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**Association of CSF, Plasma, and Imaging Markers of Neurodegeneration With Clinical Progression in People With Subjective Cognitive Decline**

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## Abstract

**Background and Objectives:** Multiple biomarkers have been suggested to measure neurodegeneration (N) in the AT(N) framework, leading to inconsistencies between studies. We investigated the association of five N biomarkers with clinical progression and cognitive decline in individuals with subjective cognitive decline (SCD).

**Methods:** We included individuals with SCD from the Amsterdam Dementia Cohort and SCIENCE project, a longitudinal cohort study (follow-up  $4\pm 3$ y). We used the following N biomarkers: CSF total (t)-tau, medial temporal atrophy visual rating on MRI, hippocampal volume (HV), serum neurofilament light (NfL) and serum glial fibrillary acidic protein (GFAP). We determined correlations between biomarkers. We assessed associations between N biomarkers and clinical progression to mild cognitive impairment or dementia (Cox regression), and MMSE over time (linear mixed models). Models included age and sex, CSF abeta (A), and CSF p-tau (T) as covariates, in addition to the N biomarker.

**Result:** We included 401 individuals ( $61\pm 9$ y, 42%F, MMSE  $28\pm 2$ , vascular comorbidities 8-19%). N biomarkers were modestly to moderately correlated (range  $r$  -0.28 – 0.58). Serum NfL and GFAP correlated most strongly ( $r$  0.58,  $p < 0.01$ ). T-tau was strongly correlated with p-tau ( $r$  0.89,  $p < 0.01$ ), although these biomarkers supposedly represent separate biomarker groups. All N biomarkers individually predicted clinical progression, but only HV, NfL and GFAP added predictive value beyond abeta and p-tau (HR 1.52 (95%CI 1.11-2.09); 1.51 (1.05-2.17); 1.50 (1.04-2.15)). T-tau, HV and GFAP individually predicted MMSE slope (range beta -0.17 – -0.11,  $p < 0.05$ ), but only HV remained associated beyond abeta and p-tau (beta -0.13 (SE 0.04),  $p < 0.05$ ).

**Discussion:** In cognitively unimpaired elderly, correlations between different N biomarkers were only moderate, indicating they reflect different aspects of neurodegeneration and should not be used interchangeably. T-tau was strongly associated with p-tau (T), which makes it less desirable to use as measure for N. HV, NfL and GFAP predicted clinical progression beyond A and T. Our results do not allow to choose one most suitable biomarker for N, but illustrate the added prognostic value of N beyond A and T.

**Classification of evidence:** This study provides Class II evidence that HV, NfL and GFAP predicted clinical progression beyond A and T in individuals with SCD.

ACCEPTED

## Introduction

In recent years, there has been a major change in the definition of Alzheimer's disease (AD). Formerly, the core criteria of AD diagnosis were based on clinical symptoms.<sup>1</sup> In 2018, a research framework has been put forward by the NIA-AA in which every individual is classified based on specific biomarkers in the AT(N) classification.<sup>2</sup> In this framework, the term 'Alzheimer's disease' refers to the presence of abnormal amyloid-beta accumulation and neurofibrillary tau tangles, i.e. 'A', measured by CSF abeta or amyloid PET, and 'T', measured by CSF phosphorylated tau (p-tau) or tau PET. The AT(N) construct is independent of the cognitive stage of the individual, which makes it possible to identify AD in cognitively normal individuals. The 'N' in the AT(N) classification represents neurodegeneration. Neurodegeneration can have many different causes and is not specific for AD. Therefore, neurodegenerative markers are not necessary for the diagnosis, but rather have been suggested to provide pathologic staging information and predictive value. Proposed biomarkers of N include atrophy on MRI, hypometabolism on fluorodeoxyglucose (FDG) PET or CSF total tau (t-tau).<sup>2</sup> In addition, blood-based biomarkers are now available and have been suggested as non-invasive alternative markers for N.<sup>2-4</sup>

Allowing different biomarkers as indicator of a biomarker group implies that they can be used interchangeably and measure the same pathological process. For the A and T biomarker group, this assumption holds fairly well, with moderate to high agreement and relatively high correlation coefficients between markers within A and T, respectively.<sup>5-7</sup> N biomarkers, however, are poorly correlated and show inadequate agreement.<sup>6, 8-11</sup> Furthermore, the fact that N biomarkers are suggested to provide staging information implies that individuals with a higher degree of neurodegeneration are assumed to deteriorate faster. However, there are only a few studies that directly compared different N biomarkers in their association with clinical progression or cognitive

decline over time. Most are hampered by small sample sizes, and none have directly compared blood-based biomarkers to CSF and imaging biomarkers yet.<sup>10, 12-15</sup>

It is difficult to determine which modality captures ‘neurodegeneration (N)’ most accurately, because there is no gold standard available. However, it should capture a different process than the accumulation of amyloid-beta (A) or fibrillary tau (T), as otherwise the addition of N would have no added value in the AT(N) classification. Furthermore, if different N biomarkers indeed capture the same process, correlations between N biomarkers should be higher than correlations between A and N, or T and N biomarkers. Finally, as N provides staging information, it should have some clinical correlate. In early disease stages especially, it is important to be able to accurately predict future deterioration, for both the individuals themselves and clinical trial recruitment, since these could still potentially benefit from disease modifying therapies. Therefore, our aims were (1) to compare the different N biomarkers CSF total (t)-tau, medial temporal atrophy (MTA) visual rating on MRI, hippocampal volume (HV), serum neurofilament light (NfL) and serum glial fibrillary acidic protein (GFAP) to each other and to markers of A and T, and (2) to determine their predictive value for clinical progression and cognitive decline beyond A and T, in a sample of cognitively normal individuals with subjective cognitive decline (SCD).

