Different AT(N) profiles and clinical progression classified by two different N markers using total tau and neurofilament light chain in cerebrospinal fluid

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ABSTRACT

Background The AT(N) classification was proposed for categorising individuals according to biomarkers. However, AT(N) profiles may vary depending on the markers chosen and the target population.

Methods We stratified 177 individuals who participated in the Japanese Alzheimer’s Disease Neuroimaging Initiative by AT(N) classification according to cerebrospinal fluid (CSF) biomarkers. We compared the frequency of AT(N) profiles between the classification using total tau and neurofilament light chain (NfL) as N markers (AT(N)tau and AT(N)nfl). Baseline characteristics and longitudinal biological and clinical changes were examined between AT(N) profiles.

Results We found that 9% of cognitively unimpaired subjects, 49% of subjects with mild cognitive impairment, and 61% of patients with Alzheimer’s disease (AD) dementia had the biological AD profile (ie, A+T+) in the cohort. The frequency of AT(N) profiles substantially differed between the AT(N)tau and AT(N)nfl classifications. When we used t-tau as the N marker (AT(N)tau), those who had T− were more frequently assigned to (N)−, whereas those who had T+ were more frequently assigned to (N)+ than when we used NfL as the N marker (AT(N)nfl). During a follow-up, the AD continuum group progressed clinically and biologically compared with the normal biomarker group in both the AT(N)tau and AT(N)nfl classifications. More frequent conversion to dementia was observed in the non-AD pathological change group in the AT(N)tau classification, but not in the AT(N)nfl classification.

Conclusions AT(N)tau and AT(N)nfl in CSF may capture different aspects of neurodegeneration and provide a different prognostic value. The AT(N) classification aids in understanding the AD continuum biology in various populations.

INTRODUCTION

As the population ages, the number of patients with dementia is expected to increase worldwide including in Asia.1 Alzheimer’s disease (AD) is pathologically characterised by β-amyloid (Aβ) deposition and fibrillar phosphorylated tau accumulation.2 Biofluid and molecular neuroimaging biomarkers have been explored to capture key aspects of the neuropathological changes of AD.

A research framework biologically defines AD by using biomarkers that reflect the brain pathology in vivo independent of clinical symptoms.3 In the framework, each individual is classified into one of eight categories by dichotomous determination according to the AT(N) system, where the cerebrospinal fluid (CSF) biomarkers of Aβ deposition (A), fibrillar tau (T) and neurodegeneration or
neuronal injury (N) are defined by the Aβ42 or Aβ42/40 ratio, phosphorylated tau (p-tau) and total tau (t-tau), respectively. Through this research framework, AD has been conceptualised as a continuum covering asymptomatic, mild cognitive impairment (MCI) and dementia stages. The prevalence of the AT(N) classification has been investigated mostly among Caucasians, although a few studies have been reported for other ethnic groups. Studies on Asian populations did not address the longitudinal clinical and biological changes among AT(N) profiles. Because the prognostic value of AT(N) profiles may vary depending on the target population, the research framework should be further investigated in various populations including Asians.

Another issue of the AT(N) system is with regard to a biofluid N marker. Currently, CSF t-tau is assigned to the N marker. Since the research framework was advocated, evidence of CSF neurofilament light chain (NFL) as an N marker have been accumulated. NIL and t-tau in CSF are not always well correlated, suggesting that these markers may reflect different aspects in neurodegeneration. Using CSF samples collected by Japanese Alzheimer’s Disease Neuroimaging Initiative (J-ADNI), this study aimed to clarify (1) the characteristics of CSF biomarkers in a J-ADNI cohort, (2) the frequencies of AT(N) profiles by comparing two different N markers (t-tau and NIL), and (3) the clinical and biological characterisations according to AT(N) profiles at both baseline and follow-up.

METHODS

Participants

J-ADNI was initiated to discover the fluid and imaging biomarkers of AD using a harmonised protocol with ADNI. Briefly, volunteer participants aged between 60 and 84 years were recruited from 38 clinical sites in Japan. Cognitively unimpaired (CU) subjects, subjects with MCI, and patients with AD dementia (ADD) were enrolled into J-ADNI using criteria consistent with those of ADNI. Their clinical and neuropsychological data were obtained from the National Bioscience Database Center (https://humanbbs.bioscienceedb.jp/en/hum0043-v1).

Out of 715 volunteers assessed for eligibility, 537 met the criteria and were enrolled. Out of 537 participants recruited in J-ADNI (CU, 154; MCI, 234; ADD, 149), 4 withdrew their consent. Of the 533 remaining participants, 194 (CU, 53; MCI, 86; ADD, 55) underwent lumbar puncture. The incidence of postdural puncture headache was 2.6%, and that of severe postdural puncture head-ache that required hospitalisation was 0.7%. All these 194 participants were analysed using AD core biomarkers including Aβ42, tau phosphorylated at threonine 181 (p-tau181), and t-tau. Due to sample availability, CSF NFL was measured in 177 participants (CU, 46; MCI, 82; ADD, 49). At 12 months, longitudinal changes in CSF biomarkers classified by AT(N) profiles were analysed in 126 participants (CU, 38; MCI, 56; ADD, 32) (online supplemental figure 1).

Lumbar puncture and biochemical analysis

CSF was collected by lumbar puncture, transferred into polypropylene tubes followed by freezing and shipped to the J-ADNI Biomarker Core at Niigata University. CSF was aliquoted at a volume of 0.5 mL and stored at −80°C until the assay. The CSF concentrations of Aβ42, p-tau181, and t-tau were examined using AlzBio3 kit (Fuji-rebio, Ghent, Belgium), and that of NFL was measured using R-PLEX Human Neurofilament L Antibody Set (Meso Scale Discovery, Rockville, MD). All analyses were conducted in duplicate by experienced laboratory personnel blinded to the clinical diagnosis. The intraclass and interassay coefficients of variation were <20% for all assays. The laboratory at Niigata University participates in the Alzheimer’s Association external quality control programme for CSF biomarkers.

We previously used CSF Aβ42<333 pg/mL as the cut-off value for Aβ positivity. Thereafter, we have established a protocol for AD core biomarker measurements unified with the ADNI Biomarker Core (PI: Leslie M. Shaw, PhD). We used this unified protocol for remeasuring the CSF biomarkers. Subsequently, we conducted the area under the receiver operating characteristic curve analysis (PET Aβ negative (PET Aβ−, n=47) vs positive (PET Aβ+, n=53); CU with PET Aβ− (n=31) vs ADD with PET Aβ+ (n=22); CU (n=53) vs ADD (n=56)), and calculated the optimal cut-off values according to Youden’s index (online supplemental figures 2 and 3). Furthermore, we used Gaussian mixture models (GMMs) for calculating the cut-off value of CSF biomarkers (n=194), excluding NIL, which was unsuitable for GMMs because of the unimodal distribution (online supplemental figure 2).

PET image acquisition and clinical evaluation

All PET images underwent the J-ADNI PET quality control process as previously described. Cognitive performance was assessed using the Mini-Mental State Examination (MMSE), Alzheimer’s Disease Assessment Scale-Cognitive Subscale (ADAS-Cog), and the sum of boxes of the Clinical Dementia Rating (CDR-SB). Instrumental activities of daily living were assessed using the Functional Assessment Questionnaire (FAQ). In this study, when the CDR changed from 0 or 0.5 to ≥1 during a follow-up, the patient was considered to have progressed to dementia.

