Association of glial and neuronal degeneration markers with Alzheimer's disease cerebrospinal fluid profile and cognitive functions

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Research

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Abstract

Background

Neuroinflammation has gained increasing attention as a potential contributing factor in the onset and progression of Alzheimer's disease (AD). The objective of this study was to examine the association of selected cerebrospinal fluid (CSF) inflammatory and neuronal degeneration markers with signature CSF AD profile and cognitive functions among subjects at the symptomatic pre- and early dementia stages.

Methods

In this cross-sectional study, 52 subjects were selected from an Icelandic memory clinic cohort. Subjects were classified as having CSF AD (n=28, age=67, 33% female, Mini-mental state examination [MMSE]=28) or non-AD (n=24, age=70, 39% female, MMSE=27) profile based on the ratio between CSF total-tau (T-tau) and amyloid-β 1-42 (Aβ 42) values (cut-off point chosen as 0.52). Novel CSF biomarkers included Neurofilament light (NFL), YKL-40, S100 calcium-binding protein B (S100B) and Glial fibrillary acidic protein (GFAP), measured with enzyme-linked immunosorbent assay (ELISA). Subjects underwent a neuropsychological assessment for evaluation of different cognitive domains including verbal episodic memory, non-verbal memory, language, processing speed and executive functions.

Results

Accuracy for distinguishing between the two CSF profiles was calculated for each CSF marker and cognitive domain. Verbal episodic memory performed the best overall (Area under curve [AUC]=0.80), with AUCs for CSF markers ranging from 0.61 to 0.64. For estimation of the relationships between CSF markers and cognitive domains (adjusted for age and education), Pearson's correlation and ridge regression analyses were performed. The ratio between NFL and YKL-40 levels correlated higher with verbal episodic memory score (r=-0.51, p <0.001) compared to single protein levels (NFL: r=-0.26, p =0.06; YKL-40: r=0.18, p =0.20). The correlation was also higher among those with CSF AD profile (r=-0.67, p <0.001) compared to those without (r=-0.46, p =0.03). GFAP levels showed weak correlation with executive functions scores (r=-0.37, p =0.007). Among those with a CSF AD profile, both S100B (r=-0.45, p =0.02) and GFAP (0.68, p <0.001) levels correlated with processing speed scores.

Conclusions

The novel CSF markers NFL, YKL-40 and GFAP show potential as markers for cognitive decline among individuals with core AD pathology at the symptomatic pre- and early stages of dementia.

Introduction

In recent years, a paradigm shift in research criteria of Alzheimer´s disease (AD) has occurred as the primary focus has shifted from clinical to biological criteria. The emphasis is now on the pathology [1] which is believed to start decades before appearance of clinical symptoms [2]. The core cerebrospinal

fluid (CSF) biomarkers reflecting the hallmarks of AD pathology, extracellular amyloid plaques (Aβ) and neurodegeneration (total tau [T-tau] and phosphorylated tau [P-tau]) have been at the center of this shift and have been extensively studied [3]. Although the diagnostic accuracies of these markers are generally satisfactory [4], their levels are relatively constant in the symptomatic stages of the disease and do not correlate well with progression of cognitive decline [5–7]. This necessitates the need for exploration of novel biomarkers that help in better understanding the different aspects of AD pathology, its progression and clinical manifestation.

Increasing evidence shows that inflammation is a contributing factor in the pathogenesis and development of AD and other neurodegenerative diseases [8, 9]. A number of studies show that Aβ toxicity and plaques induce an immune response, including activation of astrocytes and microglia, the immune cells of the brain [10–12]. Furthermore, activation of these cells is also thought to play a role in the formation and progression of neurofibrillary tangles (NFTs), contributing to neuronal dysfunction and loss [13]. Glial activation markers are therefore of high interest when it comes to exploring new biomarkers for the diagnosis of dementia.

The glial proteins YKL-40 (also known as chitinase-3-like-1 protein), calcium-binding protein S100B and glial fibrillary acidic protein (GFAP) have previously been associated with AD pathology [14]. All are expressed in astrocytes within the central nervous system (CNS), primarily (YKL-40 and S100B) [15, 16] or exclusively (GFAP) [17]. YKL-40, a chitin binding glycoprotein and a glial activation marker [18], has been identified inside reactive astrocytes in close proximity to amyloid plaques [19]. YKL-40 expression also correlates with tau pathology in AD brain tissues, demonstrating an association between glial activation and neurodegeneration [20]. S100B is a calcium binding protein, exerting both intracellular and extracellular functions and has been found to be up-regulated in AD tissues [21, 22]. GFAP is a key intermediate filament protein and marker of reactive astrocytes, whose expression has been associated with amyloid plaque load and, to a lesser extent, the number of NFTs [23–25].

Inflammation in the brain and its role in AD can be studied indirectly through the analysis of CSF proteins. Increased levels of CSF YKL-40, S100B and GFAP have been observed in AD patients compared to healthy controls, although results have not been consistent [26]. The relationship between inflammatory and core AD markers (Aβ, tau) in CSF has also been explored. Previous studies have found a strong positive association between CSF YKL-40 and tau proteins but not between YKL-40 and $A\beta_{42}$ [19, 27–29]. YKL-40 has also been shown to strongly correlate with neuronal degeneration marker neurofilament light (NFL) in CSF [30], further supporting the assocation between glial activation and neurodegeneration. NFL is mainly located in myelinated axons, therefore its levels also reflect white matter changes, with recent studies indicating a potential for this protein as both a diagnostic and progression marker in AD and other neurodegenerative diseases [26, 31]. Few studies have examined the relationship between S100B and GFAP with core AD markers in CSF. Hov et al. [32] found a correlation between S100B and P-tau but not $A\beta_{42}$ among elective surgery patients free from dementia and delirium. Ishiki et al. [33] did not find a correlation between CSF GFAP and core markers within a dementia cohort.

Loss of memory is typically among the first clinical symptoms of AD, marking the beginning of cognitive decline. The medial temporal lobe is an early site of tau accumulation and its dysfunction may underlie episodic memory decline [34]. Other cognitive domains are also involved in AD, such as language, non-verbal episodic memory and executive functions [35].

In the most recent research criteria from the International Working Group for the diagnosis of AD published in 2014 [36], the diagnosis of prodromal AD requires both the presence of cognitive symptoms and AD signature biomarker profile (increased amyloid positron emission tomography [PET] deposition or the combination of lowered CSF amyloid-$\beta_{1\text{-}42}$ and elevated CSF tau). It is important for the evaluation of novel biomarkers to examine their relationship with both entities separately, independent of diagnosis. That type of approach could both enhance understanding of the underlying pathology of AD and the sequence of events leading to cognitive impairment. The first aim of this study was to assess the ability of glial (YKL-40, S100B, GFAP) and neurodegeneration (NFL) markers in CSF to discriminate between different CSF profiles (AD vs. non-AD) among subjects at the symptomatic pre- and early stages of dementia. The second aim was to investigate the relationship between the CSF markers with neuropsychological tests reflecting different cognitive domains.

