heart disease, and stroke were evaluated during each clinical visit. *APOE* genotyping was performed on consenting participants. *APOE* ε4 allele status was classified as at least one or none. At all ADCs, either a single clinician or consensus group of clinicians made a diagnosis of normal cognition, impaired but not mild cognitive impairment (MCI), MCI, or dementia at each visit.

Statistical Analyses

Modeling clinical progression

To model longitudinal trends in cognitive domains and test associations with neuropathologies, we used multivariable regression modeling via linear mixed effects models. The primary outcome was the z score for each cognitive domain at each visit, modeled as a continuous measure. Specific primary models were run for each of 4 domains: memory, attention, language, and executive function. Following the approach of other studies,[21] we modeled longitudinal trends in cognition over time such that visits work backwards from last visit to the initial clinical visit. Empirical plots suggested change in domain scores over time was approximately linear. Models included random intercepts for both participants and ADC to account for correlation of domain scores within the same participant as well as within the same ADC. In addition, random

slopes were included for participants to allow for heterogeneity in rate of change in domain scores over time between participants.

Primary predictors were dichotomous variables for ADNC, LBD, VBI, and time. We added interaction terms between time and each primary pathology term and 3-way interactions of ADNC×LBD×time and ADNC×VBI×time to investigate differences in mean annual rate of change in each domain score by pathology groupings. The regression parameter estimate for time described the annual mean rate of change in domain scores, namely the slope, in those with low NP. The interaction between ADNC×time, for instance, described the difference in annual rate of change in those with ADNC without co-occurring LBD or VBI and those with low NP. The model estimate for 3-way interaction between ADNC×LBD×time, for instance, described the difference in annual rate of change of domain scores associated with having ADNC and LBD (ADNC+LBD) beyond the contribution of ADNC only and LBD only. If each pathology contributed independently to a cognitive domain, annual rate of change in scores for a participant with ADNC+LBD would equal the summed estimates of ADNC×time + LBD×time, namely additive effects. Primary models also included adjustment for potential confounders: age at death, sex, race/ethnicity, education, and interval between last visit and death. In secondary analyses we examined whether intermediate or high ADNC, assessed separately, interacted with VBI or LBD (focusing on cortical LBD since other subtypes are not as strongly associated with cognition[37]). In PANDA participants, we examined associations with number of cortical and subcortical microinfarcts, separately. Finally, because participants may have missing data due to severe impairment, we conducted sensitive analyses to evaluate the impact of missing data on our primary findings. We re-ran primary analyses with missing test scores imputed with the

lowest (worst) possible score when the participant had severe dementia (CDR-SB= 16-18) or if a cognitive or behavior problem was listed as the reason for missing data.

Inverse Probability Weighting

An important consideration in this study is that participants who have died and consented to autopsy may differ in characteristics from the overall study population. In some cases this selection may lead to biased results. In an attempt to account for potential selection bias, we used inverse probability weighting (IPW)[114] to adjust for differences between the overall study sample and the autopsy sample.[54] Weights were created by first modeling autopsy selection using logistic regression. Autopsy status (yes, no) was the outcome and factors associated with autopsy were the predictors. Initially, all factors potentially associated with autopsy as well as potential confounders of the relationship between neuropathology and cognition were included in models. Backward selection was used to select variables for final models. In NACC, overall factors predictive of autopsy included in the model were: baseline age, sex, race, education, early birth year (pre 1928), presence of 1 or more comorbidities, CDR-SB at last visit, atypical dementia diagnosis (not clinical AD or vascular dementia), presence of motor symptoms, and volunteering for genotype assessment. In PANDA ADCs, predictors were: baseline age, sex, race, education, presence of 1 or more comorbidities, CDR-SB at last visit, and volunteering for genotype assessment. Characteristics of autopsied and non-autopsied participants are shown in Table 4.1. Predicted probability of selection into the autopsy sample was calculated for each participant from the selection model and inverse probabilities were incorporated into the main analytic models described above as weights.[52] To stabilize the weights with low autopsy selection probability, we truncated weights at the 95th percentile,[114,115] which corresponded to 20.7 for

NACC overall and 11.7 for PANDA ADCs. Predictive model fit was assessed via ROC curves (area under the curve was 0.85 for both NACC and PANDA ADCs). We used bias-corrected and accelerated (BCa) bootstrap confidence intervals (CIs) in analyses in order to ensure that uncertainty attributable to the estimated weights was reflected in our parameter estimates.[116] Analyses were conducted using R (version 3.2.1).[89] All tests were two-sided with $\alpha = 0.05$.

RESULTS

Participant characteristics

Among 1,622 autopsied UDS participants, the mean age at death was 79.2 years (standard deviation [SD]: 9.9), the average last clinical visit - death interval was 9.4 months (SD: 6.0), and the median follow-up of participants with 2 or more clinical visits (n=1,387) was 3.3 years (IQR: 2.0-5.1). The number of participant-visits that contributed to analyses for each cognitive domain score are shown from last visit backwards (Table 4.2). ADNC, LBD, and VBI and their co-occurrence were prevalent findings at autopsy (Figure 4.2); 65% of participants had ADNC, of whom 36.7% had co-occurring LBD and 29.6% had co-occurring VBI.

Characteristics of participants grouped by ADNC, LBD, and VBI neuropathologies are described in Table 4.3. Prevalence of *APOE* ε4 allele and cognitive impairment was slightly higher in those with ADNC+LBD (with or without VBI) compared to ADNC only. Participants with LBD, in general, were more likely to be male and to have a younger age at death compared to those without LBD. Participants with VBI, with or without co-occurring pathologies, were older at death, on average, and they had a higher prevalence of other indicators of VBI, such as clinical history of stroke, severe vessel disease, and white matter disease compared to those without VBI.

Participants with mixed ADNC had a slightly lower prevalence of high ADNC with Braak stage V-VI than those with ADNC only. Over 40% of those with low NP (n=83) were cognitively impaired by the last visit; of those the majority were clinically diagnosed with Alzheimer's disease (n=54, 64.3%). Relatively few participants had LBD+VBI or ADNC+LBD+VBI; these groupings were not examined separately in analytic models.