Statistical analysis

Data were analysed statistically using GraphPad Prism (V.8.2.0; GraphPad Software, La Jolla, California, USA) and the software R. For continuous variables, we used the Mann-Whitney U test for comparing two groups and the Kruskal-Wallis test for comparing multiple groups, followed by Dunn’s multiple-comparison test. For categorical variables, groups were compared using the χ² test. The correlation between two data sets was assessed using Spearman’s rank-correlation coefficient. For the longitudinal analyses of changes in CSF biomarker, we compared slopes with zero by linear regression model analyses. The covariates included age, sex and education years. For
Additionally, higher p-42 levels showed significantly lower CSF Aβ level but not with p-42 tau levels and higher p-42 levels showed moderately positive correlations with p-tau181, t-tau, and NfL levels. As expected, p-tau181 and t-tau levels were highly correlated (r=0.7923, p<0.0001). NfL level showed moderately positive correlations with p-tau181 (r=0.2487, p=0.0008) and t-tau levels (r=0.4907, p<0.0001) (online supplemental figure 4D). Both APOE ε4 heterozygous and homozygous carriers showed significantly lower CSF Aβ42 levels and higher p-tau181, t-tau and NfL levels than non-carriers (online supplemental figure 4E).

Next, correlations among CSF biomarkers were analysed. We found that Aβ42 level moderately negatively correlated with p-tau181, t-tau, and NfL levels. As expected, p-tau181 and t-tau levels were highly correlated (r=0.7923, p<0.0001). NfL level showed moderately positive correlations with p-tau181 (r=0.2487, p=0.0008) and t-tau levels (r=0.4907, p<0.0001) (online supplemental figure 4D).

### AT(N) classification at baseline

We used CSF Aβ42 as the A marker, p-tau181 as the T marker, and t-tau or NfL as the N marker. AT(N)Aβ, AT(N)T, and AT(N)NfL were defined using t-tau and NfL as the N marker, respectively. We classified the participants into eight AT(N) categories.

The cut-off value was compared by different methods. When comparing clinical status (CU vs ADD) with PET status (PET Aβ– vs PET Aβ+), the cut-off values were

| Table 1 Cut-off values of AT(N) biomarkers based on different models | Aβ42 | p-tau181 | t-tau | NfL |
| --- | --- | --- | --- | --- |
| Analysed samples | Aβ42 | p-tau181 | t-tau | NfL |
| Aβ PET– (n=47) vs Aβ PET+ (n=53) | Area under the ROC curve (95% CI) | 0.940 (0.885 to 0.995) | 0.868 (0.794 to 0.941) | 0.898 (0.832 to 0.963) | 0.706 (0.591 to 0.821) |
| Cut-off value, pg/mL | 378.7 | 26.8 | 85.7 | 2428 |
| Sensitivity, % (95% CI) | 98.1 (90.1 to 99.9) | 83.0 (70.8 to 90.8) | 90.6 (79.8 to 95.9) | 89.1 (77.0 to 95.3) |
| Specificity, % (95% CI) | 85.1 (72.3 to 92.6) | 80.9 (67.5 to 89.6) | 80.9 (67.5 to 89.6) | 57.1 (42.2 to 70.9) |
| CU, Aβ PET– (n=31) vs ADD, Aβ PET+ (n=22) | Area under the ROC curve (95% CI) | 0.962 (0.907 to 1.000) | 0.912 (0.834 to 0.990) | 0.963 (0.917 to 1.000) | 0.852 (0.735 to 0.969) |
| Cut-off value, pg/mL | 361.6 | 29.1 | 88.8 | 2660 |
| Sensitivity, % (95% CI) | 100 (85.1 to 100) | 95.5 (78.2 to 99.8) | 95.5 (78.2 to 99.8) | 85.0 (64.0 to 94.8) |
| Specificity, % (95% CI) | 87.1 (71.2 to 94.9) | 80.7 (63.7 to 90.8) | 90.3 (75.1 to 96.7) | 80.8 (62.1 to 91.5) |
| CU (n=53) vs ADD (n=56) | Area under the ROC curve (95% CI) | 0.888 (0.821 to 0.954) | 0.805 (0.723 to 0.888) | 0.882 (0.818 to 0.947) | 0.831 (0.747 to 0.915) |
| Cut-off value, pg/mL | 288.6 | 29.0 | 91.0 | 3120 |
| Sensitivity, % (95% CI) | 82.1 (70.2 to 90.0) | 73.2 (60.4 to 83.0) | 76.8 (64.2 to 85.9) | 69.4 (55.5 to 80.5) |
| Specificity, % (95% CI) | 88.7 (77.4 to 94.7) | 79.3 (66.5 to 88.0) | 88.7 (77.4 to 94.7) | 89.1 (77.0 to 95.3) |
| Gaussian Mixture Model (n=194) | Cut-off value, pg/mL | 359.6 | 30.6 | 105.3 | NA*

The cutoffs were established at the highest Youden Index (sensitivity + specificity – 1) when comparing Aβ PET– with Aβ PET+, or comparing CU, Aβ PET– with ADD, Aβ PET+, or comparing CU with ADD. The sensitivity and specificity are for each cut-off value.

*Due to unimodal distribution.

ADD, Alzheimer’s disease dementia; Aβ, β-amyloid; CU, cognitively unimpaired subjects; NA, not available; NfL, neurofilament light chain; p-tau181, tau phosphorylated at threonine 181; ROC, receiver operating characteristic; t-tau, total tau.
### Table 2: Baseline characteristics of 8 AT(N) profile groups