Methods

Subjects

Individuals referred to The National University Hospital of Iceland Memory Clinic during a 4 year period who 1) had a score between 24–30 on the Mini Mental State Examination (MMSE) and 2) a score of 4.0 or less on the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) [37], were invited to join a prospective study on mild cognitive impairment (MCI, n = 218). The exclusion criteria were 1) cognitive impairment that without doubt could be explained by a condition other than dementia, 2) difficulties participating due to health or social issues and 3) residency outside the Reykjavík Capital Area. In entering the study, each subject underwent various assessments, including a standard clinical and neuropsychological assessment and brain magnetic resonance imaging (MRI) for evaluation of medial temporal lobe atrophy (MTA) score. Lumbar puncture for collection of CSF, which was optional by requirement of the National Bioethics Committee, was also carried out. For this particular study (Fig. 1), only subjects with CSF samples and complete neuropsychological assessment were selected from the cohort (n = 56). The final sample included 52 subjects as four were removed due to excessively high value on CSF GFAP (n = 1) or blood-contamination in CSF sample (n = 3). Clinical diagnosis of AD was based on the criteria for probable AD dementia defined by National Institute on Aging-Alzheimer's Association (NIA-AA) [38], with evidence of AD pathophysiological processes (based on MTA score or/and analysis of core CSF markers). Patients with Lewy body dementia were diagnosed based on the consensus criteria of McKeith [39]. MCI diagnosis required the fulfillment of the Winblad criteria [40], with those not fulfilling the criteria diagnosed as having subjective mild cognitive impairment (SCI).

CSF collection and analysis

CSF was collected via lumbar puncture with a 22-gauge spinal needle at the L3/4 or L4/5 interspace. Uncentrifuged samples were frozen in 2 ml polypropylene tubes and stored at -80 °C. Commercially available sandwich enzyme-linked immunosorbent assays (ELISAs) were used for measuring the concentrations of T-tau (IBL International, Hamburg, Germany), $A\beta_{42}$ (IBL International, Hamburg, Germany), NFL (Uman Diagnostics, Umeå, Sweden), YKL-40 (Quantikine ELISA Human Chitinase-3–like 1; R&D systems, MN, USA), S100B (BioVendor GmbH, Heidelberg, Germany) and GFAP (BioVendor GmbH, Heidelberg, Germany). All assays were perfomed in technical duplicates and according to manufacturer instructions. The mean Intra-assay CV was < 10% and mean Inter-assay CV < 15% for all assays.

Subject grouping based on CSF measures

Each subject was classified independently of clinical diagnosis on the basis of CSF T-tau and $A\beta_{42}$ values. T-tau/$A\beta_{42}$ ratio cut-off of 0.52 was chosen based on results from a large memory clinic cohort study [38], giving a sensitivity of 93% for AD and specificity of 83% for controls. A positive CSF AD profile was defined as T-tau/$A\beta_{42}$ ratio > 0.52. The same ratio was also used as a part of the clinical diagnosis of AD, explaining full concordance with CSF AD profile.

Neuropsychological tests

All subjects underwent a detailed neuropsychological assessment performed by licensed psychologists. Four cognitive domains commonly affected by ageing and AD were assessed using seven tests (Table 1). For evaluation of verbal episodic memory, two tests were used. The first, Rey Auditory Verbal Learning Test (RAVLT), consisted of 15 nouns read aloud by the examiner for five consecutive trials. Each trial was followed by a free-recall test. After a 30 minute delay, subjects were required to recall the words (without being read the list again) [39]. The second test was composed of a story [40] which included 25 ideas verbally presented by the examiner. Right after the story was presented (immediate recall), the subject was asked to repeat what they remembered without being given any clues (free recall). Thirty minutes later, subjects were asked to recall the story again (delayed recall). The Rey–Osterrieth complex figure test (ROCF) was used to assess non-verbal episodic memory [39]. The subject was asked to reproduce a complicated line drawing, first by copying it free-hand, second by drawing from memory (immediate recall) and third by drawing it after a 30 minute delay (delay recall). Verbal fluency [41] was evaluated with subjects having to produce as many animals names and words starting with the letters H and S as possible in 60 seconds. Two subtests were used to evaluate processing speed. Part A of The Trail Making Test (TMT-A) [42] required subjects to connect 25 numbered circles positioned randomly on a piece of paper. The first and the most simple part of Stroop test - Word reading - was also used for the evaluation of the same cognitive domain [43]. Subjects were shown a list of color names (red, green, yellow or blue), each printed in black ink, and told to read out loud as rapidly as possible. For evaluation of executive functions, The Digit Symbol Substitution Test (DSST), Trail making Test B (TMT-B) and Stroop 4th /3rd parts were used. DSST [44] is a paper-and-pencil test that requires the participant to match symbols to numbers according to a key located at the top of the page. The subject copied the symbol into spaces below a row of numbers. The number of correct symbols within 120 seconds, constituted the score. TMT-

B includes both numbers (1–13) and letters (A-L), with the subject drawing lines between circles, alternating between numbers and letters (1-A-2-B-3-C, etc.). In Stroop - part 4, subjects had to name the color of words, when color and meaning were incongruent. Part 3 – naming of squares of given colors – were used to control for speed by calculating the ratio between the two parts.

Table 1

List of neuropsychological tests administrated

Cognitive domain	Neuropsychological test	Scores (range)
Verbal episodic memory	RAVLT immediate recall	Free recall - sum of the number of words recalled from trials 1 through 5 (0 to 75)
	RAVLT delayed recall	Delayed free recall –number of words recalled after 30 minutes delay (0 to 15)
	RAVLT recognition – false positives	Recognition - number of words recognized from a list of 45 words. Number of false positives subtracted from the score (-30 to 15)
	Story immediate recall	Recall of a story containing 25 ideas (0 to 25)
	Story delayed recall	Recall of a story containing 25 ideas again after 30 minutes delay (0 to 25)
Non-verbal episodic memory	ROCF immediate recall	Complicated drawing reproduced (0 to 36)
	ROCF delayed recall	Complicated drawing reproduced again after 30 minutes delay (0 to 36)
Language	Verbal fluency animals	Number of animal names produced in 60 seconds
	Verbal fluency H + S	Number of words that begin with H/S in 60 seconds
Processing speed	TMT-A	Time in seconds to connect a set of 25 numbered dots in sequential order
	Stroop test, part I	Time in seconds to read a set of color words written in black
Executive functions	DSST	Number of symbols correctly produced in 120 seconds
	TMT-B	Time in seconds to connect 25 targets, alternating between numbers and letters
	Stroop 4th /3rd part	Part 3 – Time in seconds it takes to name squares of given colors Part 4 – Time in seconds it takes to name the color of a word
Abbreviations: RAVLT Rey Auditory Verbal Learning Test, ROCF Rey–Osterrieth complex figure, DSST Digit symbol substitution test, TMT Trail Making Test		