Associations with cognitive domains

Rates of decline by neuropathology grouping are shown in Table 4.4 for each cognitive domain. Rates of decline were the fastest for ADNC+LBD in all domains but memory (Figure 4.3); however, only decline in attention was significantly faster than ADNC only (p=0.002 for attention, p=0.4 for memory, p=0.2 for language, and p=0.2 for executive function). Based on average trajectories over follow-up, participants with ADNC+LBD generally were more impaired than those with other pathologies (Figure 4.3). ADNC+VBI had similar rates of decline to ADNC only for memory (p=0.4) and executive function (p=0.2) and slower rates of decline for attention (p=0.02) and language (p=0.01). There was a significant interaction between ADNC and LBD for decline in memory (β: 0.05; 95% confidence interval [CI]: 0.00, 0.10; p=0.04). Participants with ADNC+LBD had a slower estimated rate of decline (-0.09 points per year) than would be expected if each pathology independently contributed to memory in an additive model (e.g. the sum of estimates for ADNC only and LBD only: -0.11 + -0.05 = -0.16 points per year). Interactions between ADNC and LBD were not significant for attention (β: 0.01; 95% CI: -0.05, 0.05; p= 0.8), language (β: 0.03; 95%CI: -0.01, 0.07; p=0.1) or executive function (β: 0.04; 95%CI: -0.02, 0.09; p= 0.1). Interactions between ADNC and VBI were not significant for memory (β: 0.03; 95% CI: -0.02, 0.06; p= 0.2), attention (β: 0.04; 95% CI: -0.01, 0.08; p= 0.1),

or executive function (β: 0.03; 95% CI: -0.01, 0.07; p= 0.2). There was a significant interaction between ADNC and VBI for language (β: 0.04; 95% CI: 0.01, 0.08; p= 0.02). The estimated rate of decline in language among those with ADNC+VBI (-0.18 points) was slower than would be expected by an additive model (e.g. the summed estimate for ADNC only and VBI only: -0.22 + -0.08 = -0.30 points per year).

In secondary models (data not shown) a significant interaction between ADNC and cortical LBD for memory decline was limited to those with high ADNC (p=0.004) not intermediate ADNC (p=0.1). Interactions with cortical LBD and intermediate or high ADNC were not significant for attention or language (all, p>0.1). Decline in executive function was slower than would be expected by an additive model in those with high ADNC and cortical LBD (β: 0.13; 95% CI: 0.01, 0.25; p=0.04), but not intermediate ADNC and cortical LBD (p=0.9). Interactions between ADNC and VBI for memory decline were borderline significant for high ADNC (p=0.09) but not intermediate ADNC (p=0.7). Interactions with VBI and intermediate or high ADNC were not significant for attention or executive function (all, p>0.2). A significant interaction between VBI and ADNC for language decline was limited to those with high ADNC (p=0.047) not intermediate ADNC (p=0.7).

Microinfarcts and cognitive domains in PANDA ADCs

The PANDA ADC participants (n=257), both number of cortical microinfarcts (β: -0.03, 95% CI: -0.05, 0.00; p=0.04) and subcortical microinfarcts (β: -0.02, 95% CI: -0.05, 0.00; p=0.007) in participants without ADNC were associated with faster decline in memory compared to those with low neuropathologies. Decline in attention (in 260 PANDA ADC participants) was not

significantly associated with cortical (p=0.83) or subcortical microinfarcts (p=0.89). Among participants without ADNC, cortical (β:-0.02; 95% CI: -0.05, 0.00; p=0.05) and subcortical microinfarcts (β:-0.01; 95% CI: -0.03, 0.00; p=0.07) were borderline associated with faster decline in language compared to those with low neuropathologies in 254 PANDA ADC participants. For executive function, cortical microinfarcts (β:-0.03; 95% CI: -0.05, 0.00; p=0.03) were associated with faster decline and subcortical microinfarcts (β:-0.02; 95% CI: -0.03, 0.00; p=0.06) were borderline associated with faster decline compared to those with low neuropathology in 184 PANDA ADC participants.

Sensitivity analyses

There were 435 (26.8%) participants who were had at least one visit with a missing cognitive domain score. The number of missed visits for each neuropsychological test battery score is shown in Table 4.5. Cognitive or behavioral problem was listed as the most common reason for missed visits, particularly among the demented, however, many visits were missing all test scores without a known reason. The proportion of missed visits was higher at visits when the participant had more severe dementia by the CDR-SB (Figure 4.4); 22.1% (n=359) of participants developed severe dementia (CDR-SB 16-18) by the last visit. Those missing scores without a reason listed were primarily in participants with dementia (88.5%), from 24 ADCs, and with visit years ranging 2006-2015. We imputed missing tests scores as the worst score (generally 0) for each test at visits in which the participant had severe dementia or a cognitive problem listed as the reason for missingness. We then recalculated cognitive domains and reran primary models. Rates of decline for this sensitivity analysis are shown in Table 4.6; in general rates of decline were slightly faster but primary findings remained similar. Overall findings were

also similar between primary analyses and models that did not include weighting for autopsy selection, although rates of decline tended to be slightly faster in unweighted models (Table 4.6).

DISCUSSION

We examined trajectories of memory, attention, language, and executive function in participants with single and mixed neuropathologies using a novel approach and focusing on combinations of ADNC, LBD, and VBI. Participants with ADNC+LBD had significantly faster decline in attention compared to ADNC only. Impairment across follow-up was generally the worst for those with ADNC+LBD in all domains, especially executive function. We found significant interactions between ADNC and LBD in association with decline in memory and executive function, and between ADNC and VBI in association with decline in language. In secondary models, the significant negative interactions, in which decline was slower than expected by an additive model, were limited to those with high ADNC. In those without ADNC, participants with cortical microinfarcts had faster decline in memory, executive function, and borderline faster language compared to those with low neuropathology; participants with subcortical microinfarcts had faster decline in memory and borderline faster decline in language and executive function.