| AT(N) Profile | A-T-(N)- | A-T-(N)+ | A-T+(N)- | A-T+(N)+ | A+(N)- | A+(N)+ | A+(N)- | A+(N)+ | P value |
|---------------|----------|----------|----------|----------|--------|--------|--------|--------|---------|
| No (%)        | 52 (29.4)| 3 (1.7)  | 4 (2.3)  | 3 (1.7)  | 37 (20.9)| 4 (2.3) | 10 (5.6)| 64 (36.2)| 0.109   |
| Age, years (IQR) | 67 (9)   | 77 (4)   | 74 (5)   | 75 (3)   | 71 (10) | 74 (8)  | 73 (9)  | 74 (9)  | 0.692   |
| Female, n (%)  | 23 (44.2)| 2 (66.7) | 1 (25.0) | 2 (66.7) | 18 (48.6)| 1 (25.0) | 4 (40.0)| 36 (56.3)| 0.091   |
| Education, years (IQR) | 14 (4)   | 16 (2)   | 16 (2)   | 12 (4)   | 10 (4)  | 13 (3)  | 13 (4)  |         |         |
| APOE ε4 allele (%) |         |         |         |         |        |        |        |         | <0.001  |
| 0             | 49 (94.2)| 2 (66.7)| 3 (75.0) | 2 (66.7) | 16 (43.2)| 1 (25.0)| 3 (30.0)| 19 (29.7)|         |
| 1             | 3 (5.8) | 1 (33.3)| 1 (25.0) | 1 (33.3) | 18 (48.6)| 2 (50.0)| 6 (60.0)| 31 (48.4)|         |
| 2             | 0 (0)   | 0 (0)   | 0 (0)    | 0 (0)    | 3 (8.1) | 1 (25.0)| 1 (10.0)| 14 (21.9)|         |
| Clinical status, n (%) |         |         |         |         |        |        |        |         | <0.001  |
| CU            | 31 (59.6)| 1 (33.3)| 2 (50.0) | 1 (33.3) | 7 (18.9)| 0 (0)  | 1 (10.0)| 3 (4.7)  |         |
| MCI           | 21 (40.4)| 2 (66.7)| 1 (25.0) | 2 (66.7) | 14 (37.8)| 2 (50.0)| 5 (50.0)| 35 (54.7)|         |
| ADD           | 0 (0)   | 0 (0)   | 1 (25.0) | 0 (0)    | 16 (43.2)| 2 (50.0)| 4 (40.0)| 26 (40.6)|         |
| MMSE (IQR)    | 29 (2)  | 27 (2)  | 28 (3)   | 25 (3)   | 25 (6)  | 24 (2)  | 25 (2)  | 25 (5)  | <0.001  |
| ADAS-Cog (IQR)| 9.4 (9.1)| 20.7 (10.3)| 9.7 (7.4)| 23.0 (10.7)| 21.3 (12.7)| 25.8 (14.1)| 22.2 (10.8)| 23.3 (9.6)| <0.001  |
| CDR-SB (IQR)  | 0 (0.5) | 2.5 (1.5)| 0.5 (0.5)| 0.5 (1.5)| 1.5 (2.5)| 2.8 (2.0)| 2.0 (2.0)| 2.0 (2.5)| <0.001  |
| FAQ (IQR)     | 0 (0)   | 9 (8)   | 0 (3)    | 0 (8)    | 5 (6)   | 6 (2)   | 4 (6)   | 5 (10)  | <0.001  |
| Aβ PET, n (%) |         |         |         |         |        |        |        |         | <0.001  |
| Negative      | 34 (100)| 1 (50.0)| 1 (100)  | 1 (33.3) | 2 (14.3)| 0 (0)  | 0 (0)  | 3 (10.0) |         |
| Positive      | 0 (0)   | 1 (50.0)| 0 (0)    | 2 (66.7)| 12 (85.7)| 1 (100)| 3 (100)| 27 (90.0)|         |
| BL Aβ42, pg/mL (IQR) | 485.2 (101.7)| 373.7 (99.1)| 541.2 (144.8)| 431.5 (148.8)| 240.7 (99.9)| 198.8 (88.8)| 254.8 (73.4)| 234.0 (65.0)| <0.001  |
| BL p-tau, pg/mL (IQR)  | 19.2 (4.5)| 25.7 (2.2)| 37.8 (7.1)| 35.3 (10.4)| 22.1 (6.2)| 28.1 (1.9)| 38.2 (6.8)| 47.3 (23.4)| <0.001  |
| BL t-tau, pg/mL (IQR)  | 58.4 (29.6)| 132.0 (37.4)| 76.1 (14.3)| 140.7 (14.3)| 71.7 (37.9)| 118.2 (42.0)| 89.2 (30.1)| 151.7 (67.2)| <0.001  |
| BL NfL, pg/mL (IQR)   | 2421.6 (1344.7)| 5055.5 (3295.7)| 2663.6 (1101.9)| 2479.2 (2428.3)| 2959.0 (1508.0)| 7850.2 (13779.4)| 2874.5 (818.1)| 3515.2 (1130.2)| <0.001  |

### AT(N) Profile 2

| AT(N) Profile | A-T-(N)- | A-T-(N)+ | A-T+(N)- | A-T+(N)+ | A+(N)- | A+(N)+ | A+(N)- | A+(N)+ | P value |
|---------------|----------|----------|----------|----------|--------|--------|--------|--------|---------|
| No (%)        | 39 (20.1)| 16 (8.2) | 5 (2.6)  | 2 (1.0)  | 20 (10.3)| 21 (10.8)| 32 (16.5)| 42 (21.6)| 0.001   |
| Age, years (IQR) | 66 (8) | 76 (12) | 74 (4) | 76 (1) | 69 (7) | 75 (8) | 72 (10) | 74 (8) | 0.498   |
| Female, n (%)  | 18 (46.2)| 7 (43.8)| 3 (60.0) | 0 (0)    | 12 (60.0)| 7 (33.3)| 18 (56.3)| 22 (52.4)| 0.090   |
| Education, years (IQR) | 14 (4) | 13 (4) | 16 (0) | 20 (4) | 12 (2) | 12 (5) | 12 (4) | 14 (4) | <0.001  |
| APOE ε4 allele (%) |         |         |         |         |        |        |        |        | <0.001  |
| 0             | 37 (64.9)| 14 (87.5)| 4 (80.0)| 1 (50.0)| 11 (53.0)| 6 (28.6)| 8 (25.0)| 14 (33.3)|         |
| 1             | 2 (5.1) | 2 (12.5)| 1 (20.0)| 1 (50.0)| 9 (45.0)| 11 (52.4)| 17 (53.1)| 20 (47.6)|         |
| 2             | 0 (0)   | 0 (0)   | 0 (0)   | 0 (0)    | 4 (19.0)| 7 (21.9)| 8 (19.0)|         |         |
| Clinical status, n (%) |         |         |         |         |        |        |        |        | <0.001  |

Continued
### Table 2

| AT(N)$_{n	ext{HL}}$ | A-T-(N)$^-$ | A-T-(N)$^+$ | A-T+(N)$^-$ | A-T+(N)$^+$ | A+T-(N)$^+$ | A+T-(N)$^+$ | P value |
|---------------------|-----------|-----------|-----------|-----------|-----------|-----------|---------|
| CU                  | 27 (69.2)| 5 (31.3) | 3 (60.0) | 0 (0)     | 7 (35.0) | 0 (0)     | 4 (12.5)| 0 (0)  |
| MCI                 | 12 (30.8)| 11 (68.8)| 2 (40.0) | 1 (50.0)  | 8 (40.0) | 8 (38.1)  | 18 (56.3)| 22 (52.4) |
| ADD                 | 0 (0)    | 0 (0)    | 0 (0)    | 1 (50.0)  | 5 (25.0) | 13 (61.9) | 10 (31.3)| 20 (47.6) |
| MMSE (IQR)          | 29 (2)   | 28 (3)   | 30 (3)   | 24 (0)    | 45 (5)   | 24 (5)    | 25 (4)  | 24 (5)  | <0.001 |
| ADAS-Cog (IQR)      | 8.3 (7.5)| 13.7 (12.4)| 9.7 (15.6)| 21.9 (5.2)| 16.0 (16.0)| 24.7 (5.4)| 23.2 (9.6)| 23.7 (8.9)| <0.001 |
| CDR-SB (IQR)        | 0 (0.5)  | 1.0 (1.0)| 0.5 (1.0)| 2.0 (1.5)| 1.5 (2.5)| 2.5 (3.0)| 2.3 (2.5)| 2.0 (2.0)| <0.001 |
| FAQ (IQR)           | 0 (0)    | 2 (2)    | 0 (3)    | 4 (4)     | 5 (7)    | 6 (5)     | 5 (7)   | 5 (10)  | <0.001 |
| Aβ PET, n (%)       |          |          |          |          |          |          |        |        | <0.001 |
| Negative            | 25 (100) | 10 (90.9)| 2 (66.7) | 0 (0)    | 2 (22.2)| 0 (0)    | 1 (7.7) | 2 (10.0)|        |
| Positive            | 0 (0)    | 1 (9.1)  | 1 (33.3) | 1 (100)  | 7 (77.8)| 6 (100)  | 12 (92.3)| 18 (90.0)|        |
| BL Aβ42, pg/mL (IQR)| 479.7 (84.3)| 501.3 (139.4)| 568.2 (230.8)| 486.3 (54.9)| 214.7 (137.8)| 240.7 (66.2)| 241.9 (34.2)| 237.5 (80.7)| <0.001 |
| BL p-tau, pg/mL (IQR)| 19.2 (4.1)| 21.0 (7.2)| 35.3 (4.0)| 41.0 (9.8)| 22.4 (7.0)| 23.0 (7.0)| 40.5 (13.7)| 44.4 (21.4)| <0.001 |
| BL t-tau, pg/mL (IQR)| 54.6 (24.4)| 88.0 (30.6)| 82.8 (43.4)| 114.3 (33.7)| 59.1 (23.6)| 92.4 (15.7)| 128.3 (43.5)| 155.3 (68.3)| <0.001 |
| BL NfL, pg/mL (IQR) | 2106.9 (1118.0)| 4147.5 (1243.0)| 2464.2 (167.0)| 5922.1 (1398.6)| 2414.6 (570.9)| 3901.7 (3674.1)| 2640.4 (522.3)| 3785.2 (943.6)| <0.001 |

