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Featured Article

Frequency and longitudinal clinical outcomes of Alzheimer's AT(N) biomarker profiles: A longitudinal study

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Abstract

Introduction: We aimed to estimate the frequency of each AT(N) (β-amyloid deposition [A], pathologic tau [T], and neurodegeneration [N]) profile in different clinical diagnosis groups and to describe the longitudinal change in clinical outcomes of individuals in each group.

Methods: Longitudinal change in clinical outcomes and conversion risk of AT(N) profiles are assessed using linear mixed-effects models and multivariate Cox proportional-hazard models, respectively.

Results: Participants with A+T+N+ showed faster clinical progression than those with A−T−N− and A+T±N−. Compared with A−T−N−, participants with A+T+N± had an increased risk of conversion from cognitively normal (CN) to incident prodromal stage of Alzheimer's disease (AD), and from MCI to AD dementia. A+T+N+ showed an increased conversion risk when compared with A+T±N−.

Discussion: The 2018 research framework may provide prognostic information of clinical change and progression. It may also be useful for targeted recruitment of participants with AD into clinical trials.

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Keywords: Alzheimer's disease; Research framework; Biomarker; Prognosis

1. Background

Alzheimer's disease (AD) is characterized by amyloid plaques, tau tangles, synapse loss, neurodegeneration leading to impairments on memory, and other cognitive domains and subsequently dementia syndrome. Before the use of biomarkers, including magnetic resonance imaging (MRI) to detect atrophy, amyloid positron emission tomography (PET) scans, and cerebrospinal fluid (CSF) measurements to measure amyloid and tau, AD could only be diagnosed with certainty at autopsy. In 2011, the National Institute on Aging–Alzheimer's Association (NIA-AA) published research criteria for AD diagnosis [1]: dementia due to Alzheimer's disease, prodromal AD (mild cognitive impairment [MCI] due to AD), and preclinical AD (individuals with normal cognition who have AD pathology). Similarly, other criteria including the International Working Group-2 (IWG-2) [2] and Dubois criteria [3] were reported.

Recently, the NIA-AA published an updated research framework defining AD biologically by neuropathological biomarkers which are independent from clinical symptoms. By updating the 2011 guidelines [4], the new 2018 research framework grouped biomarkers into three categories: biomarkers of amyloid β (Aβ) plaques (labeled "A"): cortical amyloid PET ligand binding or low CSF $A\beta_{42}$; biomarkers of paired helical filament tau (labeled "T"): elevated CSF phosphorylated tau (p-tau) and cortical tau PET ligand binding; biomarkers of neurodegeneration or neuronal injury (labeled "N"): elevated CSF total tau (t-tau), 18F-fluorodeoxyglucose (FDG) PET, and brain atrophy on MRI. Dichotomizing these biomarkers as normal or abnormal results in eight AT(N) profiles. The idea of AT(N) biomarker grouping did not originate with the NIA-AA research framework. It was first proposed by an international group of investigators in 2016 [5]. The research framework indicated that if an individual presents with both biomarker evidence of Aβ and pathological tau, the term "Alzheimer's disease" would be applied. Symptoms of AD are treated as a phase of an "Alzheimer's continuum" and can be used to stage severity of the disease.

This recommendation is labeled a "research framework" because its intended use is for observational and interventional research but not for clinical use. Applying the framework in a large longitudinal cohort would help researchers to modify this framework if needed before it being adopted into actual clinical practice. Recent cross-sectional studies from the Mayo Clinic and H70 Gothenburg Birth Cohort reported the prevalence of each AT(N) profile in cognitively unimpaired individuals [6,7]. However, this study is limited by its cross-sectional design, and longitudinal data is hence necessary and urgently needed to provide important information on the clinical/cognitive outcomes of these AT(N) profiles. The Alzheimer's Disease Neuroimaging Initiative (ADNI) is a very large multisite longitudinal observational study with the objective of validating biomarkers for AD clinical trials [8–10]. ADNI makes all data available without embargo to all qualified scientists, leading to more than 1500 publications [11,12]. To date, all ADNI publications either use purely clinical classifications (dementia, MCI, subjective memory complaints, or cognitively normal), or the previous NIA-AA classifications (previously described). The objective of the present study was to estimate the frequency of AT(N) profiles in clinical diagnosis groups and to describe the longitudinal change in clinical outcomes of individuals in each group.

2. Methods

2.1. ADNI study design

We undertook cross-sectional and longitudinal analyses of participants enrolled in the ADNI database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private

partnership with the primary goal testing whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of MCI and early AD. For up-to-date information on ADNI, see www.adni-info.org. ADNI was approved by the institutional review boards of all participating institutions. Written informed consent was obtained from all participants at each site.

