Abstract

Background and Objectives: The ATN framework has been developed to categorize biological processes within the Alzheimer's disease (AD) continuum. Since AD pathology often coincides with dementia with Lewy Bodies (DLB), we aimed to investigate the distribution of ATN profiles in DLB and associate ATN-profiles in DLB to prognosis.

Methods: We included 202 DLB patients from the Amsterdam Dementia Cohort (68±7yrs, 19%F, MMSE: 24±3, DAT-SPECT abnormal: 105/119). Patients were classified into eight profiles according to the ATN framework, using CSF Aβ_{42} (A), CSF p-tau (T) and medial temporal atrophy scores (N). We compared presence of clinical symptoms in ATN profiles and used linear mixed models to analyze decline on cognitive tests (follow-up 3±2yrs for n=139). Mortality risk was assessed using Cox proportional hazards analysis. Analyses were performed on both the eight profiles, as well as three clustered categories (*normal AD biomarkers*, *non-AD pathologic change*, *AD continuum*).

Results: Fifty (25%) DLB patients had *normal AD biomarkers* (A-T-N-), 37 (18%) had *non-AD pathologic change* (A-T+N-: 10%/A-T-N+: 6%/A-T+N+: 3%) and 115 (57%) were classified within the *AD continuum* (A+T-N-: 20%/A+T+N-: 16%/A+T-N+: 10%/A+T+N+: 9%). A+T+N+ patients were older and least often had RBD symptoms. Parkinsonism was more often present in A+T-, compared to A-T+ (independent of N). Compared to patients with normal AD biomarkers, patients in A+ categories showed steeper decline on memory tests and higher mortality risk. Cognitive decline and mortality did not differ between *non-AD pathologic change* and *normal AD biomarkers*.

Discussion: In our DLB cohort, we found clinically relevant associations between ATN categories and disease manifestation. Patients within the *AD continuum* had steeper cognitive decline and shorter survival. Implementing the ATN framework within DLB patients aids in subtyping patients based on underlying biological processes and could provide targets for future treatment strategies, e.g. AD modifying treatment. Expanding the framework by incorporating markers for alpha-synucleinopathy would improve the use of the framework to characterize dementia patients with mixed pathology, which could enhance proper stratification of patients for therapeutic trials.

Introduction

The ATN research framework, developed by the NIA-AA, has been proposed to classify individuals along the Alzheimer's disease (AD) continuum (1). This framework is focused on a biological definition of AD, which includes β-amyloidosis (A), hyperphosphorylated tau (p-tau) (T) and neurodegeneration (N) assessed by specific biomarkers. Each individual can be classified into one of eight biomarker profiles. Within these profiles, the *normal AD biomarker* profile consists of negative amyloid, tau, and neurodegeneration (A−T−N−). Normal amyloid markers, combined with either positive p-tau or presence of neurodegeneration, is referred to as *non-AD pathology*. Amyloid positive profiles denote the *AD continuum*, and within this continuum, A+T-N- is defined as Alzheimer's pathologic change, while A+T+ profiles are referred to as Alzheimer's disease. This framework provides a common language in dementia research, which can aid in selecting patients for therapeutic trials and is relevant for longitudinal studies.

The ATN framework provides an unbiased descriptive classification scheme of AD pathology that could also be applied to characterize patients with mixed pathologies and other neurodegenerative diseases, such as dementia with Lewy Bodies (DLB). Although the pathological hallmark of DLB is the presence of aggregated alpha-synuclein proteins, in-vivo and pathological studies show that around 50% of DLB patients have AD co-pathology (2-6). Alzheimer's co-pathology in DLB is associated to worse clinical outcomes, such as more pronounced memory and language impairment, accelerated cognitive decline, earlier nursing home admission and higher mortality (6-8). The degree of AD co-pathology in DLB patients can differ substantially. With the ATN framework, novel possibilities emerge to characterize AD pathology in DLB and specifically investigate the impact on clinical manifestation and prognosis. This could aid in subtyping patients based on underlying biological processes, which is relevant for future treatment strategies. A previous cross-sectional study applied the A and T from the ATN framework in a DLB cohort to define concomitant AD pathology, and found associations of clinical manifestation with T+ (lower frequency of parkinsonism and REM sleep behavior disorder (RBD)), while A+ was associated with more severe cognitive dysfunction (9). In the present study, we aimed to apply the full ATN framework on a large sample of individuals with DLB in a longitudinal setting and assess the use of this framework for clinical prognosis. We investigated the distribution of the ATN profiles and studied associations between profiles and clinical manifestation, cognitive decline and mortality.