## Methods

### Study population

We included 401 individuals with SCD from the Amsterdam Dementia Cohort (ADC) and SCIENCE project (Subjective Cognitive Impairment Cohort).<sup>16,17</sup> The SCIENCE project is a substudy of ADC and prospectively follows individuals with SCD. Individuals were referred to our memory clinic because of cognitive complaints by their general physician, a geriatrist or a neurologist, and underwent an extensive diagnostic workup, including a physical, neurological and neuropsychological evaluation. In a multidisciplinary consensus meeting, all individuals received the label SCD when they performed within normal limits on a neuropsychological assessment, and criteria for mild cognitive impairment (MCI), dementia, or other neurological or psychiatric diseases that could potentially cause cognitive complaints, were not met. At follow-up, diagnoses were re-evaluated as SCD, MCI, AD dementia or other types of dementia. Clinical progression was defined as progression from SCD to MCI or dementia. Inclusion criteria for the current study were baseline SCD diagnosis, availability of follow-up information ( $\geq 2$  diagnoses), availability of CSF, and availability of MRI and/or serum biomarkers within one year of diagnosis.

MMSE was assessed annually and was used as longitudinal measure of global cognition. Education was rated using the Dutch Verhage system.<sup>18</sup>

### Biomarkers

We used all biomarkers both as continuous and dichotomous measures. We used CSF abeta (continuous and dichotomous, abnormal  $<813$  pg/mL) or amyloid PET (dichotomous, visual assessment) as biomarker for A. When both amyloid PET and CSF abeta were available, the PET result was used. We used CSF p-tau (abnormal  $>52$  pg/mL) as biomarker for T. We compared five different N biomarkers: CSF t-tau (abnormal  $>375$  pg/mL), MTA score (abnormal  $\geq 1$ ), HV, serum NfL and



serum GFAP. We used a cut-off value of  $\geq 1$  for MTA score instead of age-dependent cut-off values, to be consistent with thresholds for the other biomarkers, which are also age-independent.<sup>19</sup> For HV, NfL and GFAP, no established cut-off values were available. Because of varying rates of N+ in literature<sup>12, 20</sup>, we pragmatically took the 75<sup>th</sup> and 90<sup>th</sup> percentile for NfL and GFAP, and the 10<sup>th</sup> and 25<sup>th</sup> percentile for HV, which provides the reader with a range of possible effects sizes. Hence, for HV, NfL and GFAP, we chose two dichotomous definitions per biomarker. The following describe the procedures used to obtain these measures.

A lumbar puncture was performed between the L3/L4, L4/L5 or L5/S1 intervertebral space to obtain CSF, which was subsequently collected in polypropylene tubes.<sup>21</sup> Levels of abeta<sub>1-42</sub>, tau phosphorylated threonine 181 (p-tau) and total tau were measured using sandwich ELISA's (Innotest beta-amyloid<sub>1-42</sub>, Innotest PhosphoTAU-181p and Innotest hTAU-Ag).<sup>22</sup> CSF abeta levels were corrected for the drift that occurred over the years.<sup>23</sup>

For 79 individuals, amyloid PET was performed using the tracers [<sup>18</sup>F]Florbetapir (n=13), [<sup>18</sup>F]Florbetaben (n=48), [<sup>18</sup>F]Flutemetamol (n=7) or [<sup>11</sup>C]-PIB (Pittsburgh compound-B, n=11). An intravenous cannula was used to administer the tracers. The following systems were used to acquire the PET scans: Gemini TF PET-CT, Ingenuity TF PET-CT, and Ingenuity PET/MRI (Philips Healthcare, Best, The Netherlands). For [<sup>18</sup>F]Florbetaben<sup>24</sup> and [<sup>18</sup>F]Flutemetamol<sup>25</sup> imaging, a static scanning protocol was used, for [<sup>18</sup>F]Florbetapir<sup>17</sup> and [<sup>11</sup>C]PIB imaging<sup>26</sup>, a dynamic scanning protocol. A trained nuclear medicine physician visually rated all scans as 'positive' or 'negative', according to the radiotracer specific product guidelines.

Structural MRI 3D T1-weighted images (n=366 (89%)) were acquired as part of routine patient care from nine different systems. The acquisition parameters are described in eAppendix 1. An experienced neuroradiologist reviewed all scans. T1-weighted images were used for visual rating of

medial temporal lobe atrophy (MTA; range 0-4). Scores for the left and right sides were averaged.<sup>27</sup> Hippocampal volume was estimated using FMRIB Software Library (FSL) FIRST (v5), as described previously.<sup>28</sup> The FIRST algorithm first registers the 3D T1-weighted images to the Montreal Neurological Institute 152 template. Next, it uses a subcortical mask for segmentation based on shape models and voxel intensities to obtain hippocampal volumes. Hippocampal volumes were normalized for head size using the V-scaling factor from SIENAX<sup>29</sup>, and left and right sides were averaged. All images were visually inspected for registration or segmentation errors.

Non-fasted EDTA plasma samples (n=296 (72%)) were obtained through venipuncture and centrifuged on average within 2 hours from collection, at 1800g, 10 minutes at room temperature, before immediate storage at -80 °C until analysis. Serum GFAP and NfL levels were measured using the commercially available Simoa<sup>TM</sup> GFAP Discovery Kit (Quanterix) and the Simoa<sup>TM</sup> NF-Light Advantage Kit (Quanterix) according to manufacturer's instructions and with on-board automated sample dilution.<sup>4</sup> All samples were measured in duplicates with good average intra-assay %CV.

### **Standard protocol approvals, registrations, and patient consents**

The research is conducted in accordance with ethical consent by VU University and the Helsinki Declaration of 1975. For all individuals included in the study, written informed consent was available.

### **Statistics**

All analyses were performed in R version 4.0.3. We first used all biomarkers as continuous measures (abeta, p-tau, t-tau, MTA, HV, NfL and GFAP). Since the AT(N) classification is based on dichotomous variables, we repeated all analyses with dichotomized biomarkers (A, T,  $N_{t\text{-tau}}$ ,  $N_{MTA}$ ,  $N_{HV25}$ ,  $N_{HV10}$ ,  $N_{NfL75}$ ,  $N_{NfL90}$ ,  $N_{GFAP75}$ ,  $N_{GFAP90}$ ). CSF p-tau, t-tau, serum NfL and GFAP were log transformed due to non-normality. For Cox proportional hazards models and linear mixed models, continuous predictors

were transformed to z-scores for comparability of effect sizes, and HV was inverted, so that for all variables higher values indicates worse.