Statistical analysis

Descriptive group comparisons were performed using Mann-Whitney U tests and chi-square tests for continuous and categorical variables respectively. Raw values of CSF measures and selected neuropsychological tests (TMT, Stroop test, DSST) were naturally log-transformed to account for a non-normal distribution. Composite scores for each cognitive domain were calculated by averaging neuropsychological test z-scores and subsequently converting those score into z-scores. Before computation of composite scores, z-scores for tests measuring reaction time were reversed (TMT, Stroop test, DSST) for the purpose of test consistency (higher scores always indicating better performance). Receiver operating characteristic (ROC) curves were constructed for the differentiation between CSF AD vs. non-AD profiles. The discrimination abilities of each CSF marker and cognitive domain were compared using the area under the curve (AUC) method according to DeLong et al [45]. The AUC is the probability that a randomly selected pair of subjects from each CSF profile group is correctly classified. Ridge regression was used to estimate the independent relationship between each CSF marker and cognitive domain after adjusting for other CSF markers. Ridge regression is a parameter estimation method used to address the collinearity problem frequently arising in multiple linear regression [46]. A penalty is introduced, reducing large variance due to multicollinearity in exchange for a tolerable amount of bias. The optimal penalization parameter lambda was estimated by a 5-fold cross-validation, selecting a value between 0 and 1, using the R package glmnet. Correlations were analyzed with Pearson's correlation coefficients. For correlation and ridge regression analyses, cognitive domain measures were adjusted for age and education. For the adjustment, linear regression models were created with each test score as the dependent variable and age and education as independent variables. The residual for each subject was subsequently calculated (observed minus predicted score). Significance values were not adjusted for multiple comparisons as this study was viewed as explorative with emphasis on discovering relationships. All statistical analyses were performed using R (version 3.6.1, The R Foundation for Statistical Computing).

Results

Sample characteristics

Table 2 shows the demographic, pathophysiological and clinical characteristics of the cohort by CSF profile. There was no significant difference between the groups in gender, age, education or CSF measures. The CSF AD profile group showed significantly worse performance (lower scores) on the MMSE test (global cognition), RAVLT and Story tests measuring verbal episodic memory, ROCF immediate recall (Non-verbal episodic memory) and Verbal fluency animal (language) tests compared to the non-AD group ($p < 0.05$).

Table 2

Participant demographics, CSF marker levels and neuropsychological test scores by CSF profiles

	CSF profile		
	Non-AD T-tau/ $A\beta_{42} \leq 0.52$ (n = 24)	AD T-tau/ $A\beta_{42} > 0.52$ (n = 28)	p value □
Demographics			
Gender (M/F)	16/8	17/11	0.66
Age, years	67 (46–80)	70 (51–84)	0.17
Education, years	14.0 (9–20)	12.5 (6–17)	0.11
CSF measures			
$A\beta_{42}$ (pg/ml)	703 (374–2332)	454 (160–822)	N/A
T-tau (pg/ml)	173 (100–722)	416 (132–838)	N/A
NFL (ng/ml)	1.9 (0.9–6.5)	2.5 (1.2–4.5)	0.15
YKL-40 (ng/ml)	165 (83–399)	203 (124–367)	0.12
S100B (pg/ml)	215 (132–335)	230 (130–458)	0.17
GFAP (ng/ml)	1.0 (0.1–7.1)	1.3 (0.5–21.3)	0.09
Cognitive domains			
Global cognition			
MMSE, score	28 (24–30)	27 (24–30)	0.01
Verbal episodic memory			
RAVLT immediate recall, score	36 (23–66)	26.5 (13–51)	0.003
RAVLT delayed recall, score	6.5 (0–15)	1.5 (0–12)	< 0.001
RAVLT recognition-fp, score	9.0 (3–15)	5.5 (-3-15)	0.003

Abbreviations: AD Alzheimer's disease, CSF Cerebrospinal fluid, DDST Digit symbol substitution test, fp false positives, MMSE

Mini-Mental State – Examination, RAVLT Rey Auditory-Verbal Learning Test, ROCF Rey–Osterrieth complex figure, TMT Trail

Making Test, N/A Not applicable

Values are shown as median (range) or as numbers per group, □Mann-Whitney U non-parametric test used for continuous variables and Chi-Square test for the categorical variable (gender), p-values not applicable for $A\beta_{42}$ and T-tau due to their values used for defining CSF profiles

	CSF profile		
Story immediate recall, score	13.5 (5–17)	8 (1–18)	0.005
Story delayed recall, score	12.0 (1–19)	5.5 (0–16)	0.002
Non-verbal episodic memory			
ROCF immediate recall, score	13.3 (0–27)	7.3 (0–26)	0.04
ROCF delayed recall, score	12.8 (0–25)	8.5 (0–26)	0.07
Language			
Verbal fluency animal, score	20 (8–33)	14 (4–27)	0.02
Verbal fluency H + S, score	24.0 (14–48)	25.5 (6–63)	1.00
Processing speed			
TMT-A, sec.	43.5 (21–133)	48.0 (27–116)	0.22
Stroop – part I, sec.	23.5 (20–42)	24.5 (17–34)	0.64
Executive functions			
TMT-B, sec.	109 (44–340)	153 (60–343)	0.06
DSST, score	8.5 (3–51)	7.0 (2–16)	0.24
Stroop 4th /3rd part, sec.	2.1 (1.4-4.0)	2.1 (1.6–5.8)	0.26
Abbreviations: AD Alzheimer's disease, CSF Cerebrospinal fluid, DDST Digit symbol substitution test, fp false positives, MMSE			
Mini-Mental State – Examination, RAVLT Rey Auditory-Verbal Learning Test, ROCF Rey–Osterrieth complex figure, TMT Trail			
Making Test, N/A Not applicable			
Values are shown as median (range) or as numbers per group, Mann-Whitney U non-parametric test used for continuous variables and Chi-Square test for the categorical variable (gender), p-values not applicable for $A\beta_{42}$ and T-tau due to their values used for defining CSF profiles			

Diagnostic accuracy of CSF markers and cognitive domains distinguishing between CSF AD vs. non-AD profiles

Accuracies for distinguishing between CSF AD vs. non-AD profiles were based on ROC curves (Table 3). Neuropsychological tests reflecting verbal episodic memory had the highest accuracy compared to other measurements, with all AUCs over 0.70 which is considered fair [47]. The composite z-score (AUC = 0.80, CI: 0.69–0.92) and RAVLT delayed recall (AUC = 0.80, CI: 0.68–0.93) both distinguished the best between the CSF profile groups. Composite z-scores and tests recflecting other cognitive domains all had AUCs

below 0.70. AUC for CSF measures ranged from 0.61–0.64, with lower limit of each confidence interval below the value of 0.5.

