Comparison of variables associated with cerebrospinal fluid neurofilament, total-tau, and neurogranin.

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Manuscript word count:

Abstract

INTRODUCTION: Three cerebrospinal fluid (CSF) markers of neurodegeneration (N) (neurofilament light [NfL], total-tau [T-tau], and neurogranin [Ng]) have been proposed under the AT(N) scheme of the National Institute on Aging-Alzheimer's Association Research Framework.

METHODS: We examined, in a community-based population (N = 777, aged 50-95) (1) what variables were associated with each of the CSF (N) markers, and (2) whether the variables associated with each marker differed by increased brain amyloid. CSF T-tau was measured with an automated electrochemiluminescence Elecsys immunoassay; NfL and Ng were measured with in-house enzyme-linked immunosorbent assays.

RESULTS: Multiple variables were differentially associated with CSF NfL and T-tau levels, but not Ng. Most associations were attenuated after adjustment for age and sex. T-tau had the strongest association with cognition in the presence of amyloidosis, followed by Ng. Variables associations with NfL did not differ by amyloid status.

DISCUSSION: Understanding factors that influence CSF (N) markers will assist in the interpretation and utility of these markers in clinical practice.

Introduction

Alzheimer`s disease (AD) dementia is a progressive neurodegenerative disease characterized by the accumulation of amyloid beta (Aβ) and tau pathologies, neurodegeneration and cognitive decline (REF). Cerebrospinal fluid (CSF) Aβ42, total tau (T-tau) and phosphorylated tau (P-tau) are established diagnostic and/or prognostic biomarkers for AD (REF). In recent years, two new promising biomarkers for neurodegeneration, neurofilament light protein (NfL) and synaptic dysfunction and degeneration, neurogranin (Ng) have emerged, for review see (REF). The primary function of NfL is to determine the axonal caliber, which is crucial for morphological integrity and conduction velocity [1,2]. Therefore, NfL a putative marker of subcortical large-caliber axonal degeneration, has recently been highlighted for its potential as a biomarker of AD progression[3]. In contrast, Ng is a post-synaptic protein that is especially enriched in dendritic spines, and therefore could be a potential biomarker for detecting synaptic dysfunction and/or loss in AD (REF also here?).

Recent studies have reported that higher CSF NfL[4,5] and/or CSF Ng[4-6] are associated with greater risk of progression from MCI to AD or cognitive decline. Notably, unlike CSF NfL, which has been elevated in multiple neurodegenerative diseases, elevations of CSF Ng may be specific to AD[7], a finding recently verified in a large study also including neuropathologically verified cases (ref).

These studies primarily utilized memory clinic patients (e.g., the Amsterdam Dementia cohort study) or individuals screened to exclude cerebrovascular disease or other forms of dementia (e.g., Alzheimer`s Disease Neuroimaging Initiative (ADNI). Thus, the prognostic performance of CSF NfL and Ng in a community-based population is not well understood. In the present study, we sought to determine whether CSF NfL and CSF Ng were risk factors for

MCI, and to compare their strengths of association with MCI risk to CSF T-tau and P-tau in a population-based cohort of cognitively unimpaired (CU) participants. We also examined whether a combination of elevated NfL with elevated T-tau was more strongly associated with risk of MCI than either alone, and whether CSF Aβ42 modified the association between CSF NfL or Ng and risk of MCI.

Materials and Methods

Study participants

The MCSA is a prospective population-based study aimed at characterizing the incidence and prevalence of MCI in Olmsted County, Minnesota[8]. In 2004, Olmsted County residents between the ages of 70 and 89 were identified for recruitment using the Rochester Epidemiology Project medical records linkage system[9]. An age- and sex-stratified random sampling design was utilized to ensure that men and women were equally represented in each 10-year age strata. The study was extended to include those aged 50 and older in 2012.The present analyses included 648 CU participants aged 50 to 95 years with available CSF NfL, Ng, Aβ42, T-tau, or P-tau measures, cognitive testing, and at least one follow-up visit with cognitive testing.