Our primary results suggest that ADNC, VBI, and LBD are associated with decline in multiple cognitive domains. Prior studies have found ADNC to be strongly associated with cognitive function or decline in various domains.[19,21,37,128] We extend these findings to show associations in those with mixed pathologies compared to single pathologies. Divergence was evident in trajectories based on presence of co-occurring LBD or VBI, in particular for attention and

executive function, where ADNC+LBD had worse impairment than ADNC only, and attention and language, where rates of decline were slower for ADNC+VBI compared to ADNC only. Rates of decline in attention, language, and executive function were faster in those with LBD only compared to those with those with low neuropathology. In several studies, cortical LBD have also been associated with decline in the majority of cognitive domains,[5,32] although in other studies associations have been limited to visuospatial abilities and language.[19]

In primary models, VBI without ADNC was not associated with decline (except memory) and rates of ADNC+VBI were generally slower than ADNC only. This latter result may be because of less severe ADNC in those with VBI. Additionally our findings in PANDA ADC participants and other studies[19,82,129] suggest associations with decline may only be detectable in those with multiple VBI, particularly multiple microinfarcts. In the PANDA ADCs, numbers of cortical and subcortical microinfarcts were associated with decline in memory, language, and executive function. Although some associations were borderline significant, this could reflect a lack of power due to the small sample size. In Chapter 3 only subcortical microinfarcts were associated with overall clinical progression. In comparison, neuropsychological tests may be more sensitive to subtle deficits in specific domains.

Similar to our previous findings on clinical progression in Chapter 3, we found unexpected antagonistic interactions between ADNC and LBD or VBI, particularly in those with high ADNC. These findings may suggest that ADNC is the primary determinant of clinical progression in those with more severe ADNC, where it seems to overpower additional neuropathologies. This hypothesis is consistent with prior research that co-occurring vascular

lesions were not associated with cognitive decline beyond that of ADNC in individuals with high ADNC.[32,108,109] An alternative explanation is that severity of additional pathologies may be lower in those with high ADNC than in those with intermediate ADNC, such that effects are additive when considering quantitative measure of pathologic burden.[26] In secondary models, under Chapter 3 we found that rate of decline was much faster in those with intermediate ADNC+cortical LBD, suggestive of a synergistic interaction. We found no evidence for synergy in this study. However, in multiple domains, especially attention and executive function, trajectories were worse for those with ADNC+LBD compared to those with ADNC only, in particular for those with intermediate ADNC. This effect across multiple domains could translate into greater overall impairment across time.

There were some limitations to this study. As neuropathologic assessments are conducted at autopsy, findings may not reflect burden of pathology when clinical progression was measured. Although overall trends in cognitive domains were approximately linear over time; we did not explore non-linear trends or variability in decline; other studies suggest onset of terminal decline may differ by pathology[20] and that fluctuating cognition may be associated with LBD.[37] Many participants were missing data on neuropsychological test scores once they developed severe dementia. We used a modeling approach (linear mixed effects modeling) that is valid when missingness can be predicted entirely on observed variables or missing at random (MAR).[130,57] Our results may be biased, though, if data was missing not at random (MNAR), when missingness can only be predicted by missing data themselves or other unmeasured factors.[57] We conducted additional sensitivity analyses to assess this potential; however results were similar suggesting primary analyses were valid.

However, we used a large data set of standardized clinical evaluations that could provide power to detect statistical interactions. Our modeling approach allowed us to characterize average differences in the rate of decline between participants with mixed pathology and those with individual pathologies. We also investigated associations of microinfarcts in PANDA ADC participants whose brains underwent the same neuropathologic assessment protocols. Finally, we attempted to account for potential selection bias due to autopsy sampling.

Rate of decline in memory, attention, language, and executive function domains were associated with specific neuropathologic groupings. Trajectories of those with ADNC+LBD diverged from those of ADNC only across multiple domains, which may correspond to worse overall clinical progression and increased likelihood of dementia. In some domains, decline in those with mixed ADNC was slower than would be expected by additive or independent models, particularly in those with high ADNC. Future research may be needed to clarify effects of additional pathologic burden across the continuum of ADNC. These findings highlight the broad associations of common neuropathologies with cognitive decline in individuals with single and mixed neuropathologies.