Table 3

ROC curves – distinguishing between AD and non-AD CSF profiles

	AUC	95% CI (AUC)*
CSF measures□		
GFAP (ng/ml)	0.64	0.48–0.79
YKL-40 (ng/ml)	0.63	0.47–0.78
NFL (ng/ml)	0.62	0.45–0.78
S100B (pg/ml)	0.61	0.46–0.77
Cognitive domains		
Verbal episodic memory		
Composite z-score	0.80	0.69–0.92
RAVLT delayed recall, score	0.80	0.68–0.93
Story delayed recall, score	0.75	0.62–0.89
RAVLT immediate recall, score	0.74	0.61–0.88
RAVLT recognition-fp, score	0.74	0.61–0.87
Story immediate recall, score	0.73	0.59–0.86
Non-verbal episodic memory		
Composite z-score	0.65	0.50–0.81
ROCF immediate recall, score	0.66	0.51–0.81
ROCF delayed recall, score	0.65	0.49–0.80
Executive functions		
Composite z-score	0.64	0.49–0.80
TMT-B, sec.□	0.66	0.50–0.81

Abbreviations: AD Alzheimer's disease, AUC Area under curve, CI Confidence Intervals, CSF Cerebrospinal fluid,

DDST Digit symbol substitution test, fp false positives, RAVLT Rey Auditory-Verbal Learning Test,

ROCF Rey–Osterrieth complex figure, SE Sensitivity, SP Specificity, TMT Trail Making Test

AUC is the probability that a randomly selected pair of subjects from each CSF profile group is correctly classified,

*Confidence intervals calculated with DeLong method, □Values are natural log-transformed

	AUC	95% CI (AUC)*
DSST, score□	0.60	0.44–0.75
Stroop 4th /3rd part, sec. □	0.59	0.43–0.75
Language		
Composite z-score	0.60	0.44–0.76
Verbal fluency animals, score	0.68	0.54–0.83
Verbal fluency H + S, score	0.50	0.34–0.66
Processing speed		
Composite z-score	0.56	0.39–0.72
TMT-A, sec. □	0.60	0.44–0.76
Stroop test – part I, sec. □	0.54	0.38–0.70
Abbreviations: AD Alzheimer's disease, AUC Area under curve, CI Confidence Intervals, CSF Cerebrospinal fluid,		
DDST Digit symbol substitution test, fp false positives, RAVLT Rey Auditory-Verbal Learning Test,		
ROCF Rey–Osterrieth complex figure, SE Sensitivity, SP Specificity, TMT Trail Making Test		
AUC is the probability that a randomly selected pair of subjects from each CSF profile group is correctly classified,		
*Confidence intervals calculated with DeLong method, □Values are natural log-transformed		

Fig. 2 illustrates the ROC curves for the two cognitive domains and the CSF measure with the highest AUC from Table 3. Verbal episodic memory (AUC=0.80) was superior in distinguishing between CSF AD vs. non-AD profiles compared to non-verbal episodic memory (AUC=0.65) and CSF GFAP (0.64).

Correlations between CSF markers and cognitive domains

Pearson's correlations between the CSF markers and the cognitive domains within the whole cohort and among subjects with CSF AD profile are presented in Fig. 3a and 3b, respectively. Z-scores for cognitive domains were adjusted for age and education to control for confounding effects. Within the whole cohort, levels of inflammatory markers YKL-40 (NFL: $r = 0.62$, $p < 0.001$; T-tau: 0.46, $p = 0.001$) and S100B (NFL: $r = 0.52$, $p < 0.001$: T-tau: $r = 0.43$, $p = 0.002$) correlated significantly with neurodegeneration markers NFL and T-tau. None of the inflammatory markers correlated with $A\beta_{42}$ levels ($p > 0.05$). Higher CSF T-tau levels correlated with worse performance on verbal episodic memory ($r=-0.28$, $p < 0.04$) and higher CSF GFAP levels with worse performance on executive functions ($r=-0.37$, $p = 0.007$). Among those with CSF AD profile, higher levels of CSF NFL correlated with worse performance on verbal episodic memory ($r=-0.43$, $p = 0.02$), higher levels of CSF S100B with worse performance on processing speed ($r=-0.45$, $p =$

0.02) and higher levels of GFAP levels with worse performances on processing speed (r=-0.68, p < 0.001) and executive functions (r=-0.39, p = 0.04).

Ridge regression estimates for association between CSF marker levels and cognitive domains

Ridge regression was also performed for the estimation of the relationships between CSF measures and each cognitive domain within the whole cohort when all CSF measures were used as predictors (Table 4). Ridge regression is a penalized approach to linear multiple regression, especially useful when dealing with multicollinearity (highly correlated predictors). The method shrinks the slope coefficients towards zero as a consequence of penalization but keeps all the predictors in the model. The order of magnitude of the CSF standardized slope coefficients (st. β) and pearsons'r coefficients (Fig. 3a) were similar for non-verbal episodic memory, language, processing speed and executive functions. The CSF measure with the highest coefficient from the ridge regression was also the highest one from the Pearson's analysis for each of those cognitive domains. The order, on the other hand, differed for verbal episodic memory. Results from ridge regression placed CSF YKL-40 as having the highest coefficient, positively associating with verbal episodic memory (st. β = 0.48), while CSF NFL (st. β =-0.38) and T-tau (st. β =-0.24) ranked second and third, albeit as negative association with that same domain. The combination of NFL and T-tau with YKL-40 (by calculating ratios) was therefore also tested further.

Table 4 Ridge regression estimates for association between CSF marker levels and

cognitive domains within the whole cohort (n = 52)

	Cognitive domains - composite z-scores[b]				
	Verbal episodic memory	Non-verbal episodic memory	Language	Processing speed	Executive functions
CSF measures[a]					
$A\beta_{42}$ (pg/ml)	0.13	0.19	-0.04	0.06	0.05
T-tau (pg/ml)	-0.24	<0.01	< 0.01	0.09	0.01
NFL (ng/ml)	-0.38	-0.09	-0.12	-0.07	0.01
YKL-40 (ng/ml)	0.48	0.09	0.03	<0.01	0.04
S100B (pg/ml)	0.04	-0.14	-0.11	-0.09	-0.06
GFAP (ng/ml)	-0.06	-0.01	-0.01	-0.12	-0.17
Numbers represent standardized beta coefficients (st. β)					
[a]Values are natural log-transformed, [b]Analyses are adjusted for age and education					