Lumbar punctures were performed in the lateral decubitus position. Etc. Petersen or Mielke should add a few sentences here.

Protocol approvals Standard, registrations and patient consents

The Study was approved by the Mayo Clinic and Olmsted Medical Center institutional review boards. Written informed consent was obtained from all participants.

Participant Assessment

The MCSA visits occurred every 15 months and included physician examination, an interview by a study coordinator, and neuropsychological testing administered by a psychometrist[8]. The neuropsychological battery included 9 tests covering 4 domains: (1) memory (Auditory Verbal Learning Test Delayed Recall Trial; Wechsler Memory Scale-Revised Logical Memory II and Visual Reproduction II), (2) language (Boston Naming Test and Category Fluency), (3) executive function (Trail Making Test B and Wechsler Adult Intelligence Scale-Revised [WAIS-R] DigitSymbol subtest) and (4) visuospatial (WAIS-R Picture Completion and Block Design subtests). We calculated sample-specific z-scores for all cognitive tests, and created domain scores by averaging the z-scores within each domain. We created a global cognitive score using the z-transformation of the average of the four domains.

MCI and Dementia diagnostic Determination

For each participant, the clinical diagnosis was determined by a consensus committee including the neurologist, neuropsychologist, and the nurse who evaluated each participant. Performance in a cognitive domain was compared with the age-adjusted scores of CU individuals previously obtained using Mayo's Older American Normative Studies[10]. This approach relies on prior normative work and extensive experience with the measurement of cognitive abilities in an independent sample of subjects from the same population. Subjects with scores around 1.0 SD below the age-specific mean in the general population were considered for possible cognitive impairment. The operational definition of MCI was based on clinical judgment including a history from the patient and informant. Published criteria were used for the diagnosis: cognitive complaint, cognitive function not normal for age, essentially normal functional activities, no dementia[11]. A final decision about impairment in a cognitive domain was made after considering education, occupation, visual or hearing deficits, and reviewing all other participant information. The diagnosis of dementia[12] was based on published criteria. Participants who performed in the normal range and did not meet

criteria for MCI or dementia were deemed CU. Neither CSF findings nor imaging were considered in determining the clinical diagnoses of MCI or dementia.

CSF measurements

CSF Aβ42, T-tau, and P-tau were measured with automated electrochemiluminescence Elecsys immunoassays (Roche Diagnostics) at the Mayo Clinic Rochester MN USA. CSF NfL and Ng were measured in the Clinical Neurochemistry Laboratory at the University of Gothenburg, Mölndal, Sweden. For NfL, an in-house sandwich enzyme-linked immunosorbent assay (ELISA) with capture and detection antibodies directed against the central rod domain of the protein (NfL 21 and NfL 23, respectively) was used (REF). Nine of the 648 participants had CSF NfL levels >20,000 ng/L, which was more than three standard deviations from the mean (i.e., > 4723.5 ng/L). These 9 values were recoded as missing. For CSF Ng, an in-house ELISA method was used [13]. The Ng level of one participant was below the detection limit so this value was also recoded as missing. [could you give this sample the same value as the detection limit, and then use it ?]

Assessment of Covariates

Participant demographics (eg. Age, sex, and years of education) were ascertained at the in-clinic examination. Apolipoprotein E (*APOE*) ε4 genotyping was performed from blood drawn at the in-clinic examination. Medical conditions and the Charlson comorbidity index[14] were determined for each participant by medical record abstraction using the medical records-linkage system of the Rochester Epidemiology Project[9,15].

Statistical analysis

We tested associations between the CSF NfL or Ng and CSFAβ 42, T-tau and P-tau using Spearman Rank correlation. All CSF variables were z-log transformed in an attempt to normalize the distributions and to utilize the same units for each variable in order to adequately compare the effect size across markers. We used Cox proportional hazard regression models to determine whether the baseline CSF NfL, as a continuous variable and in quartiles, was associated with risk of MCI and dementia among CU participants. Age was used as the timescale. Participants were followed until a diagnosis of MCI/dementia, death, or last follow-up visit. The event date for those who developed MCI or dementia was defined as the midpoint between a participant's last visit defined as CU and first visit defined as MCI or dementia. Four participants went from a diagnosis of CU at one visit to a diagnosis of dementia at a subsequent visit. Analyses were repeated including and excluding these participants and the results remained the same. Thus, they were included in the presented models.