We first compared demographic and clinical variables between individuals that remained stable, and those that progressed to MCI or dementia during follow-up, using t-test, Mann-Whitney U test and chi-square where appropriate. To assess correlations between biomarkers, we used Pearson correlation analysis (CSF abeta, p-tau and t-tau, MTA score, HV, and serum NfL and GFAP). We additionally used partial correlation to adjust for age and sex.

We then investigated the associations between biomarkers and clinical progression using Cox proportional hazards analyses, with progression to MCI or dementia as outcome. We ran four different models, with a cumulative number of predictors. We first ran analyses with continuous N biomarkers as single predictors (model 1). We then added age and sex as covariates (model 2). Then we added CSF abeta as covariate (model 3), and finally, also CSF p-tau (model 4). In models with MTA and HV, scanner type was additionally added as covariate. Separate analyses were performed for each of the N biomarkers t-tau, MTA, HV, NfL and GFAP. Finally, for exploration purposes, we combined multiple N biomarkers in one model, entering all N biomarkers that were significantly associated with the outcome in model 4, simultaneously.

Next, we investigated the relationship between the different N biomarkers and MMSE over time using linear mixed models. We ran four different models with a cumulative number of covariates, similar to the models described for the Cox analyses. We first used the N biomarker, time and N biomarker\*time as predictors (model 1). Next, we added age and sex as covariates (model 2). To account for the putative modifying effect of age and sex on rate of decline, we additionally added the interaction terms age\*time and sex\*time to model 2. Then we added CSF abeta and abeta\*time as covariates (model 3) and finally, also CSF p-tau and p-tau\*time (model 4). In models with MTA and

HV, scanner type was additionally added as covariate. We included a random intercept and random slope.

We repeated the analyses with dichotomous N biomarkers. We visualized AT(N) distributions for different N biomarkers using bar graphs. We ran Cox proportional hazards models similarly to models with continuous N biomarkers, except dichotomized N biomarkers were used as predictors, as well as dichotomized A and T biomarkers when they were added as covariates in models 3 and 4. We visualized the associations between N biomarkers and clinical progression to MCI or dementia using Kaplan Meier curves. All analyses were corrected for multiple testing using the false discovery rate (FDR). FDR corrected p-values  $<0.05$  were considered significant.

#### **Data availability**

Data used within the article may be shared upon reasonable request.

## Results

### Baseline demographics

The 401 individuals were on average  $61 \pm 9$  years old, 167(42%) were female, and 153(39%) were *APOE*  $\epsilon 4$  carriers (Table 1). At follow-up, 64(16%) individuals progressed to MCI or dementia (29(7%) to MCI, 23(6%) to AD dementia and 12(3%) to non-AD dementia). Individuals who progressed to MCI or dementia were on average older, had a lower baseline MMSE score and were more often *APOE*  $\epsilon 4$  carrier. Additionally, they had lower values for abeta, higher values for p-tau, t-tau, MTA, NfL and GFAP, and smaller hippocampal volume.

### Correlations between N biomarkers

The different N biomarkers were modestly to moderately correlated (range  $r$  -0.28 – 0.58, Figure 1A). Serum markers NfL and GFAP correlated most strongly ( $r$  0.58,  $p < 0.01$ ). P-tau and t-tau, representing different AT(N) biomarker groups (T and N respectively), were very strongly correlated ( $r$  0.89,  $p < 0.01$ ). Overall, the correlation coefficients between the different biomarkers for N were in a similar range as the correlation coefficients between the different biomarkers for N on the one hand, and biomarkers for A and T on the other hand ( $r$  -0.43 – 0.33, excluding the correlation between p-tau and t-tau). After adjusting for age and sex, drastically lower coefficients were observed (Figure 1B).

### Risk of progression to MCI or dementia

We investigated the predictive value of the different N biomarkers using Cox proportional hazards analyses. The mean follow-up duration was 3.8 years ( $\pm$  2.8 years). In uncorrected models, t-tau, MTA, HV, NfL and GFAP all predicted clinical progression to MCI or dementia (Table 2, model 1). After adding covariates in model 2 (age and sex), 3 (abeta, age and sex) and 4 (abeta, p-tau, age and sex), hazard ratios were attenuated. Model 4 showed that HV, NfL and GFAP added predictive value to abeta and p-tau. T-tau also predicted MCI or dementia in models 1 to 3, but was not entered in

model 4 due to collinearity between t-tau and p-tau. In an additional explorative analysis, we added the three N markers HV, NfL and GFAP simultaneously in a model in addition to abeta and p-tau, since these biomarkers added predictive value in model 4. In this model, only HV remained significantly associated with clinical progression to MCI or dementia (HR 1.45 (SE 1.01 – 2.09)). The associations for NfL (0.94 (0.56 – 1.59)) and GFAP (1.40 (0.86 – 2.29)) were attenuated (n=258 due to varying availability rates for N biomarkers).

Results of the analyses for complete cases only (n=256) were overall similar, although not all associations survived FDR correction (eTable 1).

### **Cognitive decline over time**

We estimated change in MMSE over time using linear mixed models. In total, 1196 MMSE scores of 399 participants individuals were available, with missing values for two individuals (334  $\geq$  2 visits; range 1-17, median 3 visits). No associations between any N biomarkers and baseline MMSE scores were observed in our sample of cognitively normal elderly. Table 3 shows the results for the interaction between the N biomarkers and time, which reflects the effect of each of the N biomarkers on MMSE slope. In both uncorrected models (model 1) and models corrected for age and sex (model 2), t-tau, HV and GFAP predicted MMSE slope. T-tau and HV also added predictive value to abeta (model 3), but only HV added predictive value beyond abeta and p-tau (model 8). Results were similar for analyses with complete cases (n=256, eTable 2).

### **Dichotomous N biomarkers**

The proportion of N+ individuals, and hence the distribution of AT(N) categories, strongly depended on the definition of N (Figure 2). Proportions of N+ varied between 10% ( $N_{HV10}$ ,  $N_{NfL90}$ ,  $N_{GFAP90}$ ), and 25% ( $N_{HV25}$ ,  $N_{NfL75}$ ,  $N_{GFAP75}$ ). For  $N_{t\text{-tau}}$  and  $N_{MTA}$ , proportions of N+ were about 22%. N+ was more

common in A- compared to A+ individuals for  $N_{MTA}$  or  $N_{HV}$ , and more common in A+ compared to A- individuals for  $N_{GFAP}$ . For  $N_{NFL}$  and  $N_{t-tau}$ , frequencies of N+ were similar between A+ and A-.