TABLES AND FIGURES

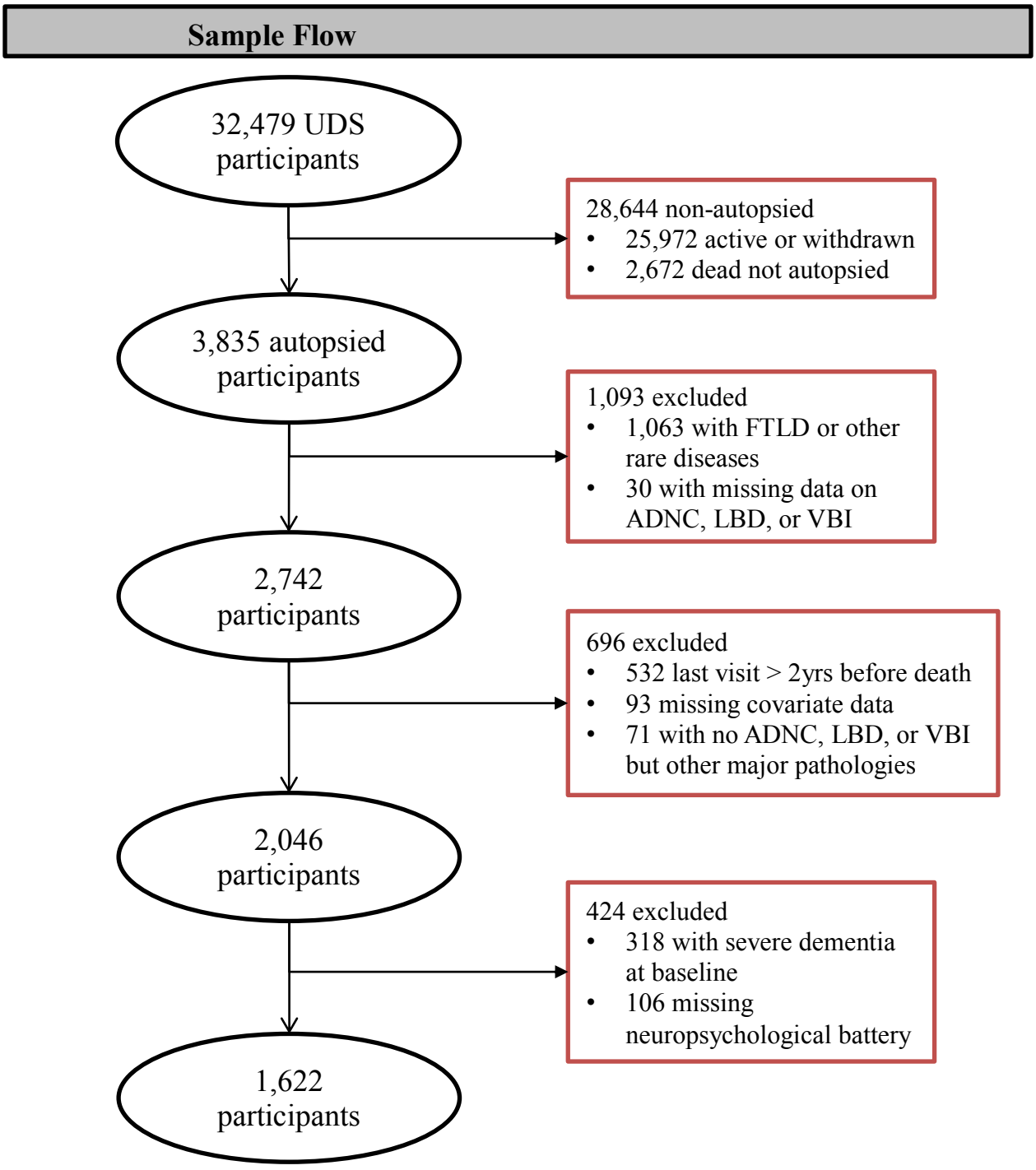

Figure 4.1 Study sample flow chart. ADNC; Alzheimer's disease neuropathologic change; FTLD, frontotemporal lobar degeneration; LBD, Lewy body disease, NACC, National Alzheimer's Coordinating Center (NACC); UDS, Uniform Data Set; VBI, vascular brain injury.

Table 4.1 Comparison of autopsied participants to non-autopsied participants

Characteristics*	Alive/Unknown	Dead, non-autopsied	Autopsied
Total, N	25,972	2,672	3,835
Age at baseline, mean (SD)	71.2 (10.1)	77.5 (9.6)	76.4 (11.4)
Age at last visit, mean (SD)	73.8 (10.6)	79.5 (9.9)	78.7 (11.8)
Birth year <1928 (25th percentile)	5,728 (22.1)	1,394 (52.2)	1,916 (50.0)
Female	15,462 (59.5)	1,275 (47.7)	1,744 (45.5)
Non-white race	5,310 (20.8)	509 (19.4)	236 (6.2)
College graduate	13,768 (53.0)	1,139 (42.6)	2,106 (54.9)
History of heart disease	5,442 (21.1)	973 (36.8)	1,288 (33.7)
History of hypertension, hypercholesterolemia or both	19,764 (76.5)	2,104 (79.0)	2,788 (73.2)
History of stroke	1,596 (6.2)	340 (12.8)	427 (11.2)
Depression	10,972 (42.6)	1,257 (47.5)	2,008 (53.1)
APOE ε4 allele	7,614 (39.9)	718 (41.1)	1,440 (44.2)
Demented at last visit	9,503 (36.6)	1,853 (69.3)	3,057 (79.7)
Rare/atypical dementia type	4,121 (15.9)	605 (22.6)	1,066 (27.8)
Motor problems	5,803 (22.4)	1,313 (49.7)	2,258 (59.7)
Genetics data available	3,967 (15.3)	463 (17.3)	1,252 (32.6)

*N,(%) unless otherwise specified. Participants missing data: race=535 (1.6%), education=230 (<1%), heart disease=224 (<1%), hypertension=176 (<1%), stroke=141 (<1%), depression=318 (1%), motor problems=170 (<1%)

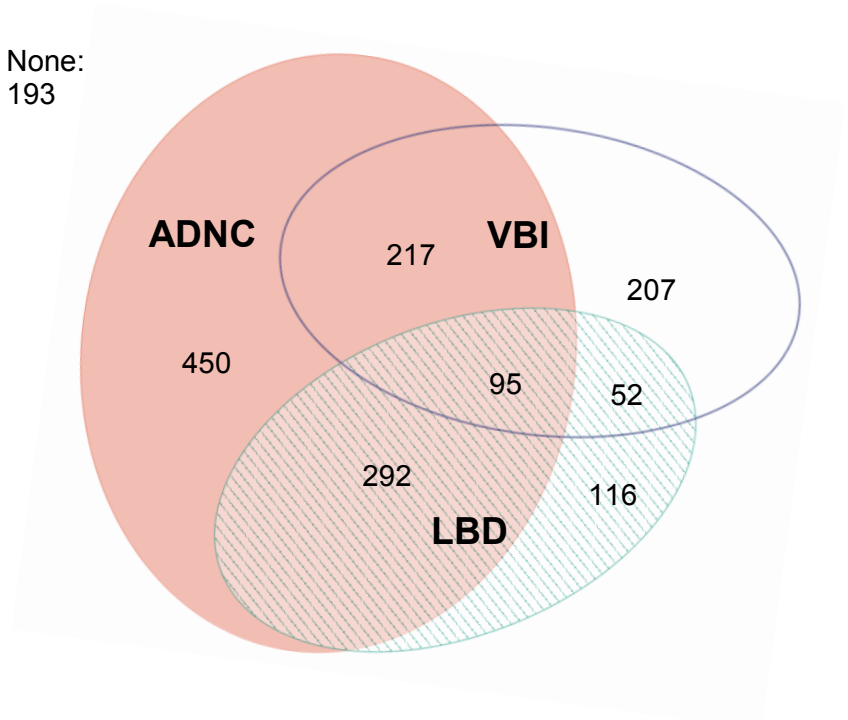

Figure 4.2 Co-occurrence of Alzheimer's disease neuropathologic change (ADNC), Lewy body disease (LBD), and vascular brain injury (VBI) in study participants (N=1,622). ADNC = moderate/frequent neuritic plaques & Braak stage III-VI; LBD = Lewy bodies in any brain region examined; VBI = gross infarcts and cortical microinfarcts.