Methods

Study population

Patients were selected from the Amsterdam Dementia Cohort (ADC) and DEvELOP (10, 11). The ADC consists of patients who visited the Alzheimer Center Amsterdam between 1999 and 2020. All patients received a standardized screening at baseline, this included a semi-structured medical history; physical, neurological and neuropsychological examinations; magnetic resonance imaging (MRI);

electroencephalography (EEG) or magnetoencephalography (MEG); and laboratory tests. Diagnoses were made during a multidisciplinary consensus meeting, based on the results obtained from the standardized screening. We selected a total of 202 DLB patients for this study based on a DLB diagnosis and the availability of CSF and MRI data for categorizing into ATN profiles. Clinical DLB diagnosis was made according to the current consensus criteria (12, 13). In 119 patients FPCIT single-photon emission computed tomography (DAT-SPECT) was performed, which supported DLB diagnoses in 105 patients. Patients with negative DAT-SPECTs were included based on fulfillment of clinical criteria for probable DLB and a consistent DLB diagnosis at follow-up. N=99 in this cohort were also included in a previous study by Ferreira et al 2020 (9).

Clinical symptoms

DLB core and suggestive clinical features were systematically assessed during the first visit or rated retrospectively from patients' medical charts when missing. Visual hallucinations were scored according to the caregiver-rated neuropsychiatric inventory (NPI, n=172) or rated retrospectively (n=30) (14). Parkinsonism was assessed through a preformatted checklist for extrapyramidal signs during the neurological exam (i.e., tremor, bradykinesia, and/or rigidity, n=179) or rated retrospectively from medical charts (n=23). For cognitive fluctuations and RBD, the semi-structured patient history interview was retrospectively reviewed. Fluctuations were rated as present when the patient or caregiver reported clear changes in attention and/or cognition during the day or between days. Information on fluctuations was available for 165 patients. RBD was scored as present, when caregivers reported that a patient would 'act out' their dreams during their sleep. Information on RBD was available for 165 patients. Duration of complaints was assessed during the patient history interview and was defined as the time difference between the moment when the patient first noticed cognitive complaints and presentation at the memory clinic. For suggestive features, we had two symptoms available that were systematically assessed; depressive symptoms and orthostatic hypotension. Depressive symptoms were assessed using the 15-items Geriatric Depression Scale (GDS), with higher scores indicating more symptoms. We used a cutoff for presence of depressive symptoms of GDS ≥ 6 (15). Orthostatic hypotension was defined as a 20 mmHg drop in systolic blood pressure or a 10 mmHg drop in diastolic blood pressure between supine and standing positions.

Neuropsychological assessment and follow-up

The Mini-Mental State Examination (MMSE) was used to assess global cognitive decline over time. In addition, we explored separate cognitive functions by looking at neuropsychological test scores. Memory was assessed with the Visual Association Test (VAT, range 0-12) and the immediate recall (range 0-75) and delayed recall (range 0-15) of the Dutch version of the verbal learning test (16, 17). Semantic memory was assessed with animal fluency (number of animals named in one minute)(16, 18). Attention and speed were assessed using Trail Making Test (TMT)-A. Executive functioning was assessed using the ratio of TMT-B/TMT-A (19). We calculated inverse scores for TMT-A and B, such

that lower scores represent worse performance. Visuospatial functioning was assessed using three subtests of the VOSP battery (number-location test (range 0-10), dot-counting (range 0-10) and fragmented letters(range 0-20) (20).

Patients were invited for annual follow-up, including repeated neuropsychological assessment. Follow-up duration varied among patients (3±2yrs), with at least one follow-up visit available for n=139 patients.