Multivariable models with age as the time scale adjusted for sex, educational level, and Charlson comorbidity index were examined. Analyses were also repeated after additional adjustment for *APOE* ε4 status. To determine whether a combination of elevated NFL with elevated T-tau was more strongly associated with risk of MCI compared to either alone, we developed a categorical variable with NfL only in the highest quartile, T-tau only in the highest quartile, both NfL and T-tau in the highest quartile, and neither in the highest quartile. We then used Cox proportional hazard models to assess the relationship between this variable and the risk of MCI after adjusting for the variables described above. We also examined whether there was an interaction between brain Aβ and CSF NFL or Ng in relation to risk of MCI. For each CSF measure, the proportional hazard assumption was tested using the Schoenfeld Residuals test. The test was non-significant, indicating that the assumption was valid. Kaplan-Meier survival curves were constructed for each outcome. Statistical analyses

were completed using SAS, version 9.4 (SAS Institute Inc). Kaplan-Meier survival curves were constructed using the survival package in R, version 3.4.1 (R Foundation). A 2-tailed, $p<0.05$ was considered significant.

Results

The baseline characteristics of the 648 CU participants are shown in Table1. The median age of the participants was 72.3 (range: 50.7-95.3) years and 366 (56.5%) were male. The median education was 14.0 (range: 8-20) years; 172 (26.6%) had an *APOE* ε4 allele. CSF NfL and Ng were correlated (Spearman $\rho=0.303$, $p<0.0001$). Notably, compared to CSF NfL, CSF Ng levels were more highly correlated with CSF T-tau (NfL Spearman's $\rho= 0.465$, $p< 0.0001$; Ng Spearman`s $\rho= 0.820$, $p< 0.0001$), P-tau (NfL Spearman $\rho=0.458$, $p<0.0001$; Ng Spearman`s $\rho= 0.805$, $p< 0.0001$) and Aβ42 (CSF NfL Spearman $\rho=0.121$, $p=0.0021$; Ng Spearman $\rho=0.392$, $p<0.0001$).

Continuous CSF measures and Risk of MCI

Participants were followed for a median of 3.8 years (range: 0.5-10.1). Of the 648 CU individuals, 96 (14.8%) progressed to MCI over the follow-up period. Each z-log-unit increase in CSF NfL, was associated with a 1.32 fold increased risk of MCI (HR 1.32, 95%CI: 1.08-1.60) after adjusting for sex, education, and the Charlson comorbidity index (**Table 2**). This association was almost identical after additional adjustment for *APOE* (HR 1.33, 95% CI: 1.09 -1.62). Neither CSF Ng,T-tau, nor P-Tau was associated with risk of MCI. However, each z-log-unit increase in CSF Aβ42 was associated with a reduced risk of MCI in multivariable models excluding (HR 0.74, 95% CI: 0.61-0.89) and including (HR 0.80, 95% CI: 0.66-0.97) adjustment for *APOE*.

CSF measures, in quartiles, and Risk of MCI

We next examined quartiles of each CSF measure to determine whether there was a dose-response effect. Compared with the lowest quartile, the highest quartile of CSF NfL was associated with a 2.9-fold increased risk of MCI (HR 2.90, 95% CI: 1.26-6.67; **Table 3**). The associated risk was even larger for the highest CSF NfL quartile after adjustment for *APOE (*HR 3.13, 95% CI: 1.36, 7.18). The second and third quartiles of CSF NfL were not significantly associated with increased risk. Kaplan-Meier plots of the association between CSF NfL quartiles and risk of MCI are shown in **Figure 1**. There were no associations between CSF T-tau, P-tau, or Ng in quartiles and risk of MCI (**Table 3**). Higher CSF Aβ42 was associated with a reduced risk of MCI (Quartile 3: HR 0.41; 95% CI 0.23-0.75), (Quartile 4: HR 0.47; 95% CI 0.27-0.81). However, after adjustment for APOE, this association was attenuated and only significant for Quartile 3 (HR 0.48, 95% CI: 0.26-0.88).