Numbers are median (IQR) for continuous variables and raw number (percentage) for categorical variables.

Differences in baseline characteristics of participants across 8 AT(N)$_{n	ext{HL}}$ profiles were first assessed using Kruskal-Wallis rank sum test for continuous variables, or a χ² test for categorical variables.

ADAS-Cog, Alzheimer’s Disease Assessment Scale-Cognitive Subscale; ADD, Alzheimer’s disease dementia; Aβ, β-amyloid; BL, baseline; CDR-SB, sum of boxes of the Clinical Dementia Rating; CU, cognitively unimpaired subjects; FAQ, Functional Assessment Questionnaire; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NfL, neurofilament light chain; p-tau181, tau phosphorylated at threonine 181; t-tau, total tau.
lower for Aβ42 and higher for p-tau181, t-tau and NfL (table 1). When the PET Aβ status and clinical status were combined (CU with PET Aβ− vs ADD with PET Aβ+), the cut-off values were intermediate between the PET Aβ status and the clinical status only, and close to GMM-calculated cut-off values (table 1). Thus, hereafter, the cut-off values used in this study were CSF Aβ42<359.6 pg/mL (A+), p-tau181>30.6 pg/mL (T+), t-tau>105.3 pg/mL (N+) and NfL>2650 pg/mL (N+).

We showed the demographic and clinical variables among the eight AT(N) biomarker categories in the AT(N)tau classification (table 2, upper half). The proportion of CU decreased from 33% to 60% in the A− groups to 25%–62% in the A+ groups.

Next, we determined the characteristics of the eight AT(N) profiles in the AT(N)nilt classification (table 2, lower half). The proportion of CU decreased from 31% to 69% in the A− groups to 0%–35% in the A+ groups, whereas that of ADD increased from 0% in the A− groups to 25%–62% in the A+ groups.

To determine the frequency of biological AD, we classified 177 subjects by using the AT system comprising CSF Aβ42 and p-tau181. The subjects were then classified into A−T− (n=55, 31.1%), A−T+ (n=7, 4.0%), A+T− (n=41, 23.2%) and A+T+ (n=74, 41.8%) (figure 1). A−T− accounted for 69.9% in CU, whereas A+T+ and A+T+ accounted for 68.3% in MCI. In ADD, A+T+ was 36.7% and A+T+ was 61.2%.

**AT(N) classification: comparison between t-tau and NfL**

We compared the frequencies of AT(N) categories between AT(N)tau and AT(N)nilt classification. In AT(N)tau, the most common was A+T+(N)+ (n=64, 36.2%), followed by A−T−(N)− (n=52, 29.4%) and A+T+(N)− (n=37, 20.9%) (figure 1). Considering the high correlation between t-tau and p-tau181 (online supplemental figure 5), CSF t-tau may not be a fully independent marker of neurodegeneration in the AD continuum.

In AT(N)nilt, the frequencies of the A−T−(N)−, A+T−(N)−, and A+T+(N)− categories decreased to 22.0% (n=39), 11.3% (n=20), and 23.7% (n=42) compared with AT(N)tau, respectively (figure 1). Thus, the subsets of participants in the A−T− and A+T− categories with neurodegeneration (A−T−(N)− and A+T−(N)−) were classified into (N)+ in AT(N)tau (figure 1). Supporting this finding, the subsets of participants in the A−T−(N)− and A+T−(N)− categories showed elevated NfL levels (online supplemental figure 6). In contrast, a subset of participants in the A+T+ category with undetectable neurodegeneration (A+T+(N)−) showed elevated t-tau levels (online supplemental figure 6); thus, they were classified into (N)+ in AT(N)nilt (figure 1).

**Longitudinal changes of AT(N) profiles**

In 126 participants with follow-up CSF examination at 12 months, changes in the levels of most of the biomarkers were not statistically significant. After 12 months, the p-tau181 level significantly elevated in the A−T−(N)− category by both AT(N) classifications, in A−T−(N)− by AT(N)tau classification, and in A+T+(N)+ by AT(N)nilt classification (online supplemental table 3, online supplemental figure 7).

We assessed the longitudinal changes of the AT(N) profiles at 12 months. The AD continuum biologically progressed, and the progression rate differed between AT(N) profiles at the baseline (figure 2). In the AT(N)nilt classification, the progression rate was 2.1% (1 of 47) among A− groups. A−T− progressed to A+T+(N)+ in five and two participants, respectively. All four participants with A−T+(N)+ progressed to A+T+(N)+. Thus, the progression rate of these A−T− to A+T+ was 42.3%. One participant with A+T+(N)+ progressed to A+T+(N)+ (14.3%) (figure 2A).

In the AT(N)nilt classification, the progression rate was 2.1% (1 of 47) in the A− groups. The progression rate from A−T− to A+T+ was 38.5%. Five A+T+(N)+ participants...
progressed to $A+T+(N)+$ (20.0%) (figure 2B). Hence, participants with the $A−$ profile rarely progressed to $A+$ within 12 months. Conversely, approximately 40% of participants with $A+T−$ progressed to $A+T+$ and 10%–20% of participants with $A+T+(N)−$ progressed to $A+T+(N)+$ within 12 months in either the AT(N)tau or AT(N) NfL classification (figure 2). Notably, longitudinal changes of AT(N) profiles were different in $A+T−(N)+$ and $A+T+(N)−$ categories between the AT(N)tau and AT(N) NfL classifications (figure 2).