Mortality

For each patient, we obtained information on mortality (deceased yes or no, date of death) from the Dutch municipal population register (accessed on June 1st, 2020). Time of survival was defined as the time between baseline visit (date of collection of clinical, CSF and MRI data) and the date of death, or if alive, the time between the baseline and June 1st, 2020.

Biological measures

All patients underwent a brain MRI scan. The protocol included 3D T1-weighted images, 3D T2-weighted images, and 3D T2-weighted fluid- attenuated inversion-recovery (FLAIR) images. Visual rating was done by raters who completed training and obtained a weighted kappa of ≥ 0.80 for MTA scores. All ratings were evaluated in a consensus meeting with an experienced neuroradiologist. Visual rating of medial temporal lobe atrophy (MTA) was performed on coronal T1-weighted images averaging scores for the left and right sides (range 0–4) (21). Posterior atrophy was rated using sagittal, axial, and coronal planes of T1 and FLAIR- weighted images averaging scores for the left and right sides (range 0–3) (22). The presence of white matter hyperintensities was rated on axial FLAIR images using the Fazekas scale (range 0-3) (23).

CSF was obtained through lumbar puncture between the L3/L4, L4/L5, or L5/S1 intervertebral space by a 25-gauge needle and syringe and collected in polypropylene tubes (24). Aβ42, total tau (t-tau), and phosphorylated tau (p-tau) were measured using sandwich ELISAs (Innotest: Aβ42 n=146, (p)tau n=151; Elecsys: Aβ42 n=56, (p)tau n=51). Innotest Aβ42 levels were adjusted for the drift in CSF biomarker analyses that occurred over the years (25).

ATN classification

To determine whether a participant was A- or A+ levels of CSF Aβ42 were used. Amyloid was considered positive when CSF concentrations indicated a drift corrected Innotest Aβ42 value of <813 pg/mL, and <1000 pg/mL for Elecsys. For T+/T-, CSF p-tau concentrations were used. P-tau was considered positive, in case of p-tau >52 pg/mL for Innotest and >19 pg/mL for Elecsys. Neurodegeneration was determined using an averaged MTA score over the left and right hemispheres (MTA ≥ 1.5 positive).

Some ATN profiles consisted of a small group of patients, we therefore performed all analyses on both the eight biomarker profiles, as well as clustered categories based on the eight biomarker profiles. The A–T–N– profile was labeled as the '*normal AD biomarkers*', the remaining A– profiles (A–T–N+, A–T+N–, and A–T+N+) as '*non-AD pathologic change*' and all A+ profiles (A+T–N–, A+T–N+, A+T+N–, A+T+- N+) as '*AD continuum*'.

Statistical analyses

All statistical analyses were conducted in Rstudio (version 4.0.3., Rstudio, Inc. Boston, MA). Demographic and clinical variables were compared among the eight ATN profiles. For continuous variables, ANOVA was used if the assumptions of normal distributed data were met or Kruskal-Wallis if the assumptions were not met. We used Chi^2 tests for categorical variables. Post hoc, we made pairwise comparisons and adjusted the p-values with the false discovery rate (FDR) correction. The association between ATN classification and cognitive decline was assessed using age and sex corrected linear mixed models (LMMs) with random intercepts. Models included time, ATN profile (A-T-N- as reference), and the interaction between time and ATN profile as independent variables. Cognitive test scores were evaluated as dependent variables in separate models. We applied the FDR method to correct for multiple testing (q set at 0.05). We assessed differences in mortality using Kaplan-Meier and Cox proportional hazard analyses, corrected for age and sex. All analyses were performed on both the eight ATN profiles as well as the clustered 3-category groups (A-T-N- as reference).

Standard protocol approvals, registrations, and patients consents

The study protocol of the Amsterdam Dementia Cohort was approved by an ethical review board. Written informed consent was obtained from all patients for the use of their clinical data and biomaterial for research purposes.

Data availability

Data not provided in the article and additional information on methods and materials may be shared upon request.