Correlations between CSF markers and cognitive domains by CSF AD profile

Relationships between the CSF measures and cognitive domains with significant Pearson‘s coefficients ($p < 0.05$), either within the whole cohort or among those with CSF AD profile, are presented in Fig. 4. CSF NFL and T-tau levels were explored as a ratio of YKL-40 levels in relation to verbal episodic memory based on the results from the ridge regression analysis (Table 4). The CSF NFL/YKL-40 ratio showed stronger correlation with verbal episodic memory ($r=-0.51$, $p < 0.001$, Fig. 4a) compared to the correlations of the proteins alone (NFL: $r=-0.26$, $p = 0.06$; YKL-40: $r = 0.18$, $p = 0.20$). Corresponding analysis based on the CSF AD profiles (Fig. 4b) also revealed stronger correlation between CSF NFL/YKL-40 ratio and the cognitive domain among those with CSF AD profile ($r=-0.67$, $p < 0.001$) than without ($r=-0.46$, $p = 0.03$). The relationship of T-tau/YKL-40 ratio with verbal episodic memory ($r=-0.44$, $p = 0.001$, Fig. 4c) was significant but weaker compared to the NFL/YKL-40 ratio within the whole cohort as well as among the CSF AD profile group ($r=-0.35$, $p = 0.07$, Fig. 4d). Correlations between individual protein levels (NFL, YKL-40, T-tau) and verbal episodic memory, both within the whole cohort and by CSF profile, are presented in Additional file 1, S1a-f. Correlations between the NFL/YKL-40 ratio and individual neuropsychological tests reflecting verbal episodic memory are presented in Additional file 1, S2a-e.

Weak negative correlation was found between CSF GFAP levels and executive functions, both within the whole cohort (r=-0.37, p = 0.01, Additional file 1, Fig. S3a) and among subjects with a CSF AD profile (r=-0.39, p = 0.04, Additional file 1, Fig. S3b). Correlation between GFAP levels and processing speed did not reach significance within the whole cohort (r=-0.27, p = 0.06, Fig. 4e) or among those with a CSF non-AD profile (r = 0.02, p = 0.94), but it did strongly correlate among those with a CSF AD profile (r=-0.68, p < 0.001, Fig. 4f). A similar albeit weaker pattern was found between CSF S100B levels and processing speed (within whole cohort: r=-0.20, p = 0.16, Fig. 4g; CSF AD profile: r=-0.45, p = 0.02; CSF non-AD profile: r = 0.03, p = 0.89, Fig. 4h). The corresponding correlations between CSF S100B and GFAP levels with individual neuropsychological tests reflecting processing speed and executive functions are presented in Additional file 1, Fig. S4a-g.

Discussion

We compared different CSF biomarkers reflecting neurodegeneration (NFL) and inflammation (YKL-40, S100B and GFAP) in relation to core CSF AD markers and cognitive functions in a cohort of subjects at the pre- and early symptomatic dementia stages. While our results indicated that these CSF markers did not improve the accuracy of distinguishing between AD and non-AD CSF profiles, they exhibited different pattern of association with certain cognitive domains, as evaluated by various neuropsychological tests. Ratios between the levels of neurodegeneration markers T-tau and NFL with YKL-40 associated with verbal episodic memory while GFAP associated with executive functions within the wole cohort. This pattern was detected more strongly among subjects with a CSF AD profile in addition to S100B and GFAP levels correlating with processing speed. Overall, these results indicate that CSF NFL, YKL-40, S100B and GFAP levels do relate to cognitive functions, specifically among those with a CSF AD profile.

Both CSF NFL and YKL-40 levels correlated with T-tau but not with $A\beta_{42}$, in accordance with previous studies [48–50], thereby NFL and YKL-40 levels most likely reflect processes that are independent of Aβ pathology [51–53]. Furthermore, neither NFL nor YKL-40 did improve the accuracy of differentiation between AD vs. non-AD CSF profiles. The putative inflammatory marker, S100B, did show similar trend as YKL-40 within the whole cohort, correlating strongly with CSF neurodegeneration markers (NFL and T-tau) but not with $A\beta_{42}$ levels. In contrast GFAP did not correlate with the CSF neurodegeneration markers nor with CSF $A\beta_{42}$ levels. Neither CSF S100B nor GFAP have been much studied in terms of correlation with CSF core AD markers. Here we found that CSF S100B and GFAP, like NFL and YKL-40, did not improve accuracy of differentiating between the CSF profiles. Hov et al. [32] found similar results among elective surgery patients free from dementia and delirium, with S100B positively correlating with P-tau but not with $A\beta_{42}$ in CSF. Ishiki et al. [33] did not find an association between GFAP and the core AD markers within a sample of healthy subjects and dementia patients. Taken together, these results further support the idea that NFL, YKL-40, S100B and GFAP are not AD specific biomarkers.

The neuropsychological tests reflecting verbal episodic memory did show the best accuracy in differentiating between the CSF profiles out of all the evaluated measures. The accuracy was good for the composite score of verbal episodic memory and RAVLT delayed recall test (80%), but fair for all the

other verbal episodic memory tests (between 70–80%). A recent meta-analysis [54] based on 47 studies has showed that immediate and delayed memory tests consistently show good accuracy (above 80%) for differentiating between AD and healthy controls, especially those involving list recall. Importantly, these studies are based on clinical diagnosis of AD, while our focus was on the signature of CSF AD biomarker profile.

CSF markers related in different ways to cognitive measures. Higher levels of T-tau and NFL in combination with lower levels of YKL-40 correlated with worse performance on verbal episodic memory. Combinations of these proteins as ratio showed stronger relationship to the cognitive domain compared to the individual protein levels. Both CSF NFL [50, 55] and YKL-40 [53] have been previously reported to associate with cognitive decline, with correlation found between CSF levels and global cognition assessed by MMSE test scores among AD patients. In the same studies, the correlation did not hold for patients with MCI. Thus, NFL and YKL-40 alone might be not sensitive to very early changes in cognition in the earliest symptomatic stages of dementia (SCI, MCI) as in more advance stages, but using their ratio could increase the overall sensitivity.

Knowledge regarding the relationship between core CSF biomarkers and cognition remains incomplete. Overall, $A\beta_{42}$ and T-tau appear to associate with memory and executive functions in some studies [56, 57], although results have not been consistent in terms of which cognitive domains they are associated with, which particular tests are most suitable and the strength of relationships in different clinical stages [56, 58, 59]. However, the levels of core CSF marker have shown evidence of reaching a plateau early in the clinical course of the disease and are therefore not considered ideal to track progression of disease at later stages [60].