Longitudinal change of cognitive functions

Owing to the small sample size of some of the AT(N) categories, we categorised eight AT(N) profiles into three groups, namely, the normal biomarker ($A−T−(N)−$), AD continuum ($A+T−(N)+$) and non-AD pathological change ($A+T−(N)−/+$, $A−T−(N)−/+$, and $A+T+(N)−$ to $A+T+(N)+$) groups. At the baseline, the AD continuum group showed significantly lower MMSE and higher ADAS-Cog, CDR-SB and FAQ scores than the normal biomarker group (figure 3, online supplemental table 2). In the AT(N)NfL classification, the AD continuum group showed significantly lower MMSE and higher ADAS-Cog, CDR-SB and FAQ scores than the non-AD pathological change group. No such significant differences were observed in the AT(N)tau classification (figure 3).

We conducted LMM analysis to evaluate cognitive decline assessed by four clinical measures (MMSE, ADAS-Cog13, CDR-SB and FAQ) during the follow-up period up to 36 months. All the clinical measures in the AD continuum and non-AD pathological change groups declined faster than in the normal biomarker group, except for the CDR-SB of the non-AD pathological change group in AT(N) NfL classification (table 3, figure 4).

Clinical conversion into dementia

Of 139 participants, 57 (41.0%) clinically converted into dementia during 36 months of follow-up. The subjects who converted to dementia exhibited significantly higher levels of t-tau and NfL at the baseline than the non-converters (t-tau, $p<0.001$; NfL, $p=0.0033$).

Cox proportional hazard analysis showed that the AD continuum and non-AD pathological change groups converted into dementia more frequently than the normal biomarker group in the AT(N)tau classification (figure 5A). In the AT(N)NfL classification, only the AD continuum group converted into dementia more frequently than the normal biomarker group in the AT(N)tau classification (figure 5A).
frequently than the normal biomarker group (figure 5B). Discordance of prognosis in the non-AD pathological change group between the AT(N) tau and AT(N) NfL classifications suggests that CSF t-tau elevation without Aβ42 reduction (A−(N) tau+) may be related to a higher rate of conversion to dementia; conversely, no such relationship was found in the case of CSF NfL elevation without Aβ42 reduction.

**DISCUSSION**

In this paper, we show the results of CSF biomarker analysis among J-ADNI participants from the preclinical stage to dementia who were longitudinally followed up for 3 years. We found that 8.7%, 48.8% and 61.2% of the CU, MCI, and ADD groups had the biological AD profile (ie, A+T+) respectively (table 2, figure 1). By comparing the N marker between t-tau and NfL, we found that the AT(N) profiles showed different frequencies. When we used...
t-tau as the N marker (AT(N)$_{tau}$), those who had T− were more frequently assigned to (N)−, whereas those who had T+ were more frequently assigned to (N)+ compared with the case of using NfL as the N marker (AT(N)$_{NfL}$) (table 2, figure 1). This finding may be explained by the high correlation between t-tau and p-tau181. Participants with A− rarely changed to A+, but approximately 40% of the participants with A+T− changed to A+T+ in 12 months (figure 2). Finally, four A+ groups, that is, the AD continuum group declined clinically and cognitively compared with the normal biomarker group. Notably, when we used AT(N)$_{tau}$ classification, the non-AD pathological change group showed a significantly higher conversion rate than the normal biomarker group (figure 5).

Since the NIA-AA Research Framework was published, the prevalence of biological AD according to CSF biomarker analysis has been reported (online supplemental table 4). In the US-ADNI study, 21%, 84% and 82% of the CU, MCI (progressed to dementia later) and ADD groups showed the A+T+ profile, respectively. A previous study with five cohorts showed biological AD in 11% of participants with CU. In the BioFINDER study, where CSF NfL was used as the N marker, 17% of the CU and 39%–86% of MCI and mild ADD groups had biological AD. Compared with these western cohorts, our Japanese cohort had slightly lower prevalence rates than those that discriminate the clinical status. Considering that both fluid and imaging biomarkers are continuous values along the course of the AD continuum, AT(N) classification defined by dichotomising the cut-off value should be cautiously interpreted. In our comparison, the cut-off value used for distinguishing PET Aβ+ individuals from PET Aβ− individuals was substantially higher than that used for distinguishing individuals with ADD from those with CU (378.7 pg/mL vs 288.6 pg/mL, table 1). Similarly, the cut-off values for the T and N markers that discriminate the PET Aβ status were lower than those that discriminate the clinical status. Considering that approximately 20% of ADD cases could be clinically misdiagnosed as dementia with the non-AD pathology and 30% of elderly people without cognitive impairment have the AD pathology, determination of the cut-off value using clinically diagnosed samples should be conducted with caution. An unbiased method has recruited participants with an earlier AD stage, or the A+T+ prevalence rate is truly low in east Asian populations.

We demonstrated the different characteristics between t-tau and NfL used as N markers. Results showed that t-tau moderately correlated with NfL ($r=0.49$; online supplemental figure 5), but highly correlated with p-tau181 ($r=0.79$), consistent with previous reports. In the AT(N)$_{tau}$ classification, participants with T− showed the (N)− profile more frequently, whereas those with T+ showed the (N)+ profile more frequently (table 2, figure 1). CSF NfL has been reported to reflect neurodegeneration more closely than t-tau in the AD continuum. Recently, it has been reported that Aβ deposition in the brain facilitates the secretion of tau fragments in CSF. Thus, the mechanism of tau elevation in CSF in the AD continuum may differ from the mechanism(s) underlying other types of neuronal injury with the non-AD pathology. It should be noted that each of the fluid and imaging biomarkers have a different prognostic value.

Figure 5 Conversion to dementia in three groups classified using AT(N)$_{tau}$ (A) and AT(N)$_{NfL}$ classification (B). Survival curves of participants without dementia at the baseline (CDR 0, n=46; CDR 0.5, n=116) illustrate the time of progression to CDR>0.5. Asterisk indicates a significantly frequent conversion to dementia compared with the normal biomarker group as a reference.

AD, Alzheimer’s disease; CDR, Clinical Dementia Rating.

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been reported to overcome this problem, because it does not depend on the clinical information of the samples. Notably, there is discrepancy in the cut-off value of CSF Aβ42 between ADNI and our study (J-ADNI). The discrepancy may be explained by the differences in the methods used to determine the cut-off value, background characteristics and ethnic background.

Our study revealed that CSF biomarkers were useful in predicting longitudinal progression in the J-ADNI cohort, as reported in western cohorts (table 3, figure 5). Conversion to dementia was most frequent in participants in the AD continuum group. Biologically, A− participants rarely converted into A+; however, approximately 40% of A+T− participants converted into A+T+ within 12 months (figure 2). In the US-ADNI study, CSF t-p tau has a faster annual rate of change than CSF Aβ42, consistent with our results. Taken together, A+ participants have a high risk of clinical and biological progression.

This study has several limitations. First, some AT(N) profiles had a small sample size, possibly yielding an insufficient statistical power for detecting significant differences between groups. Second, the follow-up period of 12 months for CSF assessment was relatively short. Thus, the longitudinal changes of biomarkers shown in previous reports could not be detected in our study. Third, participants of J-ADNI were clinically evaluated and not diagnosed by autopsy. For example, the aetiological cause in subjects with the A−T− (N)+ profile is likely to be small vessel diseases and non-tau dementia; however, this assumption needs to be confirmed by further study. Finally, to better understand the optimal N marker, further studies are required to confirm the correlation between biofluid markers and neuroimaging markers such as volumetric MRI.