Results

Figure 1 shows the distribution of ATN profiles within our DLB cohort. The *normal AD biomarker* profile (A-T-N-) was most common (n=50, 25%), the remaining n=152 patients (75%) had an ATN profile with at least one AD biomarkers abnormal. Of these profiles, A+T-N- was most common, (n=41, 20%) followed by A+T+N- (n=33, 16%). Patients in the *AD continuum* were oldest compared to other categories, with the lowest age in A-T-N- (65±7 years) and highest age in A+T+N+ (74±6 years). Hallucinations were more often present in patients within the *AD continuum* compared to patient with *normal AD biomarkers* (Figure 1). Parkinsonism was least frequently present in patients with *non-AD pathologic change* (especially A-T+N+ and A-T+N-) compared to patients within the *AD*

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continuum (especially A+T-N- and A+T-N+) (Figure 1, Table 1). RBD was least frequently reported in A+T+N+. Other clinical symptoms did not differ between ATN profiles. Patients within A+T-N- and A+T+N- groups were most frequently APOE-e4 carrier. Patients with N+ most often had a Fazekas score of 2 or higher compared to A-T-N-, indicating significant cerebrovascular lesions.

Cognitive decline

Global cognition, as measured with MMSE, did not differ between the ATN profiles at baseline. Over time, patients in the *AD continuum* had steeper decline on MMSE compared to patients with *normal AD biomarkers* (figure 4). When comparing eight categories, we found that this decline was specifically present in A+T-N- ($\beta \pm SE$ = -1.27$\pm$0.42), A+T+N- ($\beta \pm SE$ = -0.82$\pm$0.45) and A+T+N+ ($\beta \pm SE$ = -0.98$\pm$0.52).

In addition to global cognition, we investigated specific neuropsychological tests to explore differences in separate cognitive functions. Baseline neuropsychological test scores did not differ between the three ATN categories. By contrast, there were several interactions between time and ATN category, indicating different cognitive slopes over time. Patients within the *AD continuum* had steeper decline on VAT-A, indicating more severe deterioration in memory compared to *normal AD biomarkers*. In addition, AD continuum patients had steeper decline on semantic fluency and VOSP number location compared to normal AD biomarkers, but these effects did not survive FDR correction (Table 2). When inspecting the eight categories, we found that the decline on VAT-A was specifically present for A+T-N- ($\beta \pm SE$ = -0.71$\pm$0.24) and A+T+N- ($\beta \pm SE$ = -0.63$\pm$0.24), while decline on animal fluency was driven by A+T+N- ($\beta \pm SE$ = -0.84$\pm$0.35) and A+T+N+ ($\beta \pm SE$ = -1.27$\pm$0.43). The decline on the VOSP number location test was driven by the A+T-N- profile ($\beta \pm SE$ = -0.61$\pm$0.16). Last, A+T-N- had steeper decline on the TMT-B/TMT-A ratio ($\beta \pm SE$ = -0.14$\pm$0.04), indicating steeper decline in executive functioning. After FDR correction on this eight category analyses, only the effects for VOSP number location and TMT-B/TMT-A remained significant (Supplementary Table 1).

Mortality

During follow-up, n=103 patients (49%) died after a median time of 4.6 years [IQR 2.5-6.0]. The repeated survival analysis on the three groups showed increased mortality hazard (HR=1.6[1.0-2.6]) in the *AD continuum* group, while *non-AD pathologic change* did not differ from *normal AD biomarkers* (figure 5). When inspecting the eight categories, cox regression analysis showed that, compared to A-T-N-, patients in the A-T+N+ and A+T-N+ profiles had increased hazard of death (A-T+N+: HR=3.6[1.4-9.6], A+T-N+: HR=2.7[1.3-5.6]), while A-T+N- had a decreased hazard ratio (HR[CI]=0.4[0.2-1.0]) (Figure 2).

Discussion

When applying the ATN framework to our DLB cohort, the *normal AD biomarker* profile (A-T-N-) was the most common ATN profile, accounting for a quarter of all patients. The remaining 75% of

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patients had at least one abnormal AD biomarker (A, T or N) of which A+T-N- was most common. More than half of the patients fell into the *AD continuum* (A+). ATN profiles were associated to clinical symptomatology; A+T+N+ had least frequent RBD and parkinsonism was more frequently observed in A+T- profiles compared to A-T+. Patients with A+ profiles had worse prognosis, specifically steeper cognitive decline and increased mortality risk, compared to *normal AD biomarkers*. *Non-AD pathologic change* and *normal AD biomarkers* did not differ in clinical presentation or prognosis.