Risk of MCI using a combination of elevated CSF NfL and T-tau in quartiles

To determine whether the combination of CSF NfL and T-tau was associated with a greater risk of MCI compared to either measure alone, we created a categorical variable for both CSF NfL and T-tau in the highest quartile (N=68), only CSF NfL in the highest quartile (N=90), only CSF T-tau in the highest quartile (N=89), and neither CSF NfL nor T-tau in the highest quartile (N=391). Participants with only CSF NfL in the highest quartile (HR 2.24; 95% CI 1.31- 3.83) and with both CSF NfL and T-tau in the highest quartile had a similarly increased risk of MCI (HR 2.29; 95% CI 1.28- 4.09) compared to participants without both markers in the highest quartile. Participants who only had T-tau in the highest quartile did not have an increased risk of MCI (**Table 4**). These associations did not change after adjusting for *APOE*.

Assessing Effect Modification by elevated brain amyloid

Lastly, we examined the interaction between CSF Aβ42 with CSF NfL, Ng, T-tau and P-tau in relation to risk of MCI. CSF Aβ was not found to be an effect modifier in any analyses, including when examining the CSF markers as continuous variables or in quartiles.

Discussion

In the present study, we examined and compared CSF established (T-Tau and P-tau) and newer (NfL and Ng) measures of neurodegeneration for the risk of MCI among a community-based population of CU participants. Elevated levels of CSF NfL were strongly associated with risk of MCI. However, there were no associations between CSF levels of Ng, T-tau, and P-tau and risk of MCI. [could we speculate here whether the risk of cognitive decline in cognitively unimpaired individuals from a population sample is mostly associated with biomarkers reflecting non-AD type of pathophysiology (NFL), but less so with AD-type pathophysiology (P-tau and Ng) ?] Elevated levels of CSF Aβ42 were associated with a reduced risk of MCI. Examining the combination of both CSF NfL and T-tau as predictors of MCI was not better than CSF NfL alone. In all analyses, results were almost identical after adjustment for APOE. CSF Aβ42 was not an effect modifier, thus the associations between CSF NfL and risk for MCI were similar for those with versus without elevated brain amyloid. These results suggest that in a community-based sample of CU participants, CSF NfL is more strongly associated with risk of MCI compared to other CSF neurodegeneration markers, and that this association is independent of elevated brain amyloid.

Two previous studies utilizing ADNI data[3,4] reported that elevated CSF NfL was associated with faster cognitive decline among MCI patients[3,4]. Another study based on the Swedish Dementia Registry also showed that higher CSf NfL was related to shorter survival in AD[16], again suggesting that CSF NfL is a markers of clinical progression in symptomatic patients. Our results extend this finding earlier in the clinical disease spectrum by showing that high

CSF NfL is also strongly related to risk for MCI in a CU community based study. In line with our findings of a strong association between higher CSF NfL and risk for MCI are findings from ADNI who showed that NfL is associated with cortical and subcortical brain atrophy[5,3], which should translate into cognitive progression.

The association between elevated CSF NfL and risk of MCI was independent of CSF Aβ. Thus, CSF NfL is a risk factor for cognitive impairment for those on the AD pathway (with elevated brain amyloid) as well as for those who are not. This finding is also in line with previous studies that have also shown that CSF NfL reflects neurodegeneration in AD independent of Aβ[3,5,16], and studies that show CSF NfL is elevated in many different neurological diseases[16]. Thus, CSF NfL is a non-specific marker of neurodegeneration. Interestingly, all our analyses remained unchanged when adjusting the continuous and quartile models for APOE, which has been seen before[3,4].