CONCLUSION

In this study, we determined the frequency of the AT(N) profiles in the J-ADNI cohort using two different N markers in CSF. The biological AD profile (A+T+) was found in 9%, 49%, and 61% of participants with CU, MCI and AD, respectively. The AT(N) profile showed different frequencies between AT(N)tau and AT(N)NfL. Irrespective of the classification, participants with the AD continuum group progressed clinically and biologically. CSF NfL may be more reflective N-marker than t-tau in AD continuum. The AT(N) classification would aid in understanding the AD continuum biology in various populations.

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K contributed to the concept of the study, analysis of the data and wrote the manuscript. MK contributed to the concept of the study, analysis of the data and wrote the manuscript. TT contributed to acquisition of the data and analysis of the data. KS contributed acquisition of the data. AI contributed to acquisition of the data. HK contributed to acquisition of the data. AM contributed to analysis of the data. RK contributed to acquisition of the data. AI contributed to acquisition of the data. NH contributed to acquisition of the data. KS contributed acquisition of the data. RI contributed to acquisition of the data. YT contributed to conception of the study, drafting the manuscript and critical revision of the manuscript and is responsible for the overall content as guarantor.

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Competing interests

No, there are no competing interests.

Patient consent for publication

Not applicable.

Ethics approval

This study involves human participants and was approved by Niigata University Ethics CommitteeReference number: H25-636. The Ethics Committee of Niigata University approved this study (2018-0409). Participants gave informed consent to participate in the study before taking part.

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Supplemental material

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Recent publications

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## Supplementary Table 1. Baseline characteristics of each clinical category

|                  | Whole   | CU       | MCI     | ADD     |
|------------------|---------|----------|---------|---------|
| **Number (%)**   | 194 (100) | 53 (27.3) | 86 (44.3) | 55 (28.4) |
| **Age, years (IQR)** | 71 (5)   | 67 (3)   | 73 (7)   | 74 (6)   |
| **Female, n (%)** | 99 (51.0) | 28 (52.8) | 41 (47.7) | 30 (54.5) |
| **Education, years (IQR)** | 13 (1)   | 14 (2)   | 13 (1)   | 12 (2)   |
| **APOE ε4 allele (%)** |          |          |         |         |
| 0                | 101 (52.1) | 41 (77.4) | 44 (51.2) | 16 (29.1) |
| 1                | 74 (38.1)   | 12 (22.6) | 35 (40.7) | 27 (49.1) |
| 2                | 19 (9.8)    | 0 (0)    | 7 (8.1)   | 12 (21.8) |
| **MMSE (IQR)**   | 27 (3)    | 30 (1)   | 27 (2)   | 23 (2)   |
| **ADAS-Cog (IQR)** | 18.8 (8.8) | 6.7 (2)  | 20.0 (5.2) | 26.7 (3.6) |
| **CDR-SB (IQR)** | 2 (1)     | 0 (0)    | 2 (1)    | 4 (1)    |
| **FAQ (IQR)**    | 3 (3)     | 0 (0)    | 3 (2)    | 9 (3)    |
| **Aβ PET, n (%)** |          |          |         |         |
| Negative         | 47 (47.0)  | 31 (88.6) | 15 (35.7) | 1 (4.3)  |
| Positive         | 53 (53.0)  | 4 (11.4)  | 27 (64.3) | 22 (95.7) |

Numbers are median (interquartile range, IQR) for continuous variables and raw number (percentage) for categorical variables.

Abbreviations: Aβ, β-amyloid; ADAS-Cog, Alzheimer’s Disease Assessment Scale-Cognitive Subscale; ADD, Alzheimer’s disease dementia; CDR-SB, sum of boxes of the Clinical Dementia Rating; CU, cognitively unimpaired subjects; FAQ, Functional Assessment Questionnaire; MMSE, Mini–Mental State Examination; MCI, mild cognitive impairment
### Supplementary Table 2. Baseline characteristics of 3 groups

| AT(N)$_{inu}$ | Normal biomarkers | Non-AD pathologic changes | AD continuum | p-value |
|---------------|-------------------|---------------------------|--------------|---------|
| Number (%)    | 52 (29.4)         | 10 (5.6)                  | 115 (65.0)   | 0.005   |
| Age, years (IQR) | 67 (9)          | 75 (4)                    | 73 (10)      |          |
| Female, n (%) | 23 (44.2)        | 5 (50.0)                  | 59 (51.3)    | 0.698   |
| Education, years (IQR) | 14 (4)        | 16 (0)                    | 13 (4)       | 0.015   |
| APOE ε4 allele (%) | <.001          |                           |              |         |
| 0             | 49 (94.2)        | 7 (70.0)                  | 39 (33.9)    | <.001   |
| 1             | 3 (5.8)          | 3 (30.0)                  | 57 (46.9)    | <.001   |
| 2             | 0 (0)            | 0 (0)                     | 19 (16.5)    | <.001   |
| Clinical status, n (%) | <.001          |                           |              |         |
| CU            | 31 (59.6)        | 4 (40.0)                  | 11 (9.6)     | <.001   |
| MCI           | 21 (40.4)        | 5 (50.0)                  | 56 (48.7)    |         |
| ADD           | 0 (0)            | 1 (10.0)                  | 48 (41.7)    | <.001   |
| MMSE (IQR)    | 29 (2)           | 27 (5)                    | 25 (4)       | <.001   |
| ADAS-Cog (IQR) | 9.4 (9.1)       | 18.7 (15.9)               | 23.0 (10.0)  | <.001   |
| CDR-SB (IQR)  | 0 (0.5)          | 0.8 (2.8)                 | 2.0 (2.5)    | <.001   |
| FAQ (IQR)     | 0 (0)            | 3 (9)                     | 5 (8)        | <.001   |
| Aβ PET, n (%) | <.001            |                           |              |         |
| Negative      | 34 (100)         | 3 (50.0)                  | 5 (10.4)     | <.001   |
| Positive      | 0 (0)            | 3 (50.0)                  | 43 (89.6)    |         |
| BL Aβ42, pg/mL (IQR) | 485.2 (101.7) | 486.3 (185.4)             | 240.1 (70.1) | <.001   |
| BL p-tau, pg/mL (IQR) | 19.2 (4.5)     | 33.8 (7.9)                | 36.0 (23.5)  | <.001   |
| BL t-tau, pg/mL (IQR) | 58.4 (29.6)    | 122.8 (57.5)              | 119.3 (66.3) | <.001   |
| BL NfL, pg/mL (IQR) | 2421.6 (1344.70)| 3603.7 (2454.6)           | 3259.0 (1238.7) | <.001   |

