Cerebrospinal fluid neurogranin and YKL-40 as biomarkers of Alzheimer’s disease

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Abstract

Objective: Widespread implementation of cerebrospinal fluid (CSF) biomarkers of Alzheimer’s disease (AD) in clinical settings requires improved accuracy for diagnosis of prodromal disease and for distinguishing AD from non-AD dementias. Novel and promising CSF biomarkers include neurogranin, a marker of synaptic degeneration, and YKL-40, a marker of neuroinflammation.

Methods: CSF neurogranin and YKL-40 were measured in a cohort of 338 individuals including cognitively healthy controls and patients with stable mild cognitive impairment (sMCI), MCI who later developed AD (MCI-AD), AD dementia, Parkinson’s disease dementia (PDD), dementia with Lewy bodies (DLB), vascular dementia (VaD), and frontotemporal dementia (FTD). The diagnostic accuracy of neurogranin and YKL-40 were compared with the core AD biomarkers, β-amyloid (Aβ42 and Aβ40) and tau.

Results: Neurogranin levels were increased in AD and decreased in non-AD dementia compared with healthy controls. As a result, AD patients showed considerably higher CSF levels of neurogranin than DLB/PDD, VaD and FTD patients. CSF YKL-40 levels were increased in AD compared with DLB/PDD but not with VaD or FTD. Neither CSF neurogranin nor YKL-40 levels differed significantly between sMCI patients and MCI-AD patients. Both biomarkers correlated positively with CSF Aβ40 and tau. CSF neurogranin and YKL-40 could separate AD dementia from non-AD dementias (neurogranin, area under the curve [AUC] = 0.761; YKL-40, AUC = 0.604; Aβ42/neurogranin, AUC = 0.849; Aβ42/YKL-40, AUC = 0.785), but the diagnostic accuracy was not better compared to CSF Aβ and tau (Aβ42, AUC = 0.755; tau AUC = 0.858; Aβ42/tau, AUC = 0.895; Aβ42/Aβ40, AUC = 0.881). Similar results were obtained when separating sMCI from MCI-AD cases. Interpretation: CSF neurogranin and YKL-40 do not improve the diagnostic accuracy of either prodromal AD or AD dementia when compared to the core CSF AD biomarkers. Nevertheless, the CSF level of neurogranin is selectively increased in AD dementia, whereas YKL-40 is increased in both AD and FTD suggesting that synaptic degeneration and glial activation may be important in these neurodegenerative conditions.

Introduction

Amyloid-β (Aβ) containing neuritic plaques and neurofibrillary tangles composed mainly of hyperphosphorylated tau are the neuropathological hallmarks of Alzheimer’s disease (AD). According to the amyloid cascade hypothesis that has dominated the field of AD research for the last two decades, abnormal accumulation of Aβ in the
brain is the primary initiator of the disease-associated pathophysiological processes. Disappointingly, however, phase III clinical trials in patients with moderate to mild disease have failed to show clinical benefit of drugs reducing Aβ plaque burden. The failure has been partly attributed to the fact that new therapies were initiated in the late stages of the disease and to the relatively high rate of misdiagnosis in patients included in the trials thus highlighting the need for early and accurate disease biomarkers.

Cerebrospinal fluid (CSF) Aβ and tau are at present the most specific fluid biomarkers of AD reflecting amyloid plaque load and severity of neurodegeneration, respectively. Decreased CSF levels of Aβ42 (or the Aβ42/Aβ40 ratio) in combination with elevated tau (total or phosphorylated [p-tau]) levels predict with high accuracy future development of AD in patients with mild cognitive impairment (MCI) and also have some diagnostic value for differentiating AD from non-AD dementias. Nonetheless, further improvement of diagnostic accuracy, especially to differentiate AD from other dementias, would be of value in the clinic. In this context, there is a growing interest in developing novel biomarkers that would monitor other aspects of AD pathology such as for example synaptic dysfunction and neuroinflammation and two such biomarkers, neurogranin and YKL-40, have recently emerged. Neurogranin is calmodulin-binding postsynaptic protein regulating synaptic plasticity and learning. Several studies demonstrated that neurogranin levels are reduced in the brain but increased in CSF of AD patients. Interestingly, high CSF levels of neurogranin were reported in MCI patients progressing to AD compared with cognitively stable MCI (sMCI) patients and control individuals. YKL-40 (chitinase-3 like-1, cartilage glycoprotein-39) is a secreted glycoprotein considered as a potential marker of ongoing inflammations in a variety of human diseases. CSF levels of YKL-40 appear to be elevated in AD, vascular dementia (VaD) and frontotemporal dementia (FTD) but not in Parkinson’s disease (PD) or dementia with Lewy bodies (DLB).