Concomitant AD pathology has been related to unfavorable cognitive outcomes in DLB in previous studies (6, 7). By applying the ATN framework, we were able to extend on these findings by more specifically looking into various stages of AD pathology. Decline on cognitive tests was most pronounced in the A+T-N- and A+T+N- groups, and was predominantly present on tests that address memory functioning, such a semantic fluency and a visual memory task. There were no clear differences between the A+T- (*Alzheimer's pathological change*) and A+T+ (*Alzheimer's disease*) profiles in terms of cognitive decline. This underlines that amyloid pathology, more than tau or neurodegeneration, is associated to (longitudinal) memory functioning, consistent with previous work in DLB (9) and studies investigating the prognostic properties of the ATN framework in cognitively normal populations (26, 27). Patients in the *AD continuum* more often had visual hallucinations, compared to the *normal AD biomarkers* category, which is in line with previous research investigating AD co-pathology in DLB (6). Possibly, there is a relation between presence of visual hallucinations and the severity of memory impairment, that is often more pronounced in patients with A+ (28). RBD was least frequently present in patients A+T+N+ profile. In accordance with our findings, two previous studies in autopsy confirmed DLB patients also found more severe AD-related pathology in RBD negative patients (29, 30). Although longitudinal studies investigating DLB symptomatology are limited, it is possible that our results indicate that RBD becomes less prevalent in more severe disease stages, associated with more severe AD pathology. An alternative explanation is that RBD occurs more frequently in 'pure' DLB, associated with more brainstem pathology, while other symptoms such as parkinsonism and visual hallucinations become more prevalent with increasing disease severity and pathology, and thus occur more frequently in patients with concomitant AD pathology. Prospective studies are needed to confirm this hypothesis.

The largest abnormal biomarker group was A+T-N-. The meaning of isolated abnormal amyloid in DLB is not yet completely understood. Jack et al. refer to this profile as Alzheimer's pathologic change, which could be interpreted as early-stage Alzheimer's disease (1). This implies that over time, these patients will develop the full AD profile, with abnormal tau pathology. An alternative explanation could be that amyloidosis occurs in DLB as co-phenomenon to alpha-synucleinopathies, which will hence not necessarily evolve to full-blown AD-pathology over time. It is hypothesized that DLB-specific dysregulation results in abnormal accumulation of amyloid. In contrast to in AD, in

DLB not only CSF Aβ42 peptides are decreased, but lowered levels of Aβ40 and Aβ38 are also observed, independent of concomitant AD pathology, which suggests that abnormal amyloid levels in DLB could be the result of a DLB specific mechanism (31). Changes in CSF dynamics could also explain the isolated lowered Aβ42 concentrations. For example, higher volume of CSF in the brain or low Aβ producers could result in lower CSF Aβ42 concentrations (32). Presumably, all three explanations (Alzheimer's disease co-pathology; DLB related amyloid pathology and variations in CSF dynamics) play a role in DLB. Studies with repeated measurements of A and T markers could shed light on the nature and progression of amyloid pathology in DLB.

Twenty-five (12%) patients in our cohort had abnormal CSF p-tau levels, while amyloid was within the normal range (A-T+). The meaning of isolated tau pathology in DLB is not clear. These patients might reflect a different DLB subtype. Cognitive functioning in these groups did not differ from the *normal AD biomarker* group, which in line with previous studies (9, 33). On the other hand, there were associations between A-T+ categories and clinical symptomatology, as patients with A-T+ had less frequent parkinsonism. These findings are consistent with previous reports that found associations between tau pathology and parkinsonism (9, 34). Ferreira et al. also report absence of a relation with age in A-T+ profiles, which supports the suggestion of a distinguished DLB subtype and is consistent with our data. One should be cautious interpreting p-tau findings, as isolated abnormal p-tau levels are not necessarily related to neurodegeneration and could be related to processes in normal aging, such as lower CSF production or clearance (35). On the other hand, abnormal p-tau in combination with normal Aβ42 levels could still reflect amyloid-pathology due to individual differences. To overcome this, the use of ratios is recommended. When comparing the ATN framework to the commonly used CSF p-tau/Aβ42 ratio, we found some discrepancies. In 43% of patients with *non-AD pathologic change*, the p-tau/Aβ42 ratio was indicative of AD pathology (data not shown). This suggests that the ATN framework may inaccurately classify patients as non-AD pathology, while in fact, AD related processes are evolving. The use of binary outcomes instead of continuous variables is of the shortcomings of the ATN classification.