2.2. Participants

Individuals from the ADNI were included in our study if they underwent amyloid PET or CSF Aβ analysis (A), CSF p-tau examination (T), and FDG PET (N) at baseline. Detail information of the included participants was presented in the Supplementary Material. Amyloid abnormal (A+) and normal (A−) were determined by applying a cutoff value of 1.11 for the florbetapir standardized uptake value ratio (SUVr) and 192pg/ml for CSF $A\beta_{42}$ [13]. Whether tau pathology was abnormal (T+) or normal (T−) was determined by a cutoff value of 23 pg/ml for CSF p-tau level [13]. The cutoff point for FDG PET (N) (average of angular, temporal, and posterior cingulate) was 1.21 [14]. As a secondary analysis, abnormal N was defined as hippocampal volume adjusted for total intracranial volume (HVa) of less than 6723 mm^3 [13,15] (Supplementary Material). For the present study, we excluded borderline cases and reset the cutoffs that were ±5% from the original cutoffs to avoid drawing conclusions based on borderline cases (Supplementary Table 1). In our study, we stratified the MCI group into stable MCI (sMCI) with no progression to AD dementia during at least 2 follow-up years and progressive MCI (pMCI) with progression to AD dementia during at least 2 follow-up years. Controls had Mini-Mental State Examination (MMSE) scores of 24 or higher and a Clinical Dementia Rating (CDR) score of 0.

2.3. CSF measurements

CSF $A\beta_{42}$ and p-tau were measured at the ADNI Biomarker Core Laboratory (University of Pennsylvania) using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use only reagents) immunoassay kit-based reagents. All CSF biomarker assays were performed in duplicate and averaged.

2.4. Neuroimaging and cognition

Amyloid PET imaging was measured with florbetapir. Florbetapir binding images were averaged, spatially aligned, interpolated to a common voxel size (1.5 mm^3), and smoothed to a common resolution of 8 mm full width at half maximum. The global 18F-florbetapir SUVr was calculated by averaging the 18F-florbetapir retention ratio from four large cortical gray matter regions (frontal, anterior cingulate, precuneus, and parietal cortex) using the cerebellum as a reference region.

FDG-PET data were acquired and reconstructed according to a standardized protocol (http://adni.loni.ucla.edu/). Spatial normalization of each individual's PET image to the standard template was conducted using SPM529. For FDG-PET, we averaged counts of angular, temporal, and posterior cingulate regions.

Structural MRI was performed using a Siemens Trio 3.0 T scanner (n = 507) or Vision 1.5 T scanner (n = 131) (GE, Siemens, and Philips). Regional volume estimates were processed using Free-surfer software package version 4.3 and 5.1 image processing framework for the 1.5 and 3.0 T MRI images, respectively. ROIs included the hippocampus and ventricles. Estimated intracranial volume (ICV) was used to adjust ROIs for head size variation based on covariance.

General cognition was assessed by MMSE and Alzheimer Disease Assessment Scale–cognitive subscale (ADAS-Cog) 11 score (Supplementary Material).

2.5. Statistical analysis

We summarized binomial distributions with percentages and calculated 95% confidence intervals for percentages using the Wilson method. Differences across the eight biomarker profiles were tested by the Kruskal-Wallis tests for continuous variables and chi-squared tests for categorical data. To evaluate how clinical outcomes changed overtime, we included A−T−N−, A+T−N−, A+T+N−, and A+T+N+ using linear mixed-effects models. To access the risk of progression from no cognitive impairment to incident prodromal stage of AD indicated by the CDR–global score (CDR-GS) of 0.5 or greater and from MCI to incident AD dementia, we constructed unadjusted Kaplan-Meier plots. In addition, we ran multivariate Cox proportional-hazards models (Supplementary Material). All statistical analyses were performed using the R statistical software (version 3.4.4).

3. Results

Of the 645 individuals (198 CN, 310 MCI, and 137 AD dementia) who were assessed at enrollment, 541 participants had follow-up data of at least one year. A total of 283 participants were also assessed at 3 years, 238 participants at 4 years, and 80 participants at 5 years. The mean (standard deviation [SD]) duration of follow-up for each cognitive status group were presented in Supplementary Table 2. The demographic, clinical, and imaging characteristics of the included participants are shown in Table 1 and Supplementary Table 3. We added a flowchart to demonstrate the participant screening process (Supplementary Fig. 1). The mean (SD) age of the participants was 72.7 (7.3) years; 53.6% were men; 98.6% had more than 12 years of education; 47.0% had an *APOE* ε4 allele.