Table 4.2 Number of participant-visits for each cognitive domain score

Visit number (from last visit)	Memory	Attention	Language	Executive Function
0 (last visit)	1052	1073	1029	616
-1	1102	1135	1106	702
-2	947	970	958	670
-3	701	714	709	540
-4	513	522	507	408
-5	318	323	313	259
-6	156	159	158	134
-7	61	61	60	50
-8	15	17	15	13
-9	2	2	2	2

Table 4.3 Study participant characteristics stratified by ADNC, LBD, and VBI neuropathologic groupings*

Characteristics†	Low NP	VBI only	LBD only	LBD+VBI	ADNC only	ADNC+ LBD	ADNC+ VBI	ADNC+ LBD+VBI
Autopsies, N	193	207	116	52	450	292	217	95
Clinical								
Age at death, mean (SD)	87.2 (9.5)	88.4 (8.9)	79.3 (9.0)	84.8 (8.3)	81.5 (10.3)	78.1 (9.1)	85.5 (7.9)	84.2 (8.5)
Female	104 (53.9)	125 (60.4)	28 (24.1)	18 (34.6)	206 (45.8)	100 (34.2)	100 (46.1)	34 (35.8)
Non-white	12 (6.2)	10 (4.8)	4 (3.4)	4 (7.7)	17 (3.8)	13 (4.5)	18 (8.3)	9 (9.5)
College graduate	105 (54.4)	104 (50.2)	75 (64.7)	27 (51.9)	270 (60)	170 (58.2)	119 (54.8)	49 (51.6)
History of stroke	15 (7.8)	72 (34.8)	3 (2.6)	7 (13.7)	28 (6.2)	12 (4.1)	53 (24.9)	14 (14.9)
APOE ε4 allele	30 (16.9)	37 (19.6)	29 (32.6)	16 (36.4)	201 (50.6)	161 (62.6)	105 (55.3)	49 (58.3)
Cognitively Impaired	84 (43.5)	137 (66.2)	96 (82.8)	43 (82.7)	429 (95.3)	287 (98.3)	202 (93.1)	93 (97.9)
Pathological								
Braak stage V-VI	NA	12 (5.8)	6 (5.2)	1 (1.9)	349 (77.6)	208 (71.2)	137 (63.1)	65 (68.4)
Cortical LBD	NA	NA	52 (44.8)	21 (40.4)	NA	133 (45.5)	NA	32 (33.7)
Severe Atherosclerosis	16 (8.3)	58 (28.2)	11 (9.5)	7 (13.5)	45 (10.1)	22 (7.6)	31 (14.4)	11 (11.6)
Severe Arteriolosclerosis	3 (1.9)	51 (25.8)	4 (4.1)	12 (24)	33 (8.9)	20 (8.3)	40 (20.9)	24 (27.3)
Severe CAA	7 (3.7)	6 (3)	4 (3.5)	5 (9.6)	70 (15.9)	32 (11.1)	39 (18.5)	18 (20.7)
White matter disease	NA	35 (17.0)	9 (7.9)	10 (19.2)	58 (13.0)	34 (12.0)	38 (17.8)	13 (14.3)
Hippocampal Sclerosis	NA	17 (9.4)	7 (6.7)	3 (6.7)	33 (9.2)	22 (9.2)	17 (9.2)	14 (17.3)

ADNC, Alzheimer's disease neuropathologic change; CAA, cerebral amyloid angiopathy; LBD, Lewy body disease; NA, not applicable; NP, neuropathology; VBI, vascular brain injury

*ADNC = moderate/frequent neuritic plaques & Braak stage III-VI; LBD = Lewy bodies in any brain region examined; low NP = no ADNC, no VBI, no LBD, and no other major pathologies; VBI = gross infarcts and cortical microinfarcts.

†N,% unless otherwise specified. Participants missing data: stroke=10 (<1%), APOE genotype=195 (12.0%), atherosclerosis=13 (<1%), arteriolosclerosis=226 (13.9%), CAA=36 (2.2.%), white matter disease=28 (1.7%), hippocampal sclerosis=266 (16.4%).

Table 4.4 Estimated annual change in cognitive domain z scores by neuropathologies*

	Annual change in z score (95% CI)†
Memory (n=1,575)	
Low NP	-0.01 (-0.04, 0.01)
VBI only	-0.05 (-0.07, -0.02)
LBD only	-0.05 (-0.10, -0.01)
ADNC only	-0.11 (-0.13, -0.09)
ADNC+VBI	-0.12 (-0.14, -0.09)
ADNC+LBD	-0.09 (-0.12,-0.07)
Attention (n=1,592)	
Low NP	-0.04 (-0.07, -0.02)
VBI only	-0.04 (-0.07, -0.02)
LBD only	-0.12 (-0.16, -0.08)
ADNC only	-0.14 (-0.16, -0.12)
ADNC+VBI	-0.10 (-0.13, -0.08)
ADNC+LBD	-0.21 (-0.24, -0.18)
Language (n=1,582)	
Low NP	-0.07 (-0.09, -0.05)
VBI only	-0.08 (-0.10, -0.06)
LBD only	-0.12 (-0.15,-0.09)
ADNC only	-0.22 (-0.24, -0.20)
ADNC+VBI	-0.18 (-0.21, -0.16)
ADNC+LBD	-0.23 (-0.26, -0.21)
Executive Function (n=1,200)	
Low NP	-0.07 (-0.09, -0.06)
VBI only	-0.08 (-0.10, -0.06)
LBD only	-0.14 (-0.17, -0.10)
ADNC only	-0.19 (-0.21, -0.16)
ADNC+VBI	-0.16 (-0.19, -0.13)
ADNC+LBD	-0.21 (-0.25, -0.18)

ADNC, Alzheimer's disease neuropathologic change; NP, neuropathologies; LBD, Lewy body disease; VBI, vascular brain injury

*ADNC = moderate/frequent neuritic plaques & Braak stage III-VI; LBD = Lewy bodies in any brain region examined; low NP = no ADNC, no VBI, no LBD, and no other major pathologies; VBI = gross infarcts and cortical microinfarcts.