Cox proportional hazards analyses using dichotomous N biomarkers to predict clinical progression to MCI or dementia provided overall similar results to analyses with continuous biomarkers, for models 1 and 2 (Table 4). However, only  $N_{t-tau}$  and  $N_{HV25}$  added predictive value to A, and only  $N_{HV25}$  added value beyond A and T. Figure 3 visualizes the combined effect of A and N status for each N on risk of clinical progression in four-level variables (A-N-, A-N+, A+N-, A+N+).

### **Classification of evidence**

This study provides Class II evidence that HV, NFL and GFAP predicted clinical progression beyond A and T in cognitively unimpaired elderly individuals with SCD.

## Discussion

In a sample of cognitively normal individuals with SCD, we found modest to moderate correlations and low concordance between the N biomarkers t-tau, MTA, HV, NfL and GFAP. N biomarkers HV, NfL and GFAP each predicted clinical progression, and had predictive value in addition to abeta and p-tau. Therefore, we recommend HV, NfL or GFAP as biomarkers for N. The tight correlation between t-tau and p-tau precludes the use of the former as a marker of a different biomarker category than the latter.

We extend on former observations that different markers of N are not necessarily closely correlated. The low correlation between N biomarkers likely contributes to the often discordant biomarker results in the AT(N) classification.<sup>6, 9, 12, 15</sup> We add blood-based biomarkers to the comparison, showing similarly modest associations with the N biomarkers in other modalities, and also similarly strong associations with clinically relevant outcomes. Although at a population level, the overall qualitative pattern of biomarker frequencies remains rather stable regardless of the type of biomarkers used<sup>9</sup>, it becomes problematic when researchers and clinicians treat the different N biomarkers as if they were identical. For prediction modeling at the individual patient level, the prognosis for an individual will vary considerably depending on the choice of N biomarker. The choice of N biomarker will also have an effect on the design of therapeutic trials, as well as the potential implementation of the AT(N) classification in the clinic. Studies investigating the AT(N) classification that use different definitions of their biomarkers, cannot be directly compared.

We found low to modest correlations and low concordance between different N biomarkers, which is largely in line with literature.<sup>6, 10, 12, 30, 31</sup> One possible explanation for this is that although all N biomarkers capture a certain aspect of neurodegeneration, the underlying biological processes that lead to specific N biomarker abnormalities are far from identical. T-tau and NfL reflect the severity of



neuroaxonal injury, atrophy on MRI reflects loss of the neuropil, and GFAP reflects astrocyte activity.<sup>2, 32-34</sup> Literature suggests these processes all have a different longitudinal trajectory, for example, NfL and t-tau abnormality likely precede HV abnormality and t-tau eventually reaches a plateau.<sup>35-38</sup> This means correlations between N biomarkers of different processes are probably dependent on disease stage. However, MTA and HV were also poorly correlated, which is remarkable considering both HV and MTA aim to measure a similar process. We found a correlation coefficient of -0.24, which is relatively low and slightly lower than coefficients found in literature (range  $r$  -0.27 to -0.54).<sup>39-41</sup> This low correlation could be due to the fact that the MTA score is partly influenced by the volume of the surrounding CSF spaces, which means it reflects hippocampal atrophy as well as global and subcortical atrophy.<sup>42</sup> Furthermore, being cognitively normal, most individuals in our sample had an MTA score of 0, which reflects that the variability for this measure is probably too small to be a meaningful N biomarker in such a very early sample. In addition, the correlation coefficients between N biomarkers were in a similar range as the correlation coefficients between N biomarkers on the one hand, and A and T biomarkers on the other hand. This is in line with another study which found moderate correlations between biomarkers of different pathophysiological categories.<sup>6</sup> This implies that the underlying neurodegeneration processes are almost as different to each other, as they are different to processes underlying the A and T biomarker category. Overall, the low correlation coefficients illustrate that N biomarkers cannot be used interchangeably in the AT(N) classification.

We found that HV, NfL and GFAP predicted clinical progression, and HV predicted MMSE slope, beyond abeta and p-tau. Former studies that investigated the AT(N) classification often used only one biomarker for A, T and N respectively, and showed that overall, the AT(N) classification was associated with clinical progression and cognitive decline.<sup>20, 43-47</sup> From these studies, the predictive value per individual biomarker cannot be discerned and thus cannot be used to choose the optimal N biomarker. Literature regarding the comparison between different N biomarkers is more scarce. There is, however, some support that HV is associated with cognitive decline and progression more

strongly than t-tau.<sup>13-15</sup> Although in our study, we found t-tau as individual biomarker also predicted clinical progression and cognitive decline, the high correlation with p-tau hampers the addition of t-tau to a model with abeta and p-tau, making it a less desirable biomarker to use in the AT(N) classification. NfL and GFAP have both been shown to be related to baseline cognition, cognitive decline and clinical progression as individual predictors, but have not yet been studied extensively in comparison to other N biomarkers.<sup>3, 48-50</sup> In a former study, we found GFAP was more strongly related to clinical progression and cognitive decline than NfL, which is in line with our current study.<sup>4</sup> We found both GFAP and NfL predicted clinical progression beyond abeta and p-tau, but NfL was not associated with MMSE decline. A potential explanation for this difference in association is that NfL is a better marker for monitoring disease progression while its value does not lie in predicting future cognitive decline.<sup>4</sup> Differences could also be related to the fact that clinical progression to MCI or dementia is a binary outcome measure, while MMSE decline is a continuous measure with possibly a higher degree of measurement variation. Clinical progression might be a more sensitive measure with more clinical relevance. In contrast to NfL, GFAP was associated with MMSE decline, although associations were attenuated when additionally adjusting for abeta and/or p-tau. Of all N biomarkers we used, GFAP was associated most strongly with abeta, which could explain the attenuated estimates when abeta was added as covariate. MTA was not associated with clinical progression after correcting for covariates, nor with MMSE decline. Although we previously showed a dose response pattern with MTA as N<sup>20</sup>, the small variability in MTA within cognitively normal individuals makes it too crude a measure to accurately predict decline. Overall, we show there is room for improved prediction beyond abeta and p-tau, using HV, NfL and GFAP as N biomarkers.