Table 1
Characteristics of 645 participants by AT(N) biomarker classification

Variable	A−T−N−	A−T+N−	A−T−N+	A−T+N+	A+T−N−	A+T+N−	A+T−N+	A+T+N+	*P* value*
n	83	114	15	8	25	168	13	219	
Age, mean (SD), years	71.03 (6.68)	70.62 (6.97)	76.09 (8.61)	78.19 (5.31)	71.38 (6.83)	73.41 (6.80)	76.31 (6.47)	73.40 (7.58)	<.001
Male, no. (%)	38 (45.8)	60 (52.6)	10 (66.7)	5 (62.5)	15 (60.0)	78 (46.4)	11 (84.6)	129 (58.9)	.04
APOE ε4 genotype carriers, no. (%)	9 (10.8%)	24 (21.1%)	1 (6.7%)	3 (37.5%)	8 (32.0%)	99 (58.9%)	6 (46.2%)	153 (69.9%)	<.001
Education, mean (SD), years	16.51 (2.67)	16.63 (2.60)	16.33 (3.15)	15.12 (1.46)	15.48 (3.15)	15.86 (2.60)	16.15 (3.29)	15.89 (2.91)	.143
MMSE score, mean (SD)	28.66 (1.59)	28.74 (1.48)	28.13 (2.39)	26.50 (2.98)	28.84 (1.07)	28.23 (1.80)	26.54 (3.57)	24.78 (2.70)	<.001
Hippocampus, mean (SD), mm^3	7405.01 (811.68)	7579.93 (987.22)	7065.33 (911.39)	5789.17 (1802.62)	7696.72 (769.82)	7136.62 (980.27)	6026.30 (1031.97)	6131.52 (1012.89)	<.001
Amyloid PET, SUVr, mean (SD)	1.01 (0.06)	1.02 (0.05)	1.01 (0.06)	1.01 (0.08)	1.14 (0.21)	1.33 (0.19)	1.17 (0.14)	1.43 (0.17)	<.001
FDG, mean (SD)	1.38 (0.08)	1.36 (0.08)	1.10 (0.05)	1.07 (0.04)	1.39 (0.08)	1.37 (0.09)	1.04 (0.10)	1.04 (0.09)	<.001

NOTE. Data are mean (SD) or number (%) unless otherwise stated.

Abbreviations: SD, standard deviation; MMSE, Mini-Mental State Examination; FDG, 18F-fluorodeoxyglucose; A−, amyloid normal using amyloid PET or CSF Aβ; A+, amyloid abnormal using amyloid PET or CSF Aβ; T−, tau normal using CSF p-tau; T+, tau abnormal using CSF p-tau; N−, neurodegeneration or neuronal injury normal using FDG; N+, neurodegeneration or neuronal injury abnormal using FDG.

**P* values are from the Kruskal-Wallis test or Fisher's exact test.

3.1. Frequency of AT(N) profiles based on four traditional clinical diagnostic groups

The proportion of abnormal amyloid was 41.4% (95% CI: 34.5% to 48.6%) in CN group, 58.2% (95% CI: 51.5% to 64.7%) in sMCI group, 96.5% (95% CI: 90.0% to 99.3%) in pMCI group, and 94.9% (95% CI: 89.8% to 97.9%) in AD dementia group. A+T+N± accounted for 32.5% in CN group, 42.0% in sMCI group, compared with 93.0% in pMCI group and 92.0% in AD dementia group. The proportion of A+T+N+ was 2.5% in CN group, 15.1% in sMCI group, 70.7% in pMCI group, and 87.6% in AD dementia group. Suspected non-AD pathophysiology (SNAP) (A−T+N−, A−T−N+, and A−T+N+) accounted for 39.4% in CN group and 22.7% in sMCI group, compared with only 3.5% in pMCI group and 3.6% in AD dementia group (Fig. 1). AT(N) proportion by traditional clinical diagnosis was also calculated using HVa as the N measure (Supplementary Fig. 2). We found the proportion of N in the entire group varied with the methods we used (7% using FDG-PET and 41.46% using HVa). Moreover, there were no participants that had the biomarker combinations A+T−N+ and A−T−N+.

3.2. Clinical and demographic characteristics of individuals in each AT(N) profile

Among "Alzheimer's continuum" profiles (A+T−N−, A+T+N−, A+T−N+, and A+T+N+), 30.6% were clinically diagnosed with "AD dementia." For those with SNAP, 3.6% were clinically diagnosed with "AD dementia." In the A−T−N− profile, only 2.4% were clinically diagnosed with "AD dementia" (Supplementary Fig. 3). Proportion of diagnosis in each AT(N) profile was also calculated using HVa as the N measure (Supplementary Fig. 4). All MRI (hippocampal and ventricular volumes) and cognitive (ADAS-COG 11 and MMSE) measures were different among A−T−N−, A+T−N−, A+T+N−, and A+T+N+ profiles at baseline in patients with either CN or MCI after adjustment for age, gender, ICV (for MRI), and years of education (for cognitive measures) (Supplementary Fig. 5).

3.3. Longitudinal clinical outcomes in each AT(N) profiles

In individuals with CN, only A+T+N+ individuals showed changes in MMSE score. Furthermore, changes in hippocampal and ventricular volumes were observed in all four biomarker profiles (A−T−N−, A+T−N−, A+T+N−, and A+T+N+) (Fig. 2). As expected, CN individuals with A+T+N+ showed faster clinical progression than the remaining three profiles (A−T−N−, A+T−N−, and A+T+N−). However, no significant differences were detected between A+T−N− versus A−T−N− and A+T+N− versus A+T−N− (Supplementary Table 4).