Mortality risk was slightly higher for patients within the *AD continuum*, compared to *normal AD biomarkers* and *non-AD pathologic change* categories. When inspecting the eight profiles, mortality risk was higher in profiles positive for neurodegeneration (A+T-N+ and A-T+N+) and lower in a profile without neurodegeneration (A-T+N-). Although interpretation should be done with caution since based on small numbers, the common factor in these profiles was the N; it seems that mortality was most strongly associated to biomarker evidence of neurodegeneration. CSF t-tau, but not p-tau or amyloid, has previously been related to increased mortality risk in DLB (36). Since CSF t-tau is an alternative marker for neurodegeneration within the ATN framework, these findings are in line with our observations of increased mortality associated to neurodegeneration. The observation that neurodegeneration is related to mortality risk is not surprising, as the N in the ATN framework is

referred to as a pathological staging marker. Patients with more neurodegeneration were likely in a more advanced disease stage, and therefore at increased mortality risk. When applying the ATN framework in non-AD dementia, it is not completely possible to distinguish whether the neurodegeneration is attributable to AD related pathology or non-AD pathology. In this study, we used medial temporal atrophy for defining the N, since this is highly linked to AD pathology (37, 38). One could argue that the degree of neurodegeneration regardless the etiology is linked to prognosis and mortality, and other markers could have been used. Adding more generalized markers for neurodegeneration could result in different classification results (39).

The ATN framework was primarily developed to provide a common classification method for patients within the AD continuum. Our study underlines that the ATN framework could be very well applied in non-AD, especially in DLB where mixed pathology is common. Mixed pathology is rather rule than exception in most dementia cases. Characterizing pathologic burden in dementia patients is of potential interest to both clinical and research purposes. As has already been suggested by the authors of the framework, adding markers for non-AD pathology associated with dementia like Lewy Body, TDP-43 and vascular pathology, would support classification of patients by pathology alongside clinical criteria (1). This could aid in predicting disease progression but also stratification for therapeutic trials. The ATN framework is designed to be expandable with new biomarkers (ATxN) and reliable biomarkers for non-AD pathology are emerging. For Lewy body pathology, real time quaking induced conversion assays (RT-Quic) has shown promising results in detecting alpha-synuclein pathology, even in prodromal stages of DLB (40, 41). When expanding the framework with markers for non-AD pathologies, more generalized markers should be used to define the N, such as neurofilament light (NFL) or FDG-PET, as they are more sensitive in detecting neurodegeneration caused by any pathology (39).

Among the strengths of our study is the relatively large sample size with longitudinal data available. Few limitations should be noted. First, the distribution of the cohort in the eight profiles led to small sample sizes for some of the profiles. Although we expect this to be a reflection of the true distribution of biomarker profiles in a DLB setting, it hampers the interpretation of the clinical presentation and relations studied. Second, the longitudinal cognitive data might be influenced by some degree of attrition. Patients who are in a more advanced stage are likely to have shorter duration of follow-up. Although the mixed models can account for missing data, the results might still be an underrepresentation of the actual effect. Third, information on the presence of RBD and fluctuations was retrospectively rated from medical charts. Standardized measures to assess fluctuations and RBD are available, and should be incorporated in future prospective studies. A last remark is that we used cut-points for determining biomarker abnormality from AD research. Some recent studies suggest that

To conclude, applying the ATN framework in a DLB population supports our understanding of the different aspects of AD pathology in DLB and could be useful in identifying patients who are at risk for worse prognosis. Expanding the framework to ATxN would enable the use of the framework to characterize dementia patients with mixed pathology which could enhance proper stratification of patients for therapeutic trials.