†Based on models with adjustment for age at baseline, sex, non-white race, years of education, and interval between last visit and death and weighted by inverse probability of autopsy selection. Note: A negative value corresponds to decline in cognition over time

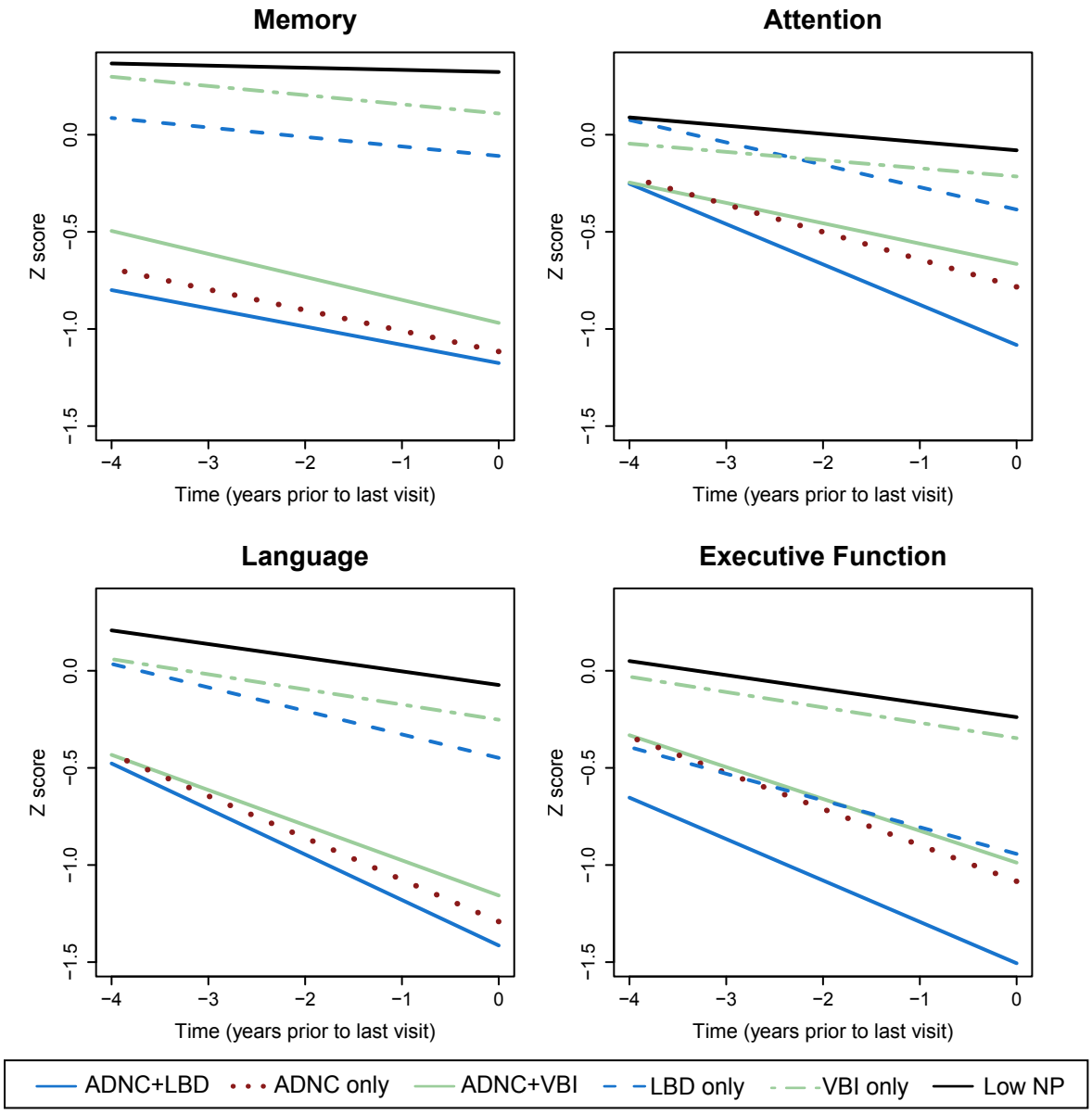

Figure 4.3 Model based population mean trajectories of cognitive domain z scores. ADNC, Alzheimer's disease neuropathologic change = moderate/frequent neuritic plaques & Braak III-VI; LBD, Lewy body disease = Lewy bodies in any brain region examined; low NP, low neuropathology = no ADNC, no VBI, no LBD, and no other major pathologies; VBI, vascular brain injury = any gross infarcts or cortical microscopic infarcts. Compared to low NP, VBI only had significantly faster rate of decline for memory (p=0.04), LBD only was significantly faster for attention (p=0.002), language (p=0.002), and executive function (p=0.003), and ADNC only was faster in all domains (all, p=0.002). There were significant interactions between LBD and ADNC for memory (p=0.04) and between VBI and ADNC for language (p=0.02), in which decline was slower than expected if these neuropathologies acted additively on the rate of decline.