Among patients with MCI, cognitive changes were observed in A+T+N±. However, changes in hippocampal and ventricular volumes were observed in all four profiles (Fig. 2). MCI patients with A+T+N+ also showed faster clinical progression than the remaining three profiles. However, no significant differences in clinical progression were detected between A+T−N− versus A−T−N− in MCI patients (Supplementary Table 4). In addition, longitudinal analysis for cognitive decline adjusted for age, gender, *APOE* ε4 status, and education years and analyses for brain atrophy adjusted for age, gender, *APOE* ε4 status, total intracranial volume, and

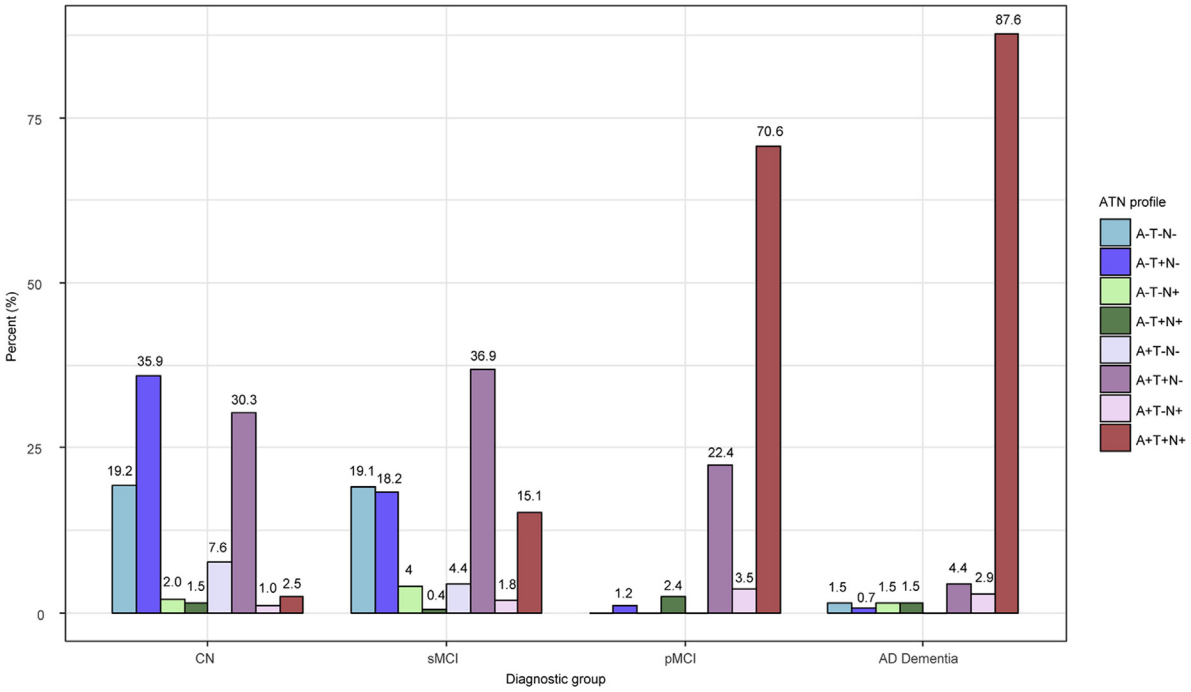

Fig. 1. Proportion of each AT(N) profile in different clinical diagnosis group. Abbreviations: AD, Alzheimer's disease, MCI, mild cognitive impairment; CN, cognitively normal; A−, amyloid normal using amyloid PET or CSF Aβ; A+, amyloid abnormal using amyloid PET or CSF Aβ; T−, tau normal using CSF p-tau; T+, tau abnormal using CSF p-tau; N−, neurodegeneration or neuronal injury normal using FDG; N+, neurodegeneration or neuronal injury abnormal using FDG.

field strength (1.5 T vs. 3.0 T) are presented in Supplementary Fig. 6. Because of the general unavailability of amyloid PET or FDG-PET for routine clinical use in many geographic locations, we added a longitudinal analysis of the data based only on CSF and MRI measures (Supplementary Fig. 7).