Limitations of the present study include that the list of N biomarkers examined is not exhaustive. For example, FDG-PET or other MRI atrophy measures have also been suggested as suitable N markers. Although the list of putative N biomarkers is long, we chose to use a variety of N biomarkers obtained by three different modalities that are widely used in literature, which makes our study

relevant to the field. Another limitation is that the sample sizes somewhat differed for each N biomarker. This might have led to differences in outcome. However, when we repeated the analyses in the sample with complete data, results were similar, indicating their robustness (eTables 1 and 2). Furthermore, our sample consisted of individuals with SCD presenting at a memory clinic, and the results might not be directly translatable to a community based setting or to other disease stages. Nonetheless, individuals with SCD can be considered an especially clinically relevant group, that might particularly benefit from the AT(N) classification system to grade their degree of underlying pathology. These are the individuals who present to a memory clinic because of worries about their cognition, and for this group AT(N) prediction modelling can make a relevant contribution. Another limitation is the lack of optimal cut-off values for HV, NfL and GFAP. Instead, we pragmatically used cut-off values obtaining a 10% and 25% N positivity rate, to provide a range of the true effect sizes. Additionally, we used continuous N biomarkers in all models. However, different cut-off values would probably have resulted in slightly different results. Last, we had a mean follow-up duration of 3.8 years and our sample had a relatively young age. Together, this could explain the low percentage of individuals with clinical progression to MCI or dementia, which limits the power to detect associations with N biomarkers. Furthermore, MMSE has a ceiling effect in cognitively normal individuals and perhaps our relatively short follow-up time hampered the finding of associations. Since all N biomarkers reflect different aspects of neurodegeneration, they could also have different associations with cognitive tests measuring specific cognitive domains. It would be interesting to investigate associations with other neuropsychological tests, but that is beyond the scope of this study since our aim was to assess the association between N biomarkers and disease progression in general. Strengths include the relatively large sample size of this well-defined cohort.

Concluding, correlations between different N biomarkers were low in a sample of cognitively normal individuals, indicating they may not reflect the same underlying pathology. T-tau was strongly associated with p-tau, and thereby disqualified as measure for N in this context. Our results show HV,

NfL and GFAP predicted clinical progression, and have added value beyond abeta and p-tau.

However, our results do not allow to choose one most suitable biomarker for N.

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**Table 1. Demographics**

	N available	Total	Stable N=337 (84%)	Progression N=64 (16%)
Age, mean±SD <sup>1</sup>	401	60.9±8.5 <sup>a,b</sup>	60±8.4	66±7.3
Sex, n female (%) <sup>2</sup>	401	167 (42%)	141 (42%)	26 (41%)
Education, median[IQR] <sup>3</sup>	398	6[5-6]	6[5-6]	6[4-6]
MMSE, mean±SD <sup>3</sup>	399	28.3±1.6 <sup>a,b</sup>	28.4±1.5	27.8±1.6
APOE carriership, n (%) <sup>2</sup>	388	153 (39%) <sup>a,b</sup>	115 (35%)	38 (61%)
Hypertension, n (%) <sup>2</sup>	401	78 (19%)	67 (20%)	11 (17%)
Hypercholesterolemia, n (%) <sup>2</sup>	401	34 (8.5%)	30 (8.9%)	4 (6.2%)
Diabetes Mellitus, n (%) <sup>4</sup>	401	31 (7.7%)	30 (8.9%)	1 (1.6%)
BMI > 30, n (%) <sup>2</sup>	317	42 (13%)	37 (14%)	5 (10%)
CSF abeta, mean±SD <sup>1</sup>	401	1031.5±259.5 <sup>a,b</sup>	1072.2±238	817.0±264.4
CSF p-tau, mean±SD <sup>3</sup>	401	49.9±24.2 <sup>a,b</sup>	46.5±20.2	67.8±33.8
CSF t-tau, mean±SD <sup>3</sup>	401	313.7±223.2 <sup>a,b</sup>	278.1±165.1	501±358.4
MTA score, median[IQR] <sup>3</sup>	364	0[0-0.5] <sup>a</sup>	0[0-0.5]	0[0-1]
N available (%)			305 (90.5%)	59 (92.2%)
HV, mean±SD <sup>1</sup>	361	4.7±0.6 <sup>a,b</sup>	4.8±0.6	4.5±0.5
N available (%)			303 (89.9%)	58 (90.6%)
Serum NfL, mean±SD <sup>3</sup>	296	10.9±5.8 <sup>a,b</sup>	10.2±5.6	14.3±5.7
N available (%)			245 (72.7%)	51 (79.7%)
Serum GFAP, mean±SD <sup>3</sup>	296	206.4±129.9 <sup>a,b</sup>	190.8±124.9	281.1±128.6
N available (%)			245 (72.7%)	51 (79.7%)
Total follow-up time, mean±SD <sup>3</sup>	401	3.8±2.8 <sup>a,b</sup>	3.6±2.7	4.5±3.2
Time to diagnosis, mean±SD	64			3.0±2.9
Number of visits,	399	3[2-4] <sup>a,b</sup>	2[2-3]	4[3-6]



median[IQR]<sup>3</sup>

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Individuals are classified in the 'Progression' group if they showed clinical progression to mild cognitive impairment or dementia during follow-up. MRI was available for n=366, there were some missing values for MTA score (n=364) and HV (n=361) due to registration and segmentation errors. MMSE = mini-mental state examination, MTA = medial temporal atrophy, HV = hippocampal volume, NfL = neurofilament light, GFAP = glial fibrillary acidic protein. <sup>1</sup> t-test, <sup>2</sup> chi-square test, <sup>3</sup> Mann-Whitney U test, <sup>4</sup> Fisher's exact test, <sup>a</sup> p <0.05, <sup>b</sup> FDR corrected p <0.05