Despite these encouraging findings it remains to be established if neurogranin and YKL-40 could provide greater diagnostic accuracy for prediction and differential diagnosis of AD than the core AD CSF biomarkers, Aβ and tau. To this end we compared the diagnostic performance of CSF neurogranin, YKL-40, Aβ42, Aβ40 and tau in a cohort of 338 individuals including cognitively healthy controls and patients with sMCI, MCI who later developed AD, AD dementia, Parkinson’s disease dementia (PDD), DLB, VaD and FTD.

## Materials and Methods

### Subjects and Methods

This study was performed at the Memory Clinic of Skåne University Hospital in Malmö, Sweden. Seventy-four patients with AD, 47 patients with DLB/PDD, 34 patients with VaD, 33 patients with FTD and 53 healthy controls were included in this study. We also included 97 individuals with a baseline diagnosis of MCI of which 35 had converted to AD (MCI-AD), while 62 remained cognitively stable (sMCI). The median clinical follow-up period for sMCI group was 5.8 years (3.0–9.6). All subjects were assessed by medical doctors with extensive experience in cognitive disorders. All patients with a clinical syndrome of dementia met the DSM-III-R criteria for dementia combined with the NINCDS-ADRDA criteria for AD, the NINDS-AIREN criteria for VaD, criteria of probable DLB according to the 2005 consensus criteria or the 1998 consensus criteria for FTD. All the individuals in the FTD group were diagnosed with behavioral variant FTD except for one patient who had semantic dementia. Patients with MCI at baseline had to fulfill the criteria advocated by Petersen. The control population consisted of healthy elderly volunteers, who were recruited in the city of Malmö, Sweden. Inclusion criteria were (1) absence of memory complaints or any other cognitive symptoms; (2) preservation of general cognitive functioning; and (3) no active or previous significant neurological or psychiatric disease. The characteristics of the study cohort are given in Table 1.

The design of this study has been approved by the Local Ethics Committee of Lund University, Sweden and the study procedure was conducted in accordance with the Helsinki Declaration. All study participants gave their informed consent to research.

### CSF sampling and biological assays

For all patients and controls, blood plasma and CSF samples were drawn at some point between 8 AM and 12 AM. The procedure and analysis of the CSF followed the Alzheimer’s Association Flow Chart for CSF biomarkers. CSF neurogranin was measured using an in-house sandwich enzyme-linked immunosorbent (ELISA) assay, as described previously. CSF levels of YKL-40 were measured using a commercial available ELISA kit (R&D Systems, Minneapolis, MN). CSF Aβ42, Aβ40 and tau were analyzed using Euroimmun immunoassay (EUROIMMUN AG, Lübeck, Germany). All measurements were performed by board-certified laboratory technicians who were blinded to clinical data.
Table 1. Demographic data, clinical characteristics and CSF levels of neurogranin, and YKL-40.