Table 4.5 Missing neuropsychological test scores

Neuropsychological Test	Physical problem	Cognitive/ behavior problem	Other	Refusal	NA
	N missed visits among demented / N total missed visits				
Memory					
Logical Memory-immediate	0 / 21	139/ 388	8 / 131	2 / 23	300 / 540
Logical Memory-delayed	0 / 23	139 / 415	9 / 129	2 / 34	300 / 540
Attention					
Digit span forward	0 / 15	134 / 326	8 / 107	2 / 20	300 / 540
Digit span backward	0 / 15	135 / 350	9 / 111	2 / 24	300 / 540
Language					
Animals	0 / 11	134 / 292	8 / 76	5 / 29	300 / 540
Vegetables	0 / 14	137 / 300	7 / 117	5 / 32	300 / 540
Boston Naming	2 / 91	133 / 319	9 / 130	4 / 42	300 / 540
Executive Function					
Trails A	4 / 247	196 / 647	10 / 114	3 / 38	300 / 540
Trails B	5 / 274	214 / 1363	13 / 151	4 / 94	300 / 540
WAIS-R Digit Symbol	7 / 291	186 / 844	10 / 250	4 / 67	300 / 540

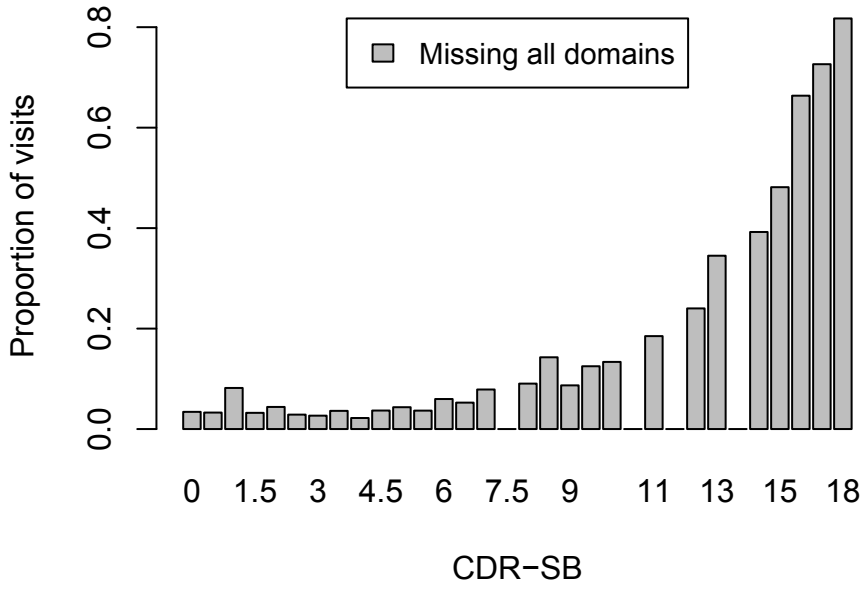

Figure 4.4 Proportion of visits missing all cognitive domains by clinical severity

Table 4.6 Estimated annual change in cognitive domain z scores by neuropathologies in sensitivity analyses: unweighted models and missing data models*

	Unweighted Models†	Missing data‡
Memory	**Annual change in z score (95% CI)**	**Annual change in z score (95% CI)***
Low NP	-0.02 (-0.05, 0.00)	-0.02 (-0.05, 0.01)
VBI only	-0.05 (-0.08, -0.03)	-0.06 (-0.08, -0.04)
LBD only	-0.07 (-0.11, -0.03)	-0.07 (-0.12, -0.03)
ADNC only	-0.12 (-0.14, -0.10)	-0.12 (-0.13, -0.10)
ADNC+VBI	-0.12 (-0.15, -0.10)	-0.12 (-0.15, -0.10)
ADNC+LBD	-0.10 (-0.12, -0.08)	-0.11 (-0.13, -0.09)
Attention		
Low NP	-0.05 (-0.07, -0.03)	-0.06 (-0.08, -0.03)
VBI only	-0.06 (-0.07, -0.03)	-0.05 (-0.08, -0.03)
LBD only	-0.18 (-0.17, -0.09)	-0.16 (-0.21, -0.12)
ADNC only	-0.17 (-0.18, -0.14)	-0.23 (-0.25, -0.20)
ADNC+VBI	-0.14 (-0.16, -0.10)	-0.16 (-0.19, -0.13)
ADNC+LBD	-0.26 (-0.25, -0.10)	-0.28 (-0.31, -0.25)
Language		
Low NP	-0.08 (-0.10, -0.06)	-0.08 (-0.10, -0.06)
VBI only	-0.08 (-0.10, -0.06)	-0.08 (-0.10, -0.06)
LBD only	-0.13 (-0.16, -0.10)	-0.16 (-0.20, -0.13)
ADNC only	-0.23 (-0.25, -0.21)	-0.25 (-0.26, -0.23)
ADNC+VBI	-0.20 (-0.22, -0.17)	-0.20 (-0.22, -0.18)
ADNC+LBD	-0.25 (-0.27, -0.22)	-0.27 (-0.29, -0.25)
Executive Function		
Low NP	-0.08 (-0.10, -0.06)	-0.08 (-0.10, -0.06)
VBI only	-0.09 (-0.12, -0.08)	-0.09 (-0.12, -0.07)
LBD only	-0.16 (-0.19, -0.13)	-0.17 (-0.20, -0.13)
ADNC only	-0.21 (-0.23, -0.19)	-0.22 (-0.24, -0.20)
ADNC+VBI	-0.18 (-0.21, -0.15)	-0.21 (-0.24, -0.18)
ADNC+LBD	-0.23 (-0.26, -0.20)	-0.22 (-0.25, -0.19)

ADNC, Alzheimer's disease neuropathologic change; NP, neuropathologies; LBD, Lewy body disease; VBI, vascular brain injury

*ADNC = moderate/frequent neuritic plaques & Braak stage III-VI; LBD = Lewy bodies in any brain region examined; low NP = no ADNC, no VBI, no LBD, and no other major pathologies; VBI = gross infarcts and cortical microinfarcts.

†Based on models that do not weight for autopsy selection but include adjustment for age at baseline, sex, non-white race, years of education, and interval between last visit and death.

‡Based on models with adjustment for age at baseline, sex, non-white race, years of education, and interval between last visit and death. Missing data was imputed as the lowest possible score for participant visits in which the participant had severe dementia or a cognitive/behavior problem listed as the reason for missingness.