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Table 2. Risk of MCI or dementia for continuous N biomarkers

		Model 1	Model 2	Model 3	Model 4
Biomarker	n				
T-tau	401	2.32 (1.86 - 2.88) <sup>a,b</sup>	2.12 (1.67 - 2.70) <sup>a,b</sup>	1.74 (1.36 - 2.23) <sup>a,b</sup>	
Abeta				1.98 (1.50 - 2.63) <sup>a,b</sup>	
P-tau					
MTA	364	1.34 (1.06 - 1.69) <sup>a,b</sup>	1.02 (0.78 - 1.34)	0.97 (0.74 - 1.28)	1.00 (0.76 - 1.33)
Abeta				2.43 (1.79 - 3.31) <sup>a,b</sup>	2.18 (1.60 - 2.96) <sup>a,b</sup>
P-tau					1.42 (1.07 - 1.89) <sup>a,b</sup>
HV	361	1.55 (1.17 - 2.07) <sup>a,b</sup>	1.36 (0.99 - 1.87)	1.43 (1.06 - 1.95) <sup>a,b</sup>	1.52 (1.11 - 2.09) <sup>a,b</sup>
Abeta				2.58 (1.88 - 3.54) <sup>a,b</sup>	2.25 (1.65 - 3.07) <sup>a,b</sup>
P-tau					1.49 (1.14 - 1.94) <sup>a,b</sup>
NfL	296	1.92 (1.51 - 2.46) <sup>a,b</sup>	1.61 (1.18 - 2.21) <sup>a,b</sup>	1.42 (1.00 - 2.01)	1.51 (1.05 - 2.17) <sup>a,b</sup>
Abeta				2.24 (1.59 - 3.15) <sup>a,b</sup>	1.96 (1.41 - 2.72) <sup>a,b</sup>
P-tau					1.52 (1.14 - 2.03) <sup>a,b</sup>
GFAP	296	2.40 (1.81 - 3.19) <sup>a,b</sup>	2.03 (1.46 - 2.82) <sup>a,b</sup>	1.58 (1.09 - 2.30) <sup>a,b</sup>	1.50 (1.04 - 2.15) <sup>a,b</sup>
Abeta				2.09 (1.46 - 3.00) <sup>a,b</sup>	1.90 (1.34 - 2.68) <sup>a,b</sup>
P-tau					1.44 (1.07 - 1.94) <sup>a,b</sup>

Data shown are hazard ratio (95% confidence interval) as estimated by Cox proportional hazards analyses

(outcome: clinical progression to mild cognitive impairment or dementia). Predictors: model 1:

neurodegeneration biomarker; model 2: neurodegeneration biomarker, age and sex; model 3: abeta,

neurodegeneration biomarker, age and sex; model 4: abeta, p-tau, neurodegeneration biomarker, age and sex.

In models with MTA and HV, scanner type was additionally added as covariate. P-tau, t-tau, NfL and GFAP were

log transformed, abeta and hippocampal volume were inverted, all biomarkers were z-transformed. MTA =

medial temporal atrophy, HV = hippocampal volume, NfL = neurofilament light, GFAP = glial fibrillary acidic

protein. T-tau was not entered in model 4 due to collinearity between t-tau and p-tau. <sup>a</sup> p < 0.05. <sup>b</sup> FDR

corrected p < 0.05.

	Model 1	Model 2	Model 3	Model 4
Biomarker	Beta (SE)	Beta (SE)	Beta (SE)	Beta (SE)
T-tau	-0.17 (0.04) <sup>a,b</sup>	-0.15 (0.04) <sup>a,b</sup>	-0.14 (0.04) <sup>a,b</sup>	
Abeta			-0.11 (0.04) <sup>a,b</sup>	
P-tau				
MTA	-0.06 (0.04)	-0.04 (0.05)	-0.04 (0.05)	-0.04 (0.04)
Abeta			-0.14 (0.04) <sup>a,b</sup>	-0.12 (0.04) <sup>a,b</sup>
P-tau				-0.12 (0.04) <sup>a,b</sup>
HV	-0.11 (0.04) <sup>a,b</sup>	-0.13 (0.05) <sup>a,b</sup>	-0.13 (0.04) <sup>a,b</sup>	-0.13 (0.04) <sup>a,b</sup>
Abeta			-0.13 (0.04) <sup>a,b</sup>	-0.12 (0.04) <sup>a,b</sup>
P-tau				-0.11 (0.04) <sup>a,b</sup>
NfL	-0.06 (0.05)	-0.05 (0.06)	-0.01 (0.06)	-0.02 (0.06)
Abeta			-0.11 (0.05) <sup>a</sup>	-0.09 (0.05)
P-tau				-0.16 (0.05) <sup>a,b</sup>
GFAP	-0.15 (0.05) <sup>a,b</sup>	-0.14 (0.06) <sup>a,b</sup>	-0.11 (0.06)	-0.10 (0.06)
Abeta			-0.08 (0.05)	-0.06 (0.05)
P-tau				-0.16 (0.05) <sup>a,b</sup>

**Table 3. Risk of cognitive decline for continuous N biomarkers**

Results shown are beta (SE) as estimated by linear mixed models. Outcome is MMSE score. Predictors: model 5: neurodegeneration, time, neurodegeneration\*time; model 6: variables included in model 5, age, sex, age\*time and sex\*time; model 7: variables included in model 6, CSF abeta and abeta\*time; model 8: variables included in model 7, CSF p-tau and p-tau\*time). In models with MTA and HV, scanner type was additionally added as covariate. Betas represent the interaction between neurodegeneration biomarker and time, which corresponds to the cognitive slope. P-tau, t-tau, NfL and GFAP were log transformed, abeta and hippocampal volume were inverted, all biomarkers were z-transformed. MTA = medial temporal atrophy, HV = hippocampal volume, NfL = neurofilament light, GFAP = glial fibrillary acidic protein. T-tau was not entered in model 4 due to collinearity between t-tau and p-tau. <sup>a</sup> p <0.05. <sup>b</sup> FDR corrected p <0.05