Increased CSF levels of inflammatory marker GFAP was found weakly associated with worse performance on tests reflecting executive functions, both within the whole cohort and among subjects with CSF AD profile. Few studies have examined the relationship between CSF GFAP levels and global cognition. Ishiki et al. [33] did not find a relationship between CSF GFAP levels and MMSE scores in a sample of healthy subjects and dementia patients. Darreh-Shori et al. [61] also reported no correlation between CSF GFAP levels and cognition (MMSE and overall cognition scores) among AD patients. Relationship between CSF S100B levels and processing speed or executive functions was not observed within the whole cohort in our study. As with CSF GFAP, little research has been conducted on the association between CSF S100B levels and cognition. Darreh-Shori et al. [61] found higher levels of CSF S100B correlating with better performances on the cognitive tests. The association between S100B levels and cognition has also been explored in serum, both in a healthy cohort of older-aged subjects [62] and in drug-free schizophrenic patients [63]. Increased levels of S100B were associated with positive outcome in the healthy cohort but a negative one among the drug-free schizophrenic patients.

Associations between selected CSF markers and cognitive domains were also examined within each CSF profile. The NFL/YKL-40 ratio was highly related to verbal episodic memory among those with CSF AD profile but not among those without. Higher levels of CSF S100B and GFAP moderately associated with

worse performance on processing speed only within the CSF AD profile group. This is of interest because the CSF markers did not directly relate to CSF AD profile (separation ability in discrimation between AD vs. non-AD profiles was poor). This outcome could possibly be explained by additive effects of distinctive processes on cognitive functions. A previous study [64] showed a similar trend where CSF YKL-40 levels associated with less preservation of global cognition only in individuals with low Aβ levels (Aβ positive). CSF Aβ levels did though not correlate with YKL-40 or cognitive decline, but to brain atropy in Aβ positive subjects.

This study has several limitations. First, the sample size was realtively small and hence present findings need to be validated in a larger study. The sample did not include healthy controls, which could underestimate associations between the studied variables. Another limitation of the study is lack of information about ApoE genotype. However, it is unlikely that the ApoE genotype affects the outcome as previous studies have suggested that ApoE ε4 status does not influence CSF NFL or YKL-40 levels [19, 65, 66].

Conclusions

Our findings suggest that levels of CSF inflammatory markers YKL-40, S100B and GFAP relate to different cognitive profiles at the symptomatic pre- and early dementia stages. Ratios between neurodegeneration markers T-tau and NFL with YKL-40 moderately associated with verbal episodic memory while GFAP weakly associated with executive functions within the whole cohort. The relationships between the levels of NFL/YKL-40 with verbal episodic memory and S100B and GFAP with processing speed were especially strong only among those with CSF AD profile, although the CSF markers did not directly relate to CSF AD profile. The CSF markers could be of potential use as progression markers, monitoring subtle cognitive changes at the earliest symptomatic stage of dementia, specifically among those with AD pathology. Further studies with bigger group sizes are needed to validate these results and to evaluate their potential in tracking changes in the more advanced stages of AD and other types of dementia.

Additional File

Additional file 1: Fig. 1. Correlations between levels of CSF NFL, YKL-40, T-tau and verbal episodic memory within the whole cohort and by CSF profile. Figure 2. Correlations between levels of CSF NFL/YKL-40 with neuropsychological tests reflecting verbal episodic memory by CSF profile. Figure 3. Correlations between levels of CSF GFAP and executive functions within the whole cohort and by CSF profile. Figure 4. Correlations between levels of CSF S100B and CSF GFAP with neuropsychological tests reflecting processing speed and executive functions by CSF profile. (DOCX 890 kb)

Declarations

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Availability of data and materials

The data which support this study are not publicly available, but may be provided upon reasonable request.

Authors‘ contributions

UDT, JS and PHP contributed to the conception and design of the study. UDT and MKJ contributed to the collection of data. UDT performed the statistical analysis and drafted the manuscript. SHL provided guidance on statistical analysis. PHP, JS, TD, SHL and MJK revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study has been approved by the National Research Ethics Committee of Iceland (VSN-14-028) and all subjects signed and informed consent. The study was conducted in accordance with the Helsinki Declaration latest revision of 2013.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