Chapter 5. Conclusions

In this dissertation we conducted an extensive analysis of mixed neuropathologies in autopsied older adults. Co-occurrence of the three most common pathologies, ADNC, LBD and VBI was prevalent in both a clinic-based and community-based autopsy sample. Our study adds to the evidence of an association between LBD and ADNC[117,93,35,64] and independence of ADNC and VBI.[33] Clinical progression was faster in those with ADNC+LBD, particularly in secondary analyses among those with intermediate ADNC. Regardless of co-occurring pathologies progression was similar for those with high ADNC in secondary analyses. Compared to participants with ADNC only, those with ADNC+LBD had faster decline in attention, and worse overall impairment in other cognitive domains, especially executive function. In a sub-sample of PANDA ADC participants, numbers of microinfarcts without co-occurring ADNC were associated with faster clinical progression and faster decline in multiple domains compared to those with low neuropathology. Using a dichotomous variable for any VBI we did not find associations with faster progression; in fact, rates of progression and cognitive decline were generally slower for those with ADNC+VBI compared to ADNC only. Together these results suggest that increased pathologic burden is associated with clinical progression and decline in multiple cognitive domains. LBD and VBI, especially multiple microinfarcts, may have stronger impact on cognition in those without severe ADNC.

While decline was faster in those with ADNC+LBD in particular, it is not clear whether this is due to independent effects of each pathology on cognitive domains or whether this group represents a subgroup of ADNC pathogenesis. Interestingly, we found intermediate ADNC was associated with cortical LBD and high ADNC was associated with limbic or amygdala only

LBD. Associations were stronger in NACC, which had a larger sample size, than in ACT. Interactions between amyloid and α-synuclein may lead to an alternative pathologic and clinical presentation than ADNC only, in which neurofibrillary tangles are more predominant.[34,36,65] *APOE* ε4 allele was slightly more prevalent in those with ADNC+LBD, suggesting genetics may influence the development of LBD in ADNC.

This study highlights the complexities of mixed neuropathologies. Our findings may be valuable to future researchers as they improve understanding of the overlap between ADNC, LBD, and VBI that could be expected at different ages and in either clinic-based or population-based samples. In both NACC and ACT, the majority of autopsied participants with ADNC also had co-occurring LBD or VBI. Younger autopsied participants with ADNC were more likely to have co-occurring LBD; while older autopsied participants with ADNC were more likely to have co-occurring VBI. Given the broad effects of each of these pathologies on cognitive domains, it may be challenging to accurately identify those with mixed neuropathologies compared to those with high ADNC.

One novel highlight of this research was the presence of negative interactions between pathologies, such that the association between LBD or VBI and clinical progression was lower in those with ADNC. In secondary models this finding was limited to those with high ADNC. These results suggest that modeling each pathology as independent factors[19–21] may overestimate differences in the rate of progression between those with single and mixed ADNC, particularly for those with high ADNC. Other research suggests that overall burden may be associated with cognition more strongly than specific combinations of pathologies,[26] which is somewhat contrary

to our findings. Future research using quantitative pathologic measures may be able to more precisely examine the distribution of additional pathologies along the continuum of ADNC and assess relationships with cognitive decline.

Some limitations exist with our study. Neuropathologies are only measured at the end of life, and findings may not reflect the pathologic burden that was present, at or prior to, clinical visits. This limits our ability to make inferences regarding the nature of any associations between clinical disease and mixed neuropathologies. It will be important to develop biomarkers that can accurately detect multiple neuropathologies prior to death. We focused on mixes of the most common pathologies to simplify our analyses. There are other less common pathologic features that may overlap as well to impact cognition. Future research on the co-occurrence of other less common pathologies may be beneficial.

Participants were often unable to complete neuropsychological testing when they developed severe dementia. Therefore data on cognitive domains was available primarily for the period of cognitive decline prior to terminal phases of dementia preceding death. To address potential issues due to missing cognitive domain scores, we used a modeling approach that is valid when missingness can be predicted entirely on observed variables[130,57] and conducted sensitivity analyses. We also attempted to adjust for selection bias based on autopsy selection in our analytic models. However, these methods are not without assumptions; misspecification of mechanisms of selection may have potentially biased results.[57]

NACC and ACT included predominantly Caucasian and well-educated older adults, which limited our ability to perform subgroup analyses by demographics. Prior research has found mixed pathologies to be more common in African Americans.[131] Although we did not find evidence that cognitive reserve modified associations between clinical progression and mixed neuropathologies, there were few participants with low education, our chosen proxy for cognitive reserve. Future research on differences in mixed pathologies in diverse populations would be beneficial. Additionally, future research on variability in clinical progression and cognitive decline may be useful as we focused on average trends in this study.

Despite the limitations, NACC data is a valuable resource for studying mixed neuropathologies. Few autopsy samples can rival the size (and thus statistical power) of NACC data and it would take decades to conduct a prospective study. Our study was enhanced by conducting a chart review of PANDA ADC participants whose brains underwent the same neuropathologic assessments and by using data from ACT to draw comparisons to a community-based autopsy sample. Furthermore, the detailed clinical data paired with neuropathology data allowed us to conduct an informative study regarding mixed neuropathologies in older adults.

Given the high prevalence of mixed pathologies in our study, it is likely that a large proportion of patients have multiple pathologies among clinical trials for ADNC. Treatments that target only part of the pathologic burden may not be effective. These data suggest that prevention and treatment of dementia may require multi-faceted strategies. Methods for identifying patients with mixed neuropathologies will be important as well. It may be difficult to differentiate individuals with mixed or single pathologies based on progression or cognitive domains alone. Future

research on mixed neuropathologies could examine whether other clinical information, such as biomarkers, behavioral and motor symptoms, or features of symptom onset may help differentiate between individuals with single and mixed neuropathologies. Our study also highlights the challenges of understanding pathologic burden in older adults as there seem to be complex relationships between pathologies and cognition. Our approaches allowed us to study mixed neuropathologies in a systematic manner that was driven by a holistic view of pathological burden in older adults.

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