We further accessed the changes in clinical outcomes of subgroups stratified by gender and *APOE* ε4 status

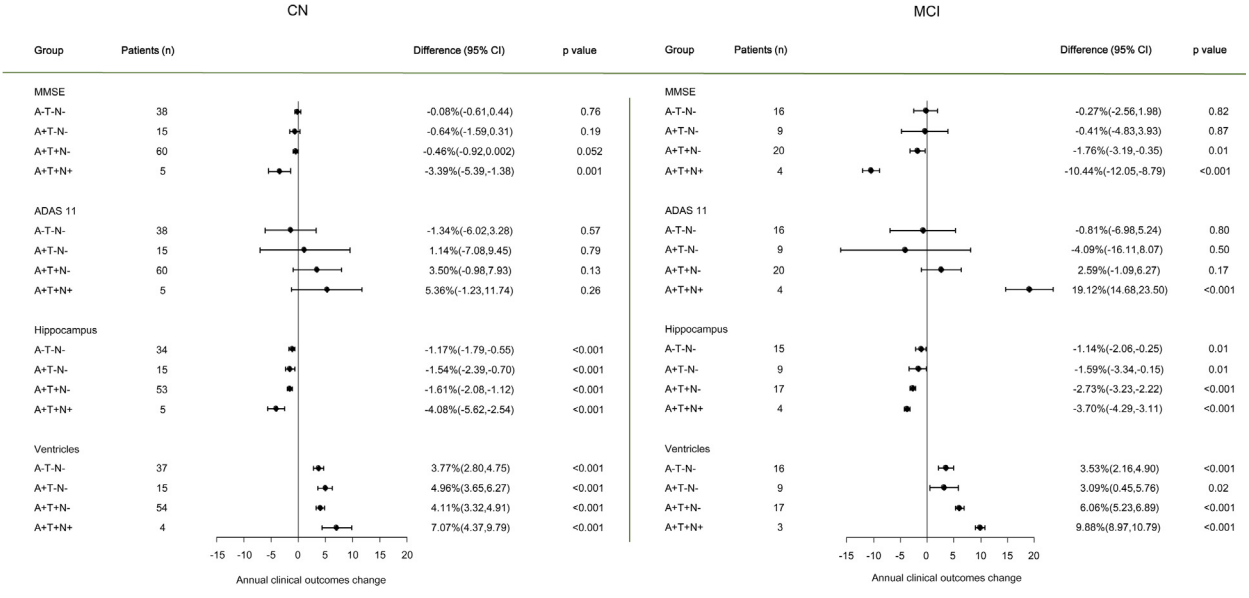

Fig. 2. Change in clinical outcomes among the four AT(N) profiles based on linear mixed-effects regression models. Analyses of cognitive decline were adjusted for age, gender, and education years. Analyses of brain atrophy were adjusted for age, gender, total intracranial volume, and field strength (1.5 T vs. 3T). Change in clinical outcomes is expressed as an annual percentage of cognitive function scores and volume change, with 95% CIs and *P* value. Abbreviations: MCI, mild cognitive impairment; CN, cognitively normal; A−, amyloid normal using amyloid PET or CSF Aβ; A+, amyloid abnormal using amyloid PET or CSF Aβ; T−, tau normal using CSF p-tau; T+, tau abnormal using CSF p-tau; N−, neurodegeneration or neuronal injury normal using FDG; N+, neurodegeneration or neuronal injury abnormal using FDG.

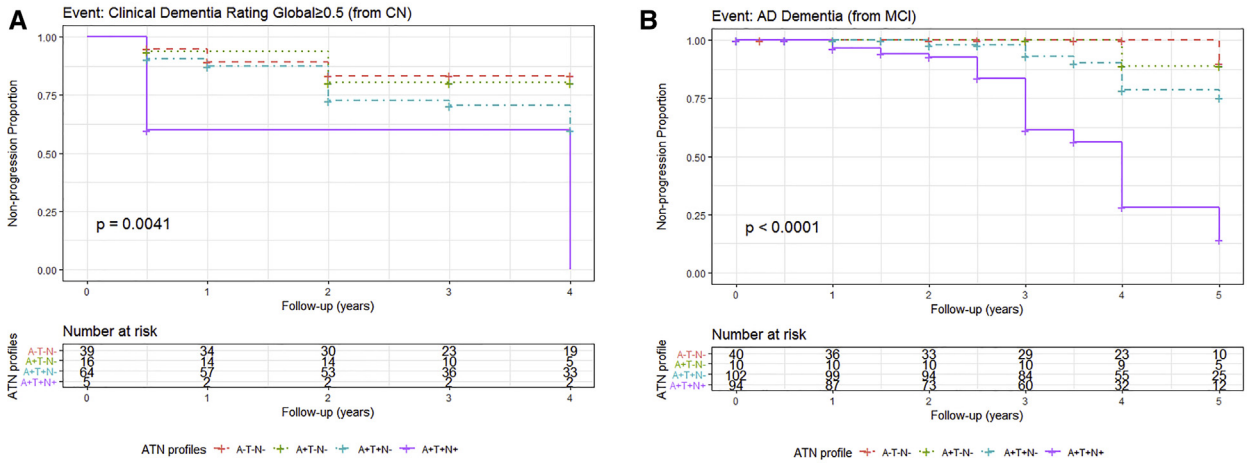

Fig. 3. Kaplan-Meier curves showing cumulative probability of disease progression. (A) Progression from cognitively normal participants to incident prodromal stage of AD indicated by a CDR–global score of 0.5. (B) Progression from mild cognitive impairment to incident AD dementia. Abbreviations: AD, Alzheimer disease; MCI, mild cognitive impairment; CN, cognitively normal; A−, amyloid normal using amyloid PET or CSF Aβ; A+, amyloid abnormal using amyloid PET or CSF Aβ; T−, tau normal using CSF p-tau; T+, tau abnormal using CSF p-tau; N−, neurodegeneration or neuronal injury normal using FDG; N+, neurodegeneration or neuronal injury abnormal using FDG.