| AT(N)$_{inu}$ | Normal biomarkers | Non-AD pathologic changes | AD continuum | p-value |
|---------------|-------------------|---------------------------|--------------|---------|
| Number (%)    | 39 (20.1)         | 23 (5.6)                  | 115 (65.0)   |       |
| Age, years (IQR) | 66 (8)          | 75 (9)                    | 73 (10)      | <.001   |
| Female, n (%) | 18 (46.2)        | 10 (43.5)                 | 59 (51.3)    | 0.723   |
| Education, years (IQR) | 14 (4)        | 16 (4)                    | 13 (4)       | 0.072   |
| APOE ε4 allele (%) | <.001          |                           |              |         |
| 0             | 37 (94.9)        | 19 (82.6)                 | 39 (33.9)    |         |
| 1             | 2 (5.1)          | 4 (17.4)                  | 57 (49.6)    |         |
| 2             | 0 (0)            | 0 (10.0)                  | 19 (16.5)    |         |
| Clinical status, n (%) | <.001          |                           |              |         |
| CU            | 27 (69.2)        | 8 (34.8)                  | 11 (9.6)     |         |
| MCI           | 12 (30.8)        | 14 (60.9)                 | 56 (48.7)    |         |
| ADD           | 0 (0)            | 1 (4.3)                   | 48 (41.7)    | <.001   |
| MMSE (IQR)    | 29 (2)           | 27 (5)                    | 25 (4)       | <.001   |
| ADAS-Cog (IQR) | 8.3 (7.5)       | 14.3 (14.7)               | 23.0 (10.0)  | <.001   |
| CDR-SB (IQR)  | 0 (0.5)          | 1.0 (1.0)                 | 2.0 (2.5)    | <.001   |
| FAQ (IQR)     | 0 (0)            | 1 (3)                     | 5 (8)        | <.001   |
| Aβ PET, n (%) | <.001            |                           |              |         |
| Negative      | 25 (100)         | 12 (0)                    | 5 (10.4)     | <.001   |
| Positive      | 0 (0)            | 3 (100)                   | 43 (89.6)    |         |
| BL Aβ42, pg/mL (IQR) | 479.7 (84.3) | 504.8 (152.3)             | 240.1 (70.1) | <.001   |
| BL p-tau, pg/mL (IQR) | 19.2 (4.1)     | 24.2 (13.5)               | 36.0 (23.5)  | <.001   |
| BL t-tau, pg/mL (IQR) | 54.6 (24.4)    | 87.6 (37.5)               | 119.3 (66.3) | <.001   |
| BL NfL, pg/mL (IQR) | 2106.9 (1118.0)| 4028.7 (1598.2)           | 3259.0 (1238.7) | <.001   |

Numbers are median (interquartile range, IQR) for continuous variables and raw number (percentage) for categorical variables.

Differences in baseline characteristics of participants across 8 AT(N) profiles were first assessed using Kruskal-Wallis rank sum test for continuous variables, or a Chi-squared test for categorical variables.
categorical variables.

Abbreviations: Aβ, β-amyloid; ADAS-Cog, Alzheimer’s Disease Assessment Scale-Cognitive Subscale; ADD, Alzheimer’s disease dementia; BL, baseline; CDR-SB, sum of boxes of the Clinical Dementia Rating; CU, cognitively unimpaired subjects; FAQ, Functional Assessment Questionnaire; MMSE, Mini–Mental State Examination; MCI, mild cognitive impairment; NfL, neurofilament light chain; p-tau181, tau phosphorylated at threonine 181; t-tau, total tau
Supplementary Table 3. Longitudinal changes of biomarkers

| AT(N) | Aβ42 slope (β) | p-value | p-tau181 slope (β) | p-value | t-tau slope (β) | p-value | NfL slope (β) | p-value |
|-------|----------------|---------|---------------------|---------|----------------|---------|--------------|---------|
| A-T(N)- | 0.005 | 0.601 | **0.014** | 0.039 | 0.001 | 0.801 | 0.003 | 0.435 |
| A-T(N)+ | NA | NA | NA | NA | NA | NA | NA | NA |
| A-T+(N)- | -0.015 | 0.692 | -0.040 | 0.279 | -0.005 | 0.467 | 0.001 | 0.660 |
| A-T+(N)+ | NA | NA | NA | NA | NA | NA | NA | NA |
| A+T-(N)- | 0.005 | 0.712 | **0.034** | 0.006 | 0.006 | 0.520 | 0.004 | 0.467 |
| A+T-(N)+ | 0.013 | 0.473 | 0.106 | 0.117 | 0.013 | 0.216 | 0.216 | 0.455 |
| A+T+(N)- | 0.002 | 0.941 | -0.024 | 0.420 | 0.020 | 0.393 | 0.103 | 0.304 |
| A+T+(N)+ | 0.002 | 0.785 | 0.009 | 0.639 | 0.006 | 0.728 | 0.009 | 0.068 |

Each statistic was calculated by linear regression model, adjusting age, sex, and education years. *Bold* indicated that the results were statistically significant. The slopes and p-values represent differences between each AT(N) profile slope relative to zero. The statistics of AT(N) profiles with a small sample size (< 3 samples who were measured the CSF biomarkers at baseline and 12 months) were not calculated and were represented as “NA”.

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### Supplementary Table 4. Prevalence of AT(N) profiles and biological AD according to CSF biomarkers across cohorts

| Report                        | Cohort                        | N marker | Clinical Status (number) | Mean Age | A- T- (N)- | A- T+ (N)+ | A- T+ (N)+ | A+ T- (N)- | A+ T+ (N)+ | A+ T+ (N)+ | Biological AD (A+T+) |
|-------------------------------|-------------------------------|----------|--------------------------|----------|------------|------------|------------|------------|------------|------------|----------------------------|
| Kern S, et al. Neurology 2018 | H70 Gothenburg                | t-tau    | CU (259)                 | 70.6     | 54         | 19         | 0          | 5          | 13         | 7          | 0           | 2           | 2           |
| Ekman U, et al. Sci Rep 2018  | ADNI                          | t-tau    | stable MCI (80)          | 74.5     | 30         | 0          | 6          | 5          | 11         | 0          | 19          | 29          | 48          |
|                               |                               |          | progressive MCI (74)     | 74.5     | 8          | 0          | 2          | 0          | 5          | 2          | 30          | 54          | 84          |
|                               |                               |          | AD (102)                 | 75.0     | 4          | 0          | 2          | 2          | 10         | 0          | 19          | 63          | 82          |
| Soldan A, et al. Neurology 2019 | ACS, AIBL, BIOCARD, IMPACT, WRAP | t-tau    | CU (814)                 | 59.6     | 39         | 6          | 6          | 17         | 19         | 2          | 2           | 9           | 11          |
| Carandini T, et al. Alzheimers Res Ther 2019 | Univ. of Milan | t-tau    | MCI (132)                | 73       | 20         | 0          | 8          | 9          | 27         | 3          | 14          | 19          | 33          |
|                               |                               |          | AD (229)                 | 72       | 0          | 0          | 2          | 3          | 25         | 3          | 15          | 52          | 67          |
| Mattsson-Carlsgen N, et al. Neurology 2020 | BioFINDER-1 | NfL | CU (53)                  | 74.5     | 40         | 4          | 4          | 2          | 25         | 8          | 17          | 0           | 17          |
|                               | BioFINDER-2                   |          | MCI (14), AD (34)        | 71.9     | 2          | 0          | 2          | 0          | 10         | 0          | 59          | 27          | 86          |
|                               |                               |          | CU (245)                 | 63.6     | 49         | 1          | 13         | 2          | 17         | 0          | 14          | 3           | 17          |
|                               |                               |          | MCI (138), AD (6)        | 70.9     | 25         | 8          | 8          | 1          | 17         | 3          | 25          | 14          | 39          |
| Lee J, et al. J Korean Med Sci 2020 | Samsung Medical Center | t-tau    | CU (51)                  | 64.1     | 73         | 0          | 14         | 2          | 8          | 2          | 0           | 2           | 2           |
|                               |                               |          | Amnestic MCI (23)        | 67.5     | 4          | 4          | 9          | 0          | 30         | 22         | 4           | 26          | 30          |
|                               |                               |          | AD (65)                  | 63.3     | 2          | 0          | 0          | 2          | 26         | 14         | 0           | 57          | 57          |
| Grontvedt GR, et al.          | Univ. Hospital of             | t-tau    | CU (61)                  | 68       | 69         | 0          | 10         | 13         | 2          | 0          | 0           | 7           | 7           |
| J Alzheimers Dis 2020 | Trondheim | Amnestic MCI (64) | 64 | 23 | 3 | 2 | 9 | 17 | 3 | 3 | 39 | 42 |
|-----------------------|-----------|------------------|----|----|---|---|---|----|---|---|----|----|
|                       |           | AD (38)          | 63.5 | 3 | 3 | 0 | 5 | 18 | 3 | 0 | 68 | 68 |
| Cousins KAQ, et al.   | U-Penn    | Amnestic AD (98) | 73.5 | 2 | 0 | 2 | 3 | 13 | 11 | 16 | 52 | 68 |
| Brain 2021             | (Autopsy) | Non-amnestic AD (20) | 63.5 | 15 | 0 | 0 | 0 | 30 | 10 | 25 | 20 | 45 |
|                       |           | Amnestic FTLD (5) | 71.0 | 40 | 0 | 0 | 0 | 40 | 20 | 0 | 0 | 0  |
|                       |           | Non-amnestic FTLD (59) | 65.0 | 64 | 12 | 3 | 5 | 12 | 3 | 0 | 0 | 0  |
| Eckerstrom C, et al.  | Gothenburg MCI | SCI (194), MCI (226) | NA | 33 | 6 | 8 | 20 | 8 | 1 | 1 | 23 | 24 |
| Alzheimers Dement     |            | t-tau CU (46)    | 71.8 | 67 | 2 | 4 | 2 | 15 | 0 | 2 | 7 | 9  |
| (Amst) 2021           |            | MCI (82)         | 71.8 | 26 | 2 | 1 | 2 | 17 | 2 | 6 | 43 | 49 |
| This study            | J-ADNI    | AD (49)          | 72.2 | 0 | 0 | 2 | 0 | 33 | 4 | 8 | 53 | 61 |
|                       |           | t-tau CU (46)    | 71.8 | 59 | 11 | 7 | 0 | 15 | 0 | 9 | 0 | 9  |
|                       |           | MCI (82)         | 71.8 | 15 | 1 | 3 | 1 | 10 | 10 | 22 | 27 | 49 |
|                       |           | AD (49)          | 72.2 | 0 | 0 | 0 | 2 | 10 | 27 | 20 | 41 | 61 |