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Conflicts of interest

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FIGURES

Figure 1: Distribution of ATN profiles across DLB patients (n=202). Pie chart illustrates the distribution of the 8 ATN profiles and corresponding 3 ATN categories.

Figure 2: Cox proportional hazards analyses to estimate survival differences between ATN profiles in DLB. Models are corrected for age and sex, A-T-N- was regarded as reference. Time to death in years is presented as median [interquartile range]. Abbreviations: HR: Hazard ratio; CI: confidence interval.

Figure 3: DLB symptom presence across three ATN categories. *p<0.05, but not significant after FDR correction. **p<0.05 and significant after FDR correction

Figure 4: Cognitive decline across ATN categories. Regression lines represent estimated MMSE scores based on linear mixed models including 95% confidence intervals, corrected for age and sex.

Figure 5: Kaplan-Meier curves for survival differences across ATN categories.

Table 1: Baseline characteristics

	A-T-N- (n=50)	A-T+N- (n=20)	A-T-N+ (n=12)	A-T+N+ (n=5)	A+T-N- (n=41)	A+T+N- (n=33)	A+T-N+ (n=21)	A+T+N+ (n=20)	p*
Age, years	65±7**[c,f,g,h]**	67±8**[c,h]**	73±4**[a,b,e]**	68±6	67±6**[c,h]**	68±5**[a,h]**	71±5**[a]**	74±6**[a,b,e,f]**	**<0.001**
Sex, n female (%)	4 (8%)	2 (10%)	3 (25%)	1 (20%)	9 (22%)	9 (27%)	3 (14%)	2 (10%)	0.305
Duration of complaints (n=199)	3 [2-4]	4 [2-5]	3 [2- 4]	4 [4-4]	3 [2-5]	3 [2-4]	3 [2-4]	2[1-3]	0.195
MMSE	24±3	25±3	24±2	23±4	24±3	24±3	22±4	24±4	0.191
Clinical symptoms, n (%)									
Visual hallucinations	23 (46%)	11 (55%)	6 (50%)	5 (100%)	23 (56%)	25 (76%)	15 (71%)	12 (60%)	0.076
Parkinsonism	33 (66%)	9 (45%)*[e,g]*	9 (75%)	1 (20%)*[e,g]*	31 (76%)**[b,d]**	24 (73%)	19 (91%)**[b,d]**	14 (70%)	**0.017**
Fluctuations (n=165)	28 (74%)	12 (75%)	6 (60%)	3 (75%)	27 (79%)	25 (86%)	15 (83%)	14 (88%)	0.686
RBD (n=165)	32 (80%)*[h]*	13 (81%)*[h]*	5 (50%)	2 (50%)	27 (79%)*[h]*	21 (70%)	10 (67%)	6 (38%)*[a,b,e]*	**0.034**
Orthostatic hypotension (n=159)	23 (62%)	8 (61%)	3 (33%)	1 (33%)	25 (67%)	17 (58%)	10 (59%)	7 (50%)	0.654
Depressive symptoms (n=184)	9 (20%)	5 (31%)	3 (25%)	0 (0%)	5 (12%)	3 (10%)	5 (25%)	4 (25%)	0.485
Biomarkers, n (%)									
Global cortical atrophy >1 (n=181)	7 (16%)	2 (12%)	3 (27%)	2 (50%)	4 (11%)	3 (10%)	3 (15%)	7 (35%)	0.149
Parietal atrophy >1 (n=174)	11 (26%)	4 (27%)	5 (50%)	1 (33%)	7 (19%)	4 (14%)	4 (22%)	8 (40%)	0.298
Fazekas >1 (n=180)	1 (2%)**[c,g,h]**	3 (18%)	5 (46%)**[a]**	0 (0%)	6 (17%)	4 (14%)	5 (25%)*[a]*	6 (30%)*[a]*	**0.015**
APOE-e4 carrier (n=178)	18 (38%)*[e,f]*	9 (50%)	1 (9%)*[e,f]*	3 (75%)	26 (70%)**[a,c]**	19 (68%)*[a,c]*	9 (53%)	8 (53%)	**0.004**
DAT-scan, n abnormal (n=119)	27 (90%)	12 (86%)	5 (71%)	2 (67%)	19 (91%)	20 (91%)	9 (90%)	11 (92%)	0.798
Ratio p-tau/abeta, n abnormal	2 (4%)**[b,d,e,f,g,h]**	12 (60%)**[a,c,f,h]**	0 (0%)**[b,d,e,f,g,h]**	4 (80%)**[a,c]**	19 (46%)**[a,c,f,h]**	32 (100%)**[a,c,g]**	10 (50%)**[a,c,f,h]**	20 (100%)**[a,b,c,e,g]**	**<0.001**
EEG, n abnormal (n=150)	38 (100%)**[b]**	13 (81%)**[a]**	9 (100%)	4 (80%)	29 (100%)	24 (100%)	14 (100%)	13 (87%)	**0.004**