(Supplementary Figs. 8 and 9). In addition, we examined the differences in the change rates of clinical outcomes in female versus male and *APOE* ε4 carriers versus *APOE* ε4 noncarriers (Supplementary Figs. 10 and 11). In the CN group, women with A+T−N− showed faster change rates of MMSE score than men. In the MCI group, women with A+T+N+ showed faster change rates of ventricular volume than men. In CN group, *APOE* ε4 carriers showed faster rates of hippocampal atrophy than did those with *APOE* ε4 noncarriers in A+T+N+ individuals. Patients with MCI who carried *APOE* ε4 showed faster ADAS-COG and ventricular volume change in A+T+N− and A+T+N+ individuals, respectively, than *APOE* ε4 noncarriers.

We also assessed how subjects move between the different ATN groups from baseline to each follow-up examination. Individuals were included if they underwent amyloid PET or CSF Aβ analysis (A), CSF p-tau examination (T), and FDG-PET (N) at baseline and with at least 2-years' follow-up data of each biomarker group. However, as time went on, the number of individuals in each group became extremely small. It is hard to conclude how subjects moved between ATN categories and how clinical outcomes changed (Supplementary Fig. 12).

3.4. Prediction of disease progression for each biomarker profile

Fig. 3 exhibits the results of a Kaplan-Meier analysis and the logrank test. Cox proportional-hazards models were developed to estimate the conversion risk from no cognitive impairment to incident prodromal stage of AD indicated by a CDR–global score of 0.5 or greater and from MCI to incident AD dementia for each biomarker profile, controlling for baseline age, gender, and years of education. Covariates of both models met proportional hazard assumptions using Schoenfeld residuals technique (Global Schoenfeld Test $P = .23$ and .51, respectively). CN individuals with A+T+N+ and A+T+N− had an increased risk of conversion to the prodromal stage of AD (CDR-GS ≥ 0.5) compared with A−T−N−. CN individuals with A+T+N+ also had an increased risk of conversion to prodromal stage of AD compared with A+T−N− and A+T+N−. However, we did not detect any differences in conversion risk among individuals with CN between A+T+N− and A+T−N−, and between A+T−N− and A−T−N−.

In MCI patients, compared with A−T−N−, participants with A+T+N+ and A+T+N− had an elevated risk of conversion to AD dementia. MCI patients with A+T+N+ also had an increased risk of conversion to AD dementia compared with A+T−N− and A+T+N−. However, we did not detect any differences in conversion risk among MCI individuals between A+T+N− and A+T−N−, and between A+T−N− and A−T−N− (Table 2). We also accessed the changes in clinical outcomes and the conversion risk by using HVa to define N (Supplementary Figs. 13 and 14).

4. Discussion

Our findings suggest that as the disease progresses, abnormal (β-amyloid deposition [A], pathologic tau [T], and neurodegeneration [N]) A/T/N biomarkers accumulate. Cognitive decline was observed only in A+T+N±. However, brain atrophy was observed in A−T−N−, A+T−N−, and A+T+N±, in elders with both MCI and CN. Individuals with abnormal amyloid had faster change rates of clinical outcomes and faster progression rates if they had abnormal phospho-tau, with or without neurodegeneration. Brain β-amyloidosis alone (without tauopathy and neurodegeneration) did not predict clinical outcomes change and disease progression. A faster change rate of clinical outcomes was observed in female versus male and *APOE* ε4 carriers versus *APOE* ε4 non-carriers but only in amyloid positive profiles.

Table 2
Progression risk from CN to prodromal stage of AD and from MCI to AD

Biomarkers	5-year progression rate	Hazard ratio (95% CI)*	*P* value	Hazard ratio (95% CI)*	*P* value	Hazard ratio (95% CI)*	*P* value
Progression from CN to prodromal stage of AD							
A−T−N−	10.7%	Reference		/	/	/	/
A+T−N−	45.4%	1.84 (0.50-6.8)	.36	Reference		/	/
A+T+N−	44.4%	2.79 (1.14-6.9)	.03	1.50 (0.52-4.4)	.46	Reference	
A+T+N+	100%	11.21 (2.83-44.4)	<.001	6.90 (1.16-29.6)	.009	4.68 (1.39-15.7)	.01
Progression from MCI to AD							
A−T−N−	10%	Reference		/	/	/	/
A+T−N−	11.1%	2.92 (0.18-47.3)	.45	Reference		/	/
A+T+N−	24.5%	9.89 (1.30-75.2)	.03	3.33 (0.44-25.2)	.24	Reference	
A+T+N+	85.95%	53.66 (7.22-398.8)	<.001	17.34 (2.33-128.9)	.005	5.20 (3.13-8.6)	<.001

Abbreviations: CI, confidence interval; CN, cognitively normal; MCI, mild cognitive impairment; AD, Alzheimer's disease; A−, amyloid normal using amyloid PET or CSF Aβ; A+, amyloid abnormal using amyloid PET or CSF Aβ; T−, tau normal using CSF p-tau; T+, tau abnormal using CSF p-tau; N−, neurodegeneration or neuronal injury normal using FDG; N+, neurodegeneration or neuronal injury abnormal using FDG.