All cohorts use CSF Aβ42 as A maker and CSF p-tau181 as T marker. The number for each AT(N) profile and biological AD indicates percentage.

Abbreviations: AD, Alzheimer’s disease dementia; CU, cognitively unimpaired subjects; FTLD, frontotemporal lobar degeneration; MCI, mild cognitive impairment; NA, not applicable; SCI, subjective cognitive impairment.
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Supplementary figure 1

715 assessed for eligibility (170 CU, 349 MCI, 196 ADD)
- 178 not met criteria (16 CU, 115 MCI, 47 ADD)
- 4 withdrew consent (3 MCI, 1 ADD)
- 339 not obtained CSF (101 CU, 145 MCI, 93 ADD)

194 with BL CSF (53 CU, 86 MCI, 55 ADD)
- Cutoff for Aβ42, p-tau181, t-tau
- Correlation for Aβ42, p-tau181, t-tau

17 without BL NfL (7 CU, 4 MCI, 6 ADD)

177 with BL NfL (46 CU, 82 MCI, 49 ADD)
- Cutoff for NfL
- Correlation for NfL
- Cross-sectional ATN
- Longitudinal cognitive function change, Conversion

51 without 12M NfL (8 CU, 26 MCI, 17 ADD)

126 with 12M NfL (38 CU, 56 MCI, 32 ADD)
- Longitudinal CSF biomarker change, ATN change

Supplementary Figure 1. Flowchart showing the number of participants used for each analysis
Supplementary figure 2

A. Aβ42

B. p-tau181

C. t-tau

D. NfL
Supplementary figure 2. Determination of cutoff values for each of the biomarkers by different methods

In the first column, the cutoff value was determined between Aβ PET negative (Aβ PET−) and positive (Aβ PET+) participants. In the second column, the cutoff value was determined between CU participants with Aβ PET− (CU, Aβ−) and ADD patients with Aβ PET+ (AD, Aβ+). In the third column, the cutoff value was determined between CU subjects and ADD participants. The fourth column shows the cutoff value by GMM (except NfL, which is not suitable because of the unimodal distribution). The dotted lines in upper panels in each biomarker represent the cutoff values calculated according to Youden’s index. The lower panels in each biomarker show the ROC curves to determine the cutoff values. In GMM, the cutoff values are estimated as the crossing point (vertical lines) of the prevalence-weighted densities.
Supplementary Figure 3. ROC curves of different CSF biomarkers

(A) ROC curves that distinguish Aβ PET negative (Aβ PET−) from PET-positive (Aβ PET+) participants are shown. (B) ROC curves that distinguish CU participants with Aβ PET− (CU, Aβ PET−) from ADD patients with Aβ PET+ (ADD, Aβ+) are shown. (C) ROC curves that distinguish CU participants from ADD patients.
Supplementary figure 4

A. Clinical status

B. Age

C. Education

D. Sex

E. APOE ε4 allele
**Supplementary Figure 4. Analysis of various parameters by CSF biomarkers**

Parameters including clinical status (A), age (B), years of education (C), sex (D), and numbers of *APOE* ε4 allele (E) at baseline were analyzed by CSF biomarkers. Orange: Aβ42, blue: p-tau, green: t-tau, violet: NFL.
Supplementary figure 5

|       | Aβ42   | p-tau  | t-tau  | NfL    |
|-------|--------|--------|--------|--------|
| Aβ42  | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) | ![Image](image4.png) |
| p-tau | $r = -0.4690$ | $r = 0.7923$ | $r = 0.4907$ | $r = 0.2487$ |
|       | $p < 0.0001$ | $p < 0.0001$ | $p < 0.0001$ | $p = 0.0008$ |

Supplementary Figure 5. Correlations between different CSF biomarkers

Scatterplots (shown in upper diagonal) and correlation coefficients (shown in lower diagonal) are presented among CSF biomarkers including Aβ42, p-tau, total tau and NfL.
Supplementary Figure 6. CSF biomarker levels at baseline among 8 AT(N) profiles

Upper panels show CSF biomarker levels of each of AT(N) groups stratified by AT(N)\textsubscript{tau} classification. Lower panels show CSF biomarker levels of each of AT(N) groups stratified by AT(N)\textsubscript{NFL} classification.
Supplementary Figure 7. Longitudinal changes of CSF biomarkers in 8 AT(N) profiles
Linear regression model adjusted for age, sex, and education years predicts the changes of each CSF biomarker over time among participants classified into eight AT(N) categories classified into AT(N)$_{tau}$ (upper panel) and AT(N)$_{NFL}$ (lower panel). Asterisk shows a significant change of slope relative to zero.