AD: Alzheimer‘s disease

AUC: Area under curve

$A\beta_{42}$: Amyloid-β_{1-42}

CSF: Cerebrospinal fluid

DSST: Digit Symbol Substitution Test

FP: False positives

GFAP: Glial fibrillary acidic protein

IQCODE: Informant Questionnaire on Cognitive Decline in the Elderly

MCI: Mild cognitive impairment

MMSE: Mini-mental state examination

MTA: Medial temporal lobe atrophy

NFL: Neurofilament light

NFTs: Neurofibrillary tangles

PET: Positron emission tomography

P-tau: Phosphorylated tau

RAVLT: Rey Auditory Verbal Learning Test

ROC: Receiver operating characteristic

ROCF: Rey–Osterrieth Complex Figure

S100B: S100 calcium-binding protein B

SCI: Subjective cognitive impairment

TMT: Trail Making Test

T-tau: Total-tau

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Figures

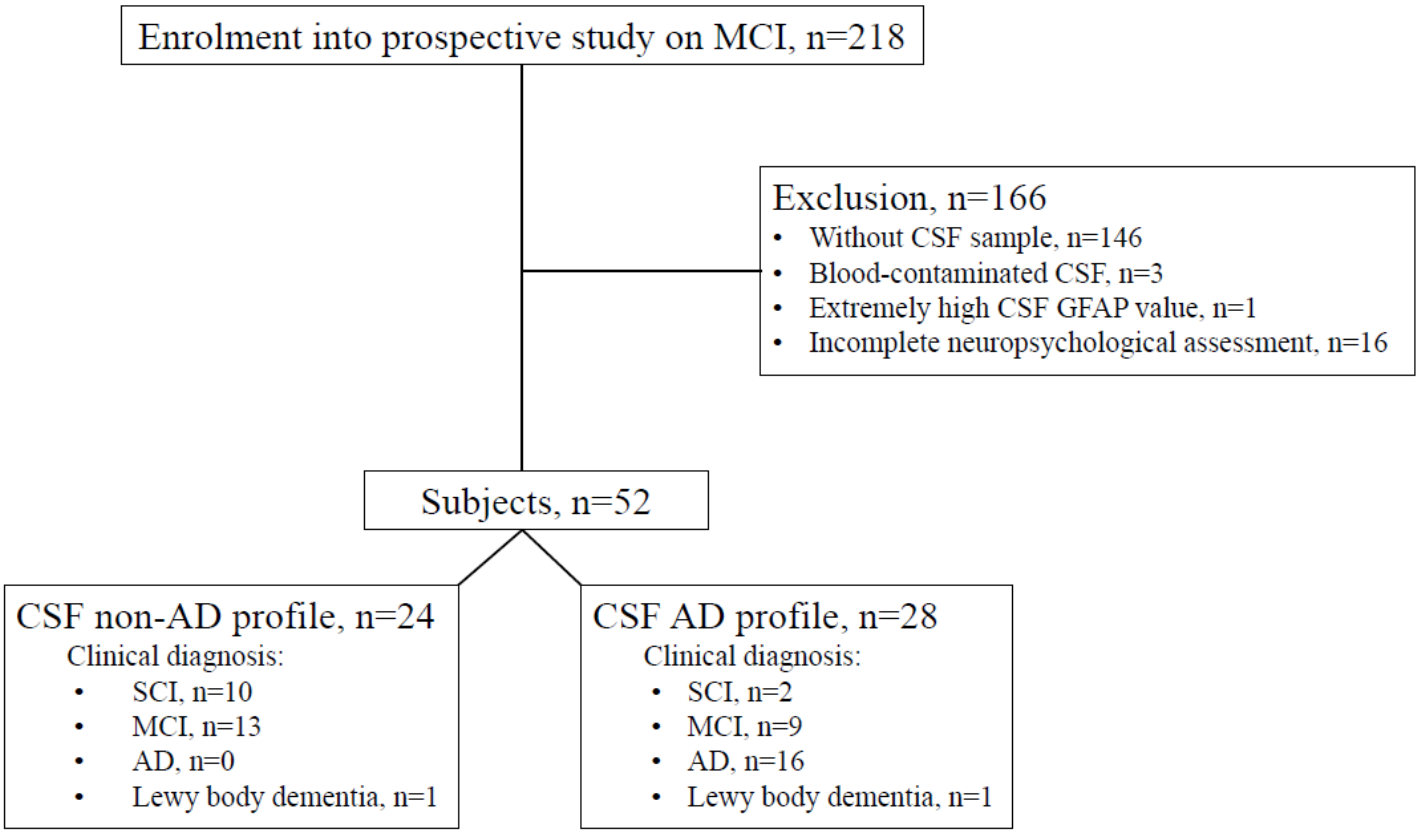

Figure 1

Flow diagram of sample selection

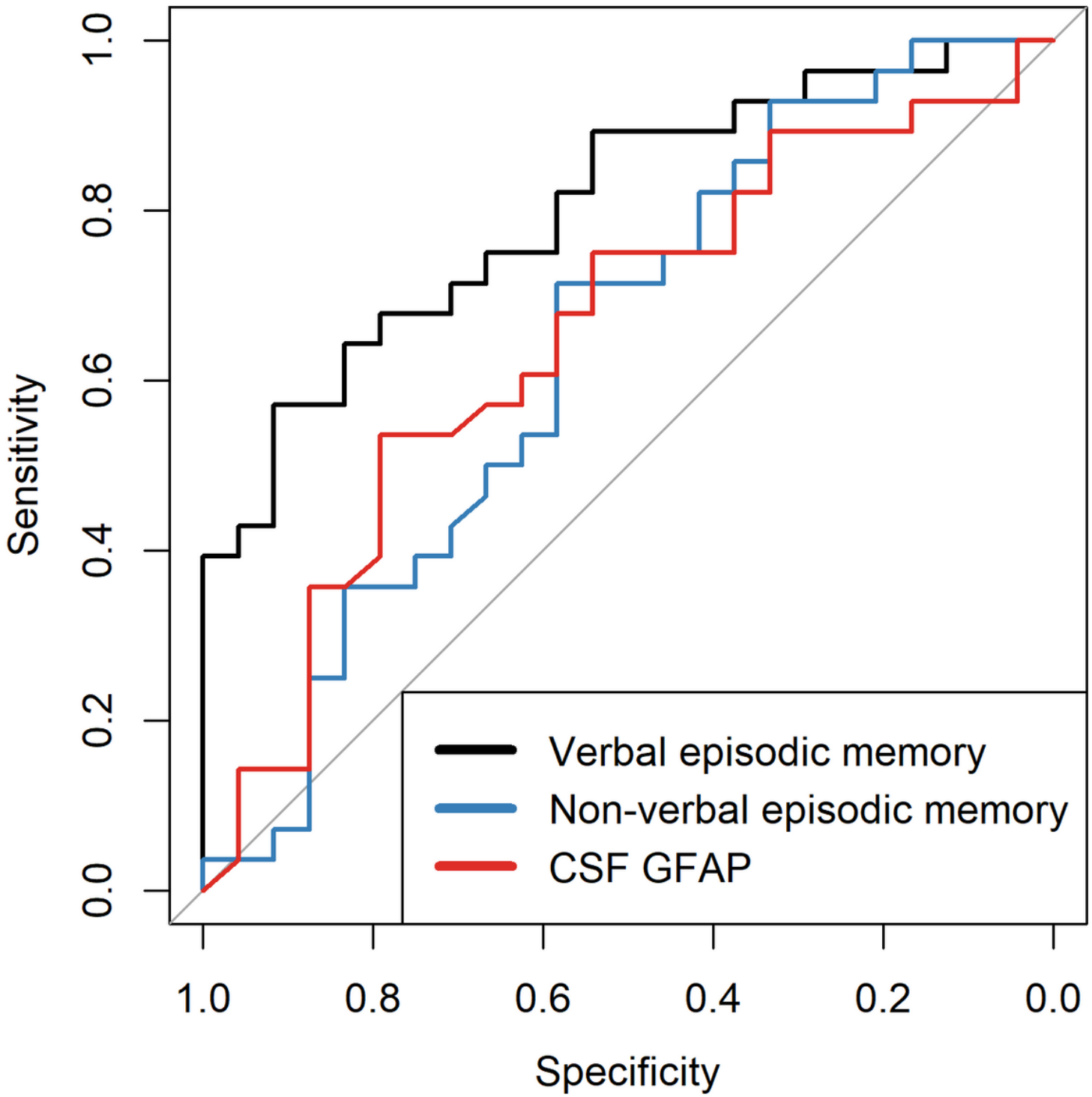

Figure 2

Comparison between ROC curves of the two cognitive domains and the CSF measure with the highest area under the curve (AUC)