|                      | Control (n = 53) | sMCI (n = 62) | MCI-AD (n = 35) | AD (n = 74) | DLBL/PDD (n = 47) | VaD (n = 34) | FTD (n = 33) |
|----------------------|-----------------|---------------|-----------------|-------------|------------------|-------------|-------------|
| **Age**              | 75.3 (6.4)      | 69.2 (7.5)\textsuperscript{a} | 75.0 (7.6)\textsuperscript{b} | 76.4 (7.4)\textsuperscript{b} | 74.5 (6.3)\textsuperscript{b} | 75.7 (7.8)\textsuperscript{b} | 71.7 (6.7)\textsuperscript{c,d,e} |
| **Sex (% female)**   | 70%             | 56%           | 66%             | 68%         | 40%\textsuperscript{f,g} | 47%\textsuperscript{h} | 51%         |
| **MMSE**             | 28.6 (1.8)      | 28.2 (1.2)    | 26.4 (1.7)\textsuperscript{b} | 19.4 (3.3)\textsuperscript{h,i} | 21.9 (5.1)\textsuperscript{h,i} | 21.5 (4.4)\textsuperscript{h,i} | 21.8 (6.6)\textsuperscript{h,i} |
| **APOE 1 or 2 e4 alleles** | 31%             | 28.2 (1.2) | 65%\textsuperscript{d} | 54%\textsuperscript{d,g} | 24%\textsuperscript{h,i,k} | 27%\textsuperscript{f} |
| **Neurogranin, pg/mL** | 557 (328)      | 542 (279)     | 652 (348)       | 711 (404)\textsuperscript{f} | 480 (312)\textsuperscript{f,g} | 313 (150)\textsuperscript{f,g} | 370 (194)\textsuperscript{f,g} |
| **YKL-40, ng/mL**    | 200 (64)        | 184 (69)      | 219 (59)        | 248 (70)\textsuperscript{h} | 217 (65)\textsuperscript{h} | 221 (69) | 222 (59)\textsuperscript{h} |
| **Aβ42, pg/mL**      | 668 (287)       | 486 (200)\textsuperscript{a} | 314 (79)\textsuperscript{b} | 260 (106)\textsuperscript{b} | 340 (173)\textsuperscript{b,h} | 397 (187)\textsuperscript{b,k} | 676 (289)\textsuperscript{p,q} |
| **Aβ40, pg/mL**      | 5136 (1531)     | 3821 (1377)\textsuperscript{a} | 4219 (1227)\textsuperscript{f} | 3892 (1383)\textsuperscript{a} | 3170 (1327)\textsuperscript{a} | 3209 (1383)\textsuperscript{a} | 4470 (1550)\textsuperscript{h,o,a} |
| **Tau, pg/mL**       | 467 (191)       | 437 (175)     | 643 (224)\textsuperscript{b} | 768 (267)\textsuperscript{h,r} | 472 (171)\textsuperscript{i} | 496 (191)\textsuperscript{i} | 382 (205)\textsuperscript{f} |
| **Aβ42/neurogranin** | 1.57 (0.99)     | 1.10 (0.60)\textsuperscript{i} | 0.65 (0.46)\textsuperscript{a,k} | 0.50 (0.40)\textsuperscript{a,b} | 0.91 (0.55)\textsuperscript{a,h} | 1.62 (1.20)\textsuperscript{i,l,o} | 2.40 (2.03)\textsuperscript{i,l,o} |
| **Aβ42/YKL-40**      | 3.82 (2.23)     | 2.83 (1.18)\textsuperscript{a} | 1.57 (0.76)\textsuperscript{a,b} | 1.10 (0.47)\textsuperscript{a,b} | 1.69 (0.90)\textsuperscript{a,b,h} | 1.93 (0.95)\textsuperscript{a,b,k} | 3.08 (1.24)\textsuperscript{i,l,o,p} |
| **Aβ42/Aβ40**        | 0.13 (0.04)     | 0.13 (0.04)    | 0.08 (0.02)\textsuperscript{a,b} | 0.07 (0.02)\textsuperscript{a,b} | 0.11 (0.04)\textsuperscript{a,k,l} | 0.13 (0.04)\textsuperscript{i} | 0.15 (0.04)\textsuperscript{a,j,l,o} |
| **Aβ42/tau**         | 1.66 (0.82)     | 1.25 (0.56)\textsuperscript{c} | 0.54 (0.26)\textsuperscript{a,k} | 0.38 (0.23)\textsuperscript{b} | 0.82 (0.50)\textsuperscript{a} | 1.02 (0.54)\textsuperscript{c,h} | 2.57 (3.45)\textsuperscript{b,i,l,o,a} |

Data are shown as mean (SD) unless otherwise specified. CSF, cerebrospinal fluid; sMCI, stable mild cognitive impairment; MCI-AD, mild cognitive impairment that subsequently converted to AD; AD, Alzheimer’s disease; DLBL/PDD, dementia with Lewy bodies or Parkinson’s diseases dementia; VaD, vascular dementia; FTD, frontotemporal dementia; MMSE, Mini Mental State Examination.