*Hazard ratios (95% CI) calculated using Cox regression analyses and corrected for baseline age, gender, *APOE* ε4 status, and years of education.

In CN group, the proportion of A−T−N− was only 19.2%. This proportion is consistent with other publications that have shown that biomarkers change years before symptom onset [16]. A recent study from the Mayo Clinic reported the proportion of each AT(N) profile in CN individuals [6]. Unlike the Mayo Clinic study, which is likely to be representative of a community-type sample of older people, the ADNI samples exclude people with comorbidities such as stroke and other neurodegenerative disease. However, the proportions of N+ were highly similar when we used HVa to define N (37% from Mayo vs. 41.46% from our study) and were consistent with a recent ADNI study [17]. Furthermore, it is of interest that SNAP accounts for a sizable proportion of the CN group. How the pathology of individuals with this biomarker category developed needs further scrutiny in longitudinal studies. In CN group, frequency of A/N scheme were also conducted in Knight Alzheimer's Disease Research Center [18]. Frequency of A/N scheme were also performed previously in ADNI and Mayo Clinic Study of Aging in patients with MCI [19]. The results were nearly identical with ours. In patients with AD dementia, the proportion of A+T+N±, which could be termed "Alzheimer's disease" according to the NIA-AA research framework, reached 92.0%. The "AD diagnosis" of the remaining 8% individuals was pathologically proven to be false.

There is face validity to the observation that the proportion of A+T+N+ was increasing and the proportion of A−T−N− was decreasing from CN to AD dementia. A+T+N− increased from 30.3% (in CN group) to 36.9% (in sMCI group) and then decreased to 4.4% (in AD dementia group), and A+T−N− were concentrated in CN and sMCI groups. This supports that A+T−N− to A+T+N− to A+T+N+ is the temporal sequence of biomarkers toward AD. We postulate SNAP consist of primary age-related tauopathy (PART), non-Alzheimer's degeneration and their combination. From our findings, SNAP, especially PART, usually range from normal to mild cognitive changes.

By using different methods to define "N," we detected that the proportion of "N" was 7% using FDG-PET and 41.46% using HVa. A previous study also detected a poor agreement between FDG-PET and hippocampal volume in the ADNI database [20], reflecting discordance among biomarkers in the N group. In fact, the two methods for defining N track distinct aspects of the AD pathophysiological process: Atrophy on MRI reflects dendritic and neuronal losses, and FDG PET likely indicates synaptic activity and loss of synapses [21,22]. Beyond that, the discordance may be partly explained by the dynamic character of biomarker changes and suboptimal cutoff values. While FDG may (and almost certainly does) reflect something about progression, its group differences even in much younger normals make its meaning less clear than structural volume changes.

In the longitudinal analyses, changes in hippocampal and ventricular volumes were observed in all the four profiles in both CN and MCI groups. This might partly relate to aging not captured by these biomarkers. However, general cognition (MMSE score and ADAS-COG 11 score) decline was only seen in A+T+N±. This might be partly interpreted as brain atrophy occurring earlier than cognitive decline [23,24]. However, one limitation should be noted that MMSE and ADAS-COG may not be the most sensitive markers for change (vs. longer, more difficult episodic memory tests). Noncognitive functions such as function (Alzheimer's disease cooperative study-activities of daily living) and behavior (neuropsychiatric inventory) could also be accessed in the future which be informative for clinical trials. On comparing the change rates of clinical outcomes, it is reasonable that A+T+N+ showed the fastest and A−T−N− showed the slowest clinical progression among the four profiles. Note that previous work with CN individuals adopting a two-class biomarker construct, A+N− versus A−N− [15], found no differences in cognitive decline. However, an another A/N study reported contrary results [25]. CSF p-tau specifically reflects the phosphorylation

state of tau, a different form of t-tau, which in turn reflects nonspecific neurodegeneration or neuronal injury. Therefore, the 2018 research framework separates biomarkers for pathologic tau from measures of neurodegeneration or neuronal injury. However, no significant differences were detected between A+T−N− versus A−T−N−. In line with our findings, there was no association between CSF $A\beta_{42}$ status and cognitive decline or volume loss among CSF p-tau negative individuals. This association only occurred among CSF p-tau positive individuals [26,27]. To further examine this result, comparisons of clinical outcomes between A−T+N± and A+T+N± would be warranted in future studies. There is considerable prior work indicating that cerebral amyloidosis is associated with longitudinal clinical decline before the clinical diagnosis of AD [28,29]. However, whether there were interactions between Aβ and tau is not clear, although the evidence points toward interactions between Aβ and tau being implicated in AD-related clinical decline [30]. Longitudinal analyses of Alzheimer's continuum profiles could facilitate participant selection and prediction of trial outcomes [31]. Moreover, our longitudinal study examined the extent to which relationships of signs/symptoms and biomarkers improved study validity if they were to be adopted into clinical practice.