When we compared elevated CSF NfL to T-tau and P-tau, NfL was more strongly associated with risk of MCI. In fact, there was no association between T-tau or P-tau and risk of MCI. Further, when we evaluated whether the combination of elevated T-tau and NfL was a greater risk factor than NfL alone, the combination was not any better in predicting risk of MCI. These findings suggest that NfL is a stronger predictor of cognitive deterioration than T-tau in a population-based sample of CU. In addition, our results support the hypothesis that CSF NfL and CSF T-tau may convey at least partly different information in neurodegeneration. CSF NfL is a measure of axonal injury whereas CSF T-tau probably reflects the intensity of neuronal injury at a given time point[17-19]. These differences may explain our discordant findings between NfL and T-tau.

We did not find any associations between CSF Ng and risk for MCI. This parallels findings from the memory clinic based Amsterdam Dementia Cohort, where Ng did not predict

progression from CU to MCI, but did predict progression from MCI to AD dementia [6]. Another recent study conducted in ADNI showed that elevated Ng was associated with cognitive decline in participants with MCI but not among CU[20]. These findings together with our finding that CSF Ng was not associated with risk for MCI may reflect that CSF Ng may be a later marker for cognitive decline first evident when cognitive deterioration has occurred. We found a strong correlation between CSF Ng and T-tau and P-tau, which has been described before[4]. This strong correlation may explain the lack of findings for CSF Ng and risk for MCI/dementia. We speculate that our findings of a lack of association between CSF T-tau, P-tau and CSF Ng suggest, that CSF NfL could change before the other markers, but additional data and analyses are needed to understand the temporal changes in these markers.

Strengths of the study include the community-based population and the large number of CU individuals. There are however a few limitations. First, we did not differentiate between different cognitive subtypes of MCI in our analyses as the subgroups would have become quite small. Second, all CSF markers were measured at one point in time. Thus, we could not incorporate time-varying measures and the relationship between intra-individual change in these markers and cognition could not be assessed. Third, when examining CSF Aβ 42 as an effect modifier, we considered the lowest tertile as indicating elevated brain amyloid. It is possible that subtle effects of emerging Aβ pathology in subjects with slightly higher CSF Aβ 42 levels have been overlooked. Fourth, participants who progressed to MCI may have been unhealthier than participants who remained CU. However, we controlled in all our analyses for Charlson Comorbidity index.

Taken together, our results might indicate that NfL could become a good prognostic biomarker in AD [but we discuss above that it is not a biomarker for AD-type pathology?]. NFL may emerge as a preferable biomarker to T-tau as T-tau and P-tau are so highly correlated in AD that they may not seem to provide independent information. In the ongoing

revisions of the new NIA-AA criteria 2018 (REF), NfL is discussed as a Neurodegeneration marker in the N+ group in the future, a suggestion that is strongly supported by our findings.

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Table 1. Baseline participant characteristics

Characteristics	N	median (IQR)/N(%)
Age	648	72.3 (63.4, 78.3)
Male	648	366 (56.5%)
Education	648	14.0 (12.0, 16.0)
APOE E4	647	172 (26.6%)
Hypertension	642	386 (60.1%)
BMI	642	28.2 (25.2, 31.6)
Charlson comorbidity index	642	2.0 (1.0, 4.0)
Cognitive z-scores		
Global	626	0.05 (-0.65, 0.73)
Memory	645	0.05 (-0.76, 0.69)
Visualspatial	632	0.03 (-0.60, 0.69)
Attention	635	0.09 (-0.68, 0.73)
Language	638	0.11 (-0.62, 0.73)
CSF measures		
NfL, pg/ml	639	484.7 (359.5, 700.9)
Neurogranin, pg/ml	647	164.7 (131.0, 217.5)
Total tau	647	211.3 (166.1, 269.7)
Phosphorylated tau	647	17.8 (14.1, 23.3)

Aβ42	647	1105.0 (786.2, 1537.0)