\textsuperscript{1}APOE data was only available from 11 FTD patients.

Demographic factors and clinical characteristics were compared using Students t-test, one-way ANOVA and chi-square tests. CSF biomarkers were analyzed with univariate general linear models controlling for age and gender; \textsuperscript{a}compared with controls, \textit{P} < 0.001; \textsuperscript{b}compared with sMCI, \textit{P} < 0.001; \textsuperscript{c}compared with controls, \textit{P} < 0.05; \textsuperscript{d}compared with AD, \textit{P} < 0.01; \textsuperscript{e}compared with VaD, \textit{P} < 0.05; \textsuperscript{f}compared with controls, \textit{P} < 0.01; \textsuperscript{g}compared with MCI-AD, \textit{P} < 0.05; \textsuperscript{h}compared with AD, \textit{P} < 0.05; \textsuperscript{i}compared with MCI-AD, \textit{P} < 0.001; \textsuperscript{j}compared with sMCI, \textit{P} < 0.01; \textsuperscript{k}compared with sMCI, \textit{P} < 0.05; \textsuperscript{l}compared with AD, \textit{P} < 0.001; \textsuperscript{m}compared with DLBL/PDD, \textit{P} < 0.01; \textsuperscript{n}compared with VaD, \textit{P} < 0.01; \textsuperscript{o}compared with VaD, \textit{P} < 0.001; \textsuperscript{p}compared with MCI-AD, \textit{P} < 0.01.
Statistical analysis

SPSS (IBM, Armonk, NY) and R version 3.1.2 were used for statistical analysis. Neurogranin and YKL-40 levels were not normally distributed and therefore ln-transformed before analysis. Neurogranin levels were below the detection limit of the assay for 9 cases (3%), which were assigned concentration of 120 pg/mL, equal to the lower detection limit of the assay. YKL-40 levels were above the detection limit of the assay for nine cases (3%), which were assigned concentration of 400 ng/mL, equal to the higher detection limit of the assay.

We used Students t-test, one-way analysis of variance (ANOVA) and chi-square tests to compare demographic factors and clinical characteristics (age, gender, Mini Mental State Examination [MMSE], APOE ε4). There were significant differences in age and gender between the diagnostic groups (Table 1). Therefore, for group-wise comparisons of neurogranin and YKL-40, we used univariate general linear models controlling for age and gender. sMCI patients, MCI patients who later progressed to AD (MCI-AD) and AD dementia patient were included in all the analysis as separate diagnostic categories. The diagnostic accuracy of CSF biomarkers was assessed with the receiving operating characteristic (ROC) curve analysis.

Differences in the area under the ROC curve (AUC) of two ROC curves were compared using bootstrap method. *P* ≤ 0.05 was considered statistically significant.

Results

The demographics are given in Table 1. CSF levels of YKL-40 correlated positively with age in controls \(r = 0.382, P = 0.005\) as well as in AD patients \(r = 0.309, P = 0.007\). In the AD group, women showed slightly higher neurogranin levels than men \(t(72) = 2.18, P = 0.033\), but this was not the case in the controls. We did not find any differences in either neurogranin or YKL-40 concentrations between APOE ε4 allele carriers and non-carriers (data not shown).