**Table 4. Risk of MCI or dementia for dichotomous N biomarkers**

		Model 1	Model 2	Model 3	Model 4	
Biomarker	n					
T-tau	401	4.95 (2.99 - 8.22) <sup>a,b</sup>	3.68 (2.16 - 6.25) <sup>a,b</sup>	2.47 (1.40 - 4.36) <sup>a,b</sup>	#	
MTA	364	1.74 (0.98 - 3.08)	0.90 (0.47 - 1.71)	0.84 (0.42 - 1.66)	0.85 (0.43 - 1.68)	
HV	25	361	2.60 (1.49 - 4.54) <sup>a,b</sup>	2.03 (1.13 - 3.67) <sup>a,b</sup>	2.22 (1.22 - 4.04) <sup>a,b</sup>	2.27 (1.24 - 4.16) <sup>a,b</sup>
	10	361	1.94 (0.91 - 4.12)	1.31 (0.57 - 3.01)	1.89 (0.84 - 4.26)	1.96 (0.87 - 4.39)
NfL	75	296	3.50 (2.00 - 6.11) <sup>a,b</sup>	1.98 (1.04 - 3.78) <sup>a</sup>	1.40 (0.73 - 2.68)	1.42 (0.74 - 2.71)
	90	296	2.54 (1.30 - 4.96) <sup>a,b</sup>	1.48 (0.72 - 3.04)	1.04 (0.51 - 2.11)	1.07 (0.53 - 2.17)
GFAP	75	296	4.01 (2.26 - 7.10) <sup>a,b</sup>	2.32 (1.20 - 4.49) <sup>a,b</sup>	1.10 (0.53 - 2.29)	1.03 (0.49 - 2.16)
	90	296	4.69 (2.52 - 8.74) <sup>a,b</sup>	2.89 (1.48 - 5.66) <sup>a,b</sup>	1.68 (0.86 - 3.27)	1.68 (0.87 - 3.25)

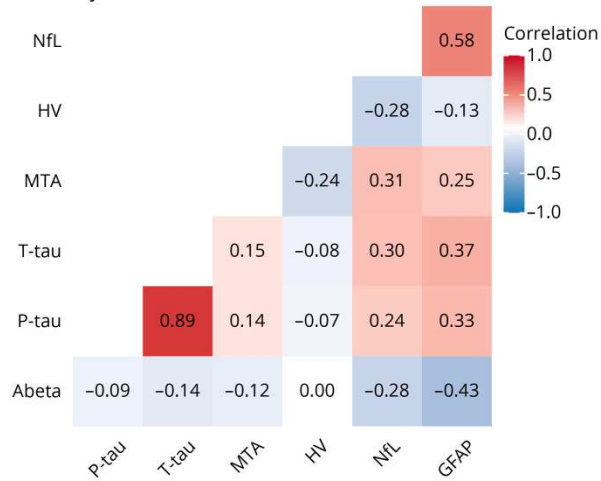
Data shown are hazard ratio (95% confidence interval) as estimated by Cox proportional hazards analyses (outcome: clinical progression to mild cognitive impairment or dementia). Predictors: model 1: dichotomized N biomarker; model 2: dichotomized N, age and sex; model 3: dichotomized A, N, age and sex; model 4: dichotomized A, T, N, age and sex. In models with MTA and HV, scanner type was additionally added as covariate. MTA = medial temporal atrophy, HV 25 = hippocampal volume, threshold 25<sup>th</sup> percentile, HV 10 = hippocampal volume, threshold 10<sup>th</sup> percentile, NfL 75 = neurofilament light, threshold 75<sup>th</sup> percentile, NfL 90 = neurofilament light, threshold 90<sup>th</sup> percentile, GFAP 75 = glial fibrillary acidic protein, threshold 75<sup>th</sup> percentile, GFAP 90 = glial fibrillary acidic protein, threshold 90<sup>th</sup> percentile. T-tau was not entered in model 4 due to collinearity between t-tau and p-tau. <sup>a</sup> p <0.05. <sup>b</sup> FDR corrected p <0.05.

### Figure 1. Correlations between N biomarkers

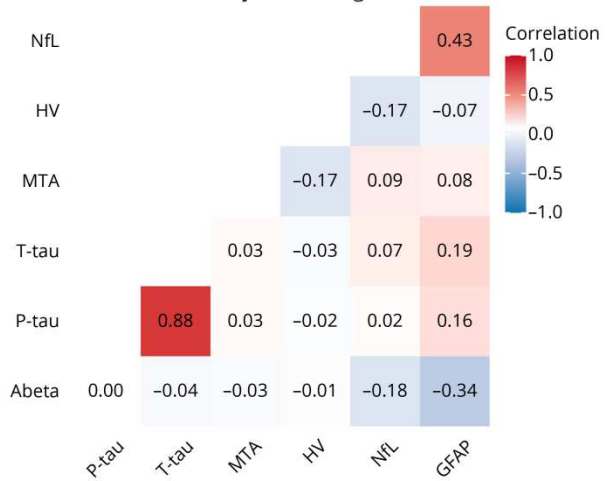
Heatmaps showing correlations between different biomarkers. A. Correlation coefficients (Pearson), B.

Correlation coefficients (partial correlation, adjusted for age and sex). P-tau, t-tau, NfL and GFAP were log-transformed. MTA = medial temporal atrophy, HV = hippocampal volume, NfL = neurofilament light, GFAP = glial fibrillary acidic protein.