Table 2. Continuous CSF measures and risk of Mild Cognitive Impairment

	Model 1				Model 2			
z-Log CSF	N	events	HR (95% CI)	p-value	N	events	HR (95% CI)	p-value
NfL	633	94	1.32 (1.08,1.60)	0.006	632	94	1.33 (1.09, 1.62)	0.005
Neurogranin	641	95	1.03 (0.83,1.29)	0.786	640	95	1.03 (0.83, 1.27)	0.814
Total tau	641	95	1.07 (0.85,1.34)	0.580	640	95	1.06 (0.85, 1.33)	0.591
P-tau	641	95	1.12 (0.90,1.39)	0.298	640	95	1.10 (0.89, 1.37)	0.373
Aβ 42	641	95	0.74 (0.61,0.89)	0.001	640	95	0.80 (0.66, 0.97)	0.023

Model 1 with age as time scale and adjusted for sex, education, and the Charlson Comorbidity index.

Model 2 with age as time scale and adjusted for the variables in Model 1 and *APOE* genotype.

Table 3. CSF measures, in quartiles, and risk of Mild Cognitive Impairment

Quartiles of			Model 1				Model 2	
Log CSF	N	events	HR (95% CI)	p-value	N	events	HR (95% CI)	p-value
NfL	633	94			632	94		
2 vs. 1			1.52 (0.64,3.64)	0.345			1.69 (0.71, 4.03)	0.238
3 vs. 1			1.43 (0.60,3.42)	0.420			1.58 (0.66, 3.75)	0.302
4 vs. 1			2.90 (1.26,6.67)	0.012			3.13 (1.36, 7.18)	0.007
Neurogranin	641	95			640	95		
2 vs. 1			0.82 (0.45,1.47)	0.498			0.82 (0.45, 1.48)	0.505
3 vs. 1			0.79 (0.43,1.43)	0.436			0.75 (0.41, 1.37)	0.354
4 vs. 1			0.89 (0.50,1.59)	0.701			0.88 (0.49, 1.56)	0.650
Total tau	641	95			640	95		
2 vs. 1			1.08 (0.54,2.16)	0.822			1.19 (0.59, 2.38)	0.628
3 vs. 1			0.71 (0.35,1.44)	0.338			0.77 (0.38, 1.57)	0.478
4 vs. 1			1.13 (0.58,2.20)	0.714			1.14 (0.59, 2.21)	0.701
P-tau	641	95			640	95		
2 vs. 1			1.88 (0.87,4.10)	0.111			1.96 (0.90, 4.27)	0.091
3 vs. 1			1.56 (0.72,3.36)	0.258			1.62 (0.75, 3.50)	0.216
4 vs. 1			1.72 (0.81,3.68)	0.160			1.67 (0.78, 3.55)	0.187
Aβ42	641	95			640	95		
2 vs. 1			0.65 (0.37,1.13)	0.128			0.74 (0.42, 1.30)	0.289
3 vs. 1			0.41 (0.23,0.75)	0.003			0.48 (0.26, 0.88)	0.018
4 vs. 1			0.47 (0.27,0.81)	0.006			0.58 (0.33, 1.02)	0.058

Model 1 with age as time scale and adjusted for sex, education, and the Charlson Comorbidity index.

Model 2 with age as time scale and adjusted for the variables in Model 1 and *APOE* genotype.

Table 4. CSF measures, in quartiles, and risk of Mild Cognitive Impairment, using a combination of elevated CSF Neurofilament light protein and T-tau

Quartiles of Log CSF	Model 1 N	events	HR (95% CI)	p-value	Model 2 N	events	HR (95% CI)	p-value
Neither T-tau nor NfL in top quartile	632	94	REF		631	94	REF	
T-tau in top quartile			1.28 (0.68,2.41)	0.440			1.23 (0.65,2.31)	0.530
NfL in top quartile			2.24 (1.31,3.83)	0.003			2.29 (1.34,3.94)	0.003
NfL and T-tau in top quartile			2.29 (1.28,4.09)	0.005			2.12 (1.18,3.81)	0.012

Model 1 with age as time scale and adjusted for sex, education, and the Charlson Comorbidity index.

Model 2 with age as time scale and adjusted for the variables in Model 1 and *APOE* genotype.