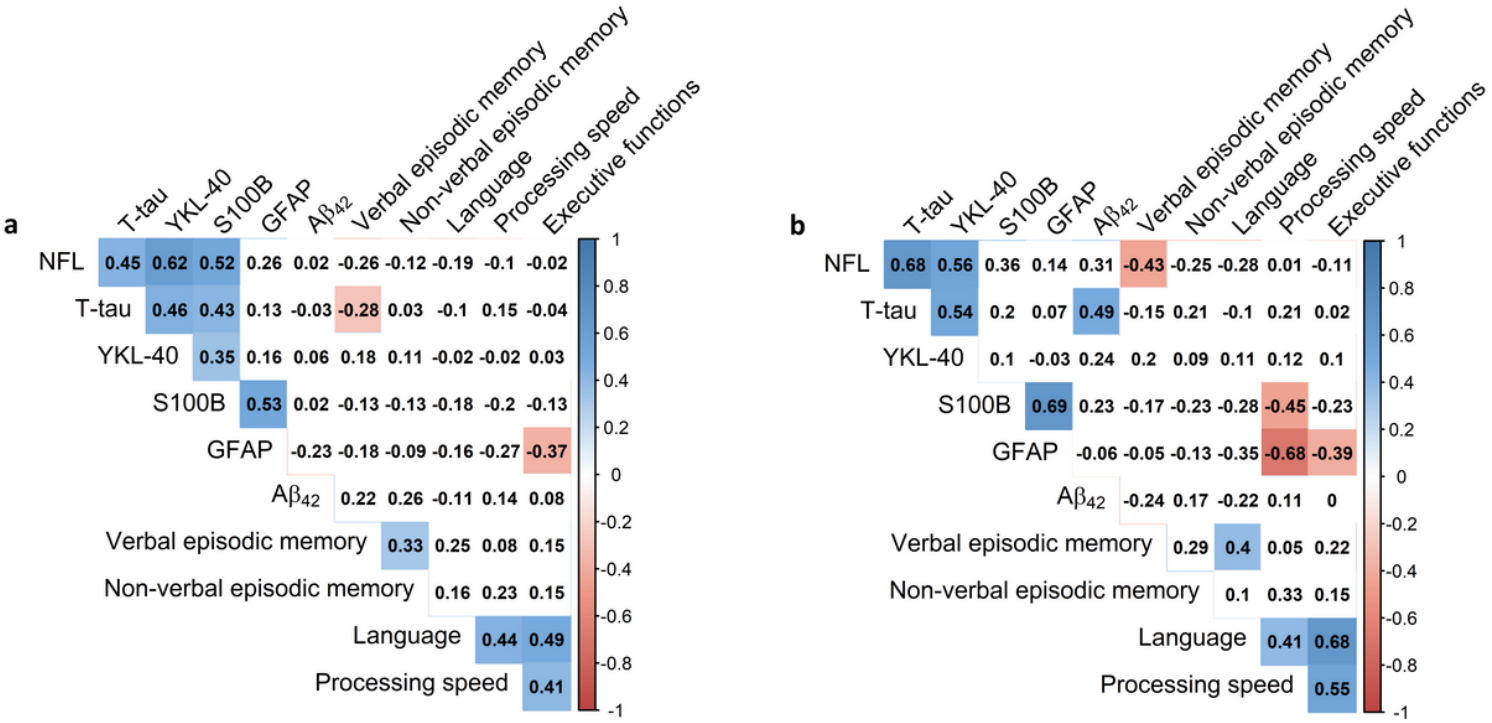

Figure 3

Pearson‘s correlation matrix between CSF markers and cognitive domains a) within the whole cohort (n=52) and b) among subjects with CSF AD profile (n=28). Colored squares indicate statistical significance ($p<0.05$). Z-scores for cognitive domains were adjusted for age and education and CSF measures were naturally log-transformed

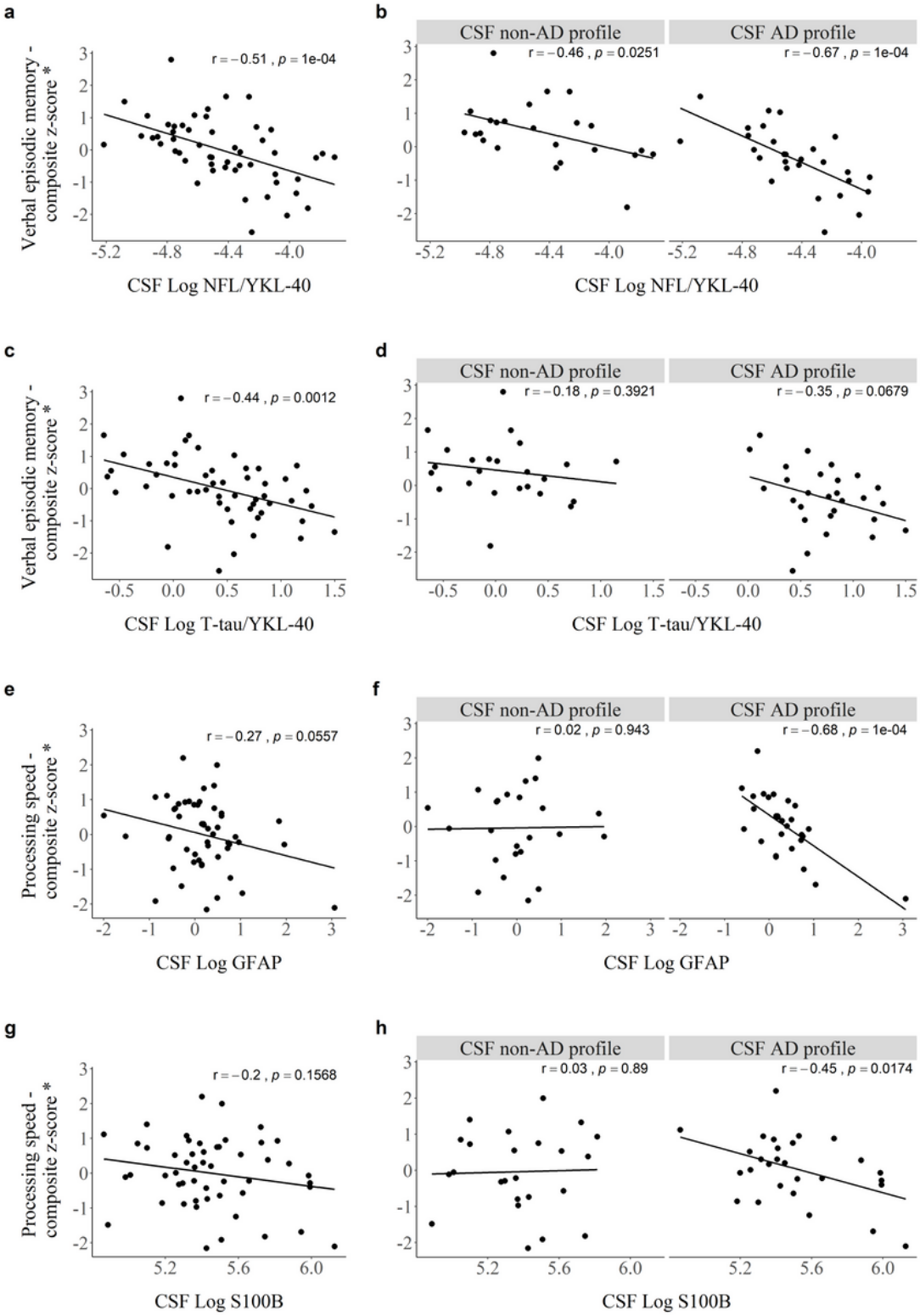

Figure 4

Simple regression illustrations of the pearson's correlations between CSF NFL/YKL-40 ratio and verbal episodic memory (a,b), T-tau/YKL-40 ratio and verbal episodic memory (c,d), GFAP and processing speed (e,f) and S100B and processing speed (g,h) within the whole cohort and by CSF profile. *Cognitive domains were adjusted for covariates (age and education)

Supplementary Files

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