A. Unadjusted correlation



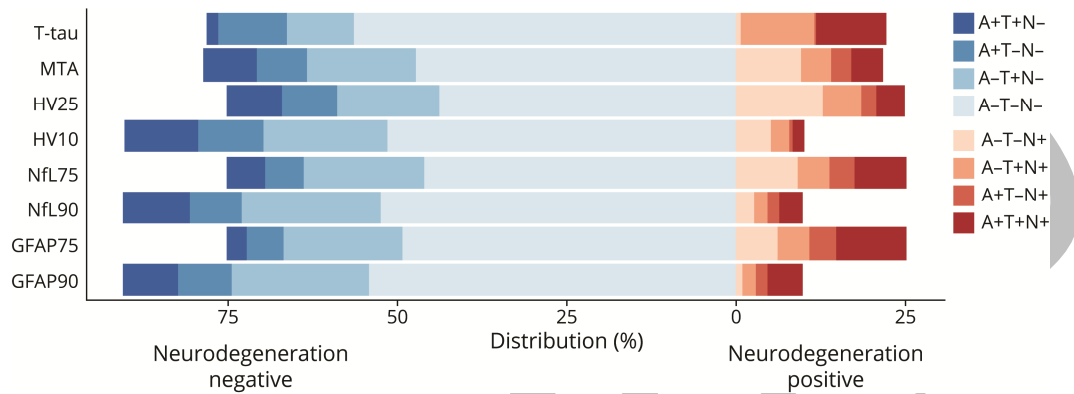
B. Partial correlation adjusted for age and sex



ACCEPT

**Figure 2. Distribution of AT(N) profiles according to different definitions of neurodegeneration**

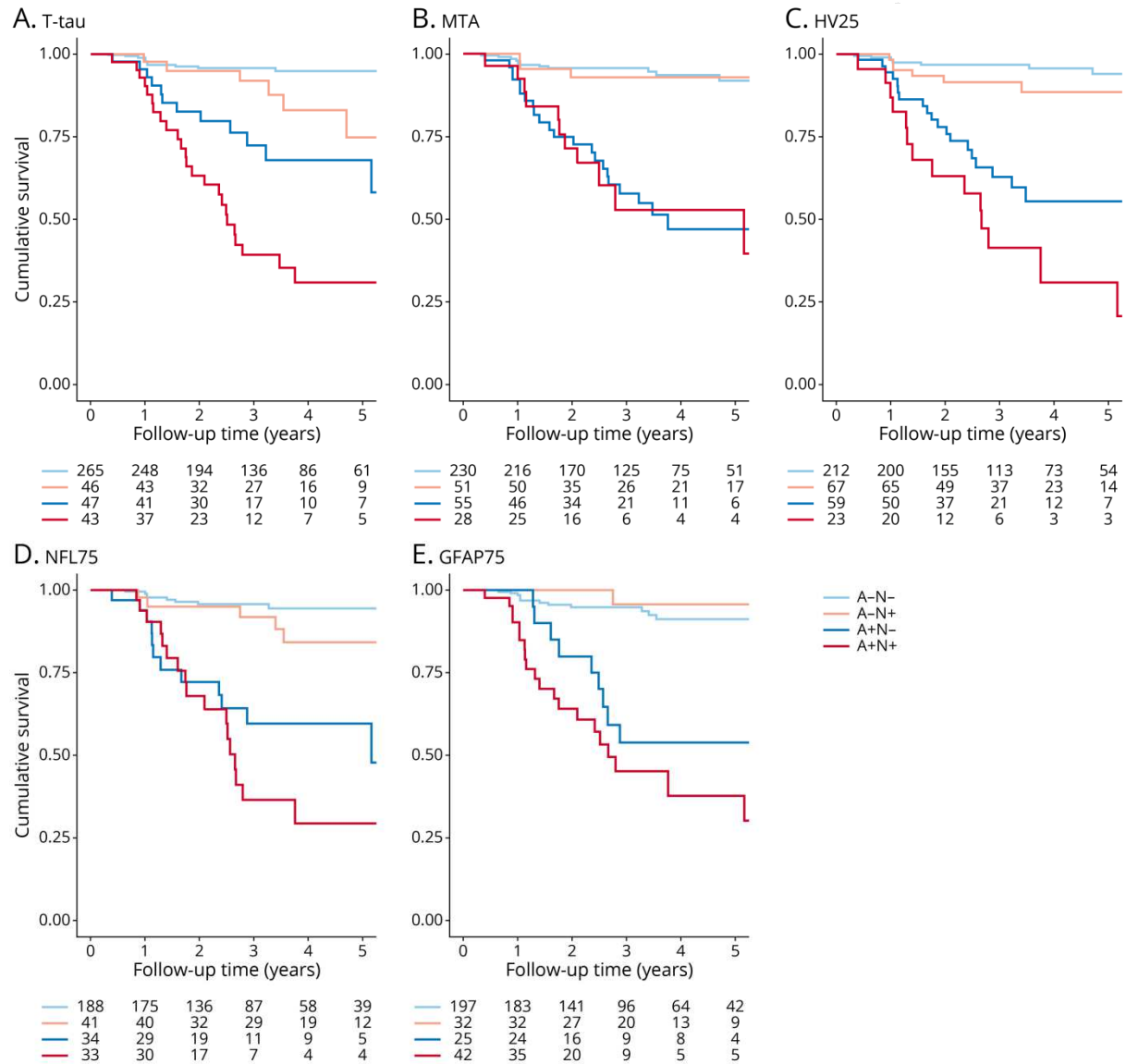
Distribution of AT(N) profiles for different definitions of neurodegeneration. MTA = medial temporal atrophy, HV 25 = hippocampal volume, threshold 25<sup>th</sup> percentile, HV 10 = hippocampal volume, threshold 10<sup>th</sup> percentile, NfL 75 = neurofilament light, threshold 75<sup>th</sup> percentile, NfL 90 = neurofilament light, threshold 90<sup>th</sup> percentile, GFAP 75 = glial fibrillary acidic protein, threshold 75<sup>th</sup> percentile, GFAP 90 = glial fibrillary acidic protein, threshold 90<sup>th</sup> percentile.



ACCEPTED

**Figure 3. Kaplan Meier curves visualizing clinical progression within AN classification**

Kaplan Meier curves visualizing clinical progression to mild cognitive impairment or dementia for different definitions of neurodegeneration (A. T-tau, B. MTA, C. HV 25, D. NFL 75, E. GFAP 75). Survival is visualized by constructing a four-level variable of dichotomous amyloid and neurodegeneration status (A-N-, A-N+, A+N-, A+N+). MTA = medial temporal atrophy, HV 25 = hippocampal volume, threshold 25<sup>th</sup> percentile, NfL 75 = neurofilament light, threshold 75<sup>th</sup> percentile, GFAP 75 = glial fibrillary acidic protein, threshold 75<sup>th</sup> percentile.







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## Association of CSF, Plasma, and Imaging Markers of Neurodegeneration With Clinical Progression in People With Subjective Cognitive Decline

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