Overall, women had a faster change rate of clinical outcomes than men but only in amyloid positive profiles according to our findings. These results were consistent with previous studies, which found that women with AD pathology are more likely to be expressed clinically as dementia than men [32]. Our findings indicated that the increased change rate of clinical outcomes in women might be Alzheimer's continuum-specific. Whether the same results are observed among individuals with SNAP will need further study.

Many studies have reported that *APOE* ε4 carriage increases the rate of Aβ-related cognitive decline occurring in the preclinical AD [33]. We found *APOE* ε4 carriage could accelerate clinical decline among individuals with Aβ positive. However, this effect only existed in the presence of phospho-tau with or without neurodegeneration (A+T+N− or A+T+N+). Again, these findings point to phospho-tau as an important marker of Aβ-associated clinical decline. We also confirmed that this effect is greater in *APOE* ε4 carriers.

In line with our findings, a recent study reported that among cognitively healthy elderly participants with subjective memory complaints, brain β-amyloidosis alone (negative for raised tau protein concentrations) did not predict progression to prodromal Alzheimer's disease [34]. Our present findings are also consistent with our previous meta-analysis, which indicated that the combination of low CSF Aβ and high CSF tau levels could significantly predict the progression from MCI to AD dementia, whereas abnormal CSF Aβ alone had no significant association with the progression to AD dementia in patients with MCI [35]. Our findings support the hypothesis that Aβ accumulation is necessary but not sufficient to produce the clinical decline of AD [4].

Our study had limitations. The number of individuals within each profile was relatively small as eight possible AT(N) combinations exist. The numbers were smaller still when stratifying by gender and *APOE* ε4. Focusing on the biomarker sequence of preclinical AD, small sample sizes precluded A−T−N+, A−T+N−, A−T+N+, and A+T−N+ groups in longitudinal and survival analyses. The cutoff points we used should be thoroughly examined using the methods described previously [36]. A single cut point approach lacks accuracy when research questions require high diagnostic certainty. Finally, the present study did not analyze how vascular factors (e.g. small vessel disease), which commonly co-occur in elderly, may affect the clinical manifestation and rate of cognitive decline in this framework. Reproducibility of findings in different patient groups from different centers would be beneficial for the clinical applicability of these biomarkers.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jalz.2019.05.006.

RESEARCH IN CONTEXT

1. Systematic review: We reviewed available English language literature in PubMed for studies related to A/T/N classification. Recent cross-sectional studies reported the prevalence of each (β-amyloid deposition [A], pathologic tau [T], and neurodegeneration [N]) ATN profile in cognitively unimpaired individuals. However, this study is limited by its cross-sectional design, and longitudinal data is hence necessary and urgently needed to provide important information on the clinical/cognitive outcomes of these ATN profiles.
2. Interpretation: Participants with A+T+N+ showed faster clinical progression than those with A−T−N− and A+T±N−. Compared with A−T−N−, participants with A+T+N± had an increased risk of conversion from cognitively normal (CN) to incident prodromal stage of AD, and from mild cognitive impairment (MCI) to AD dementia. A+T+N+ showed an increased conversion risk when compared with A+T±N−.
3. Future directions: Applying the 2018 research framework in participants screening for AD clinical trials may be beneficial.

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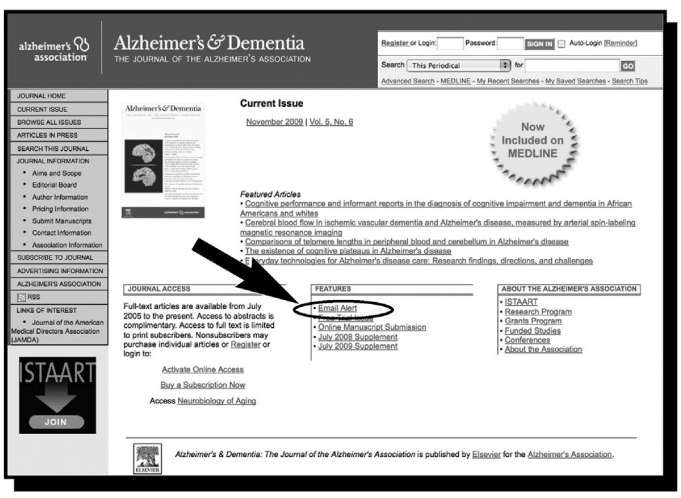

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