Data represent mean± standard deviation, median [interquartile range] or n (%).

*p-value represents the overall group difference between all eight profiles. In case of p<0.05, post hoc pairwise analyses analyzed which profiles differed (indicated with letters); [A] p<0.05 compared to A-T-N-, [B] p<0.05 compared to A-T+N-, [C] p<0.05 compared to A-T-N+, [D] p<0.05 compared to A-T+N+, [E] p<0.05 compared to A+T-N-, [F] p<0.05 compared to A+T+N-, [G] p<0.05 compared to A+T-N+, [H] p<0.05 compared to A+T+N+. Bold: pairwise comparison remaining significant after false discovery rate correction with q<0.05. Italics: pairwise comparison did not survive FDR correction. Abbreviations: MMSE: Mini-Mental State Examination; RBD: Rapid eye movement sleep behavior disorder; GDS: Geriatric Depression Scale.

Table 2: Slopes of cognitive test scores over time per ATN category

		non-AD pathology vs normal AD biomarkers	*AD continuum* vs normal AD biomarkers
MMSE	*Baseline*	0.78 (0.71)	-0.22 (0.58)
	Slope	0.06 (0.41)	-0.87 (0.33)**
TMT-A	*Baseline*	0.11 (0.14)	0 (0.11)
	Slope	0.04 (0.03)	-0.05 (0.03)
TMT-B/TMT-A	*Baseline*	0.19 (0.44)	0.46 (0.39)
	Slope	0.04 (0.14)	0.06 (0.14)
RAVLT immediate recall	*Baseline*	1.68 (1.92)	0.57 (1.53)
	Slope	0.31 (0.45)	-0.68 (0.41)
RAVLT delayed recall	*Baseline*	0.64 (0.59)	0.1 (0.47)
	Slope	0.02 (0.12)	-0.14 (0.11)
VAT-A	*Baseline*	0.26 (0.73)	-0.33 (0.59)
	Slope	0.01 (0.2)	-0.55 (0.18)**
Animal fluency	*Baseline*	0.68 (1.15)	0.09 (0.92)
	Slope	-0.34 (0.27)	-0.67 (0.26)*
VOSP - dot counting	*Baseline*	-0.01 (0.36)	-0.24 (0.29)
	Slope	0.29 (0.12)*	-0.03 (0.11)
VOSP - fragmented letters	*Baseline*	0.51 (1.08)	-0.38 (0.86)
	Slope	0.33 (0.24)	-0.14 (0.22)
VOSP - number location	*Baseline*	0.08 (0.4)	0.06 (0.32)
	Slope	0.03 (0.13)	-0.29 (0.12)*

Data represent β(SE) as estimated by linear mixed models, corrected for age and sex (normal AD biomarkers (A-T-N-) as reference). β baseline = Association between ATN category and baseline test result. β Slope = association with annual decline. TMT data are log-transformed and inversed. *$p<0.05$. ** $p<0.05$ and remaining significant after false discovery rate correction with $q<0.05$. Abbreviations: RAVLT: Dutch version of the Rey Auditory Verbal Learning test; TMT: Trail-Making Test; VAT-A: Visual Association Test version A; VOSP: Visual Object and Space Perception battery. Total number of observations per model: MMSE: 597, TMT-A: 454, TMT-B/TMT-A: 211, RAVLT: 477, VAT-A: 485, Animal fluency: 479, VOSP dot counting: 383, fragmented letters: 401, number location: 421.