CSF levels of neurogranin and YKL-40 in different diagnostic groups

The CSF levels of neurogranin were increased in patients with AD dementia \(P = 0.027\) and at the same time decreased in patients with VaD \(P < 0.001\) and FTD \(P = 0.006\) compared to cognitively healthy controls (Fig. 1A). Neurogranin levels were higher in AD dementia
than in non-AD dementias, that is, DLB/PDD (P = 0.002), VaD (P < 0.001) and FTD (P < 0.001).

When compared with cognitively healthy controls, the CSF levels of YKL-40 were increased in patients with AD dementia (P < 0.001) and FTD (P = 0.004). CSF YKL-40 levels were also increased in patients with AD dementia compared with cognitively stable cases with MCI (P < 0.001) and DLB/PDD (P = 0.018). The patients with MCI, who subsequently developed AD dementia (MCI-AD), did not have higher levels of YKL-40 when compared to the cognitively stable patients with MCI (P = 0.085) or AD patients (P = 0.146) (Fig. 1B).

**Associations with CSF Aβ and tau**

In order to establish if changes in CSF levels of neurogranin and YKL-40 are related to amyloid and/or tau pathology in AD, we examined associations between these biomarkers and CSF Aβ and tau in cognitively healthy controls and in patients with AD dementia or MCI who later developed AD (MCI-AD). Both neurogranin and YKL-40 correlated with tau as well as with Aβ40 in all studied diagnostic groups (Table 2). While we also found that neurogranin and YKL-40 positively correlated with Aβ42 in AD patients, this was not the case in any other diagnostic groups (Table 2). Finally, there were significant negative associations between the Aβ42/Aβ40 ratio and both neurogranin and YKL-40 in MCI-AD patients whereas in control and AD groups the ratio only correlated with neurogranin.

**CSF neurogranin and YKL-40 as clinical biomarkers of AD dementia**

We next sought to determine whether CSF neurogranin and YKL-40 could improve the differential diagnosis of AD dementia when compared to the standard AD biomarkers, CSF Aβ and tau. Given that the ratios of Aβ42/Aβ40 or Aβ42/tau perform better than Aβ42 or tau alone, we also assessed the ability of Aβ42/neurogranin and Aβ42/YKL-40 ratios to distinguish different dementia groups.

The results of the ROC analysis are summarized in Table 3. For all examined diagnostic groups, the Aβ42/neurogranin and Aβ42/YKL-40 ratios showed improved accuracy (larger AUC) in comparison with neurogranin and YKL-40, respectively. When comparing individual AUC, we found that the Aβ42/neurogranin ratio was not significantly different from the Aβ42/Aβ40 ratio and performed poorer than the Aβ42/tau ratio when separating patients with AD dementia from patients with non-AD dementias (Table 3). The results were similar for differentiating patients with AD dementia from MCI patients, who later developed AD dementia (MCI-AD).

The diagnostic accuracy of the Aβ42/YKL-40 was not improved compared with the Aβ42/Aβ40 and Aβ42/tau ratios when differentiating patients with AD dementia from those with non-AD dementias. Furthermore, the Aβ42/YKL-40 ratio was not significantly different from either the Aβ42/Aβ40 ratio or the Aβ42/tau ratio in distinguishing AD from MCI-AD.

**CSF neurogranin and YKL-40 as clinical biomarkers of AD during the MCI stage**

Finally, we studied whether neurogranin or YKL-40 could improve the prediction of future development of AD in patients with MCI. The Aβ42/neurogranin ratio performed poorer than either the Aβ42/Aβ40 or the Aβ42/tau ratio in distinguishing patients with sMCI from MCI patients who later developed AD. The accuracy of the Aβ42/YKL-40 ratio was not significantly different from either the Aβ42/Aβ40 ratio or the Aβ42/tau when differentiating sMCI from MCI-AD (Table 3).

**Discussion**

Patient care and drug development in AD are in critical need of accurate early disease biomarkers. These biomarkers will reduce the costs and failure rate of clinical trials by guiding the selection of patients who will benefit from a given treatments and by effectively evaluating patient response to new drugs. The complexity of AD provides a strong rationale for use of multiple biomarkers that

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**Table 2.** Associations between CSF neurogranin, YKL-40 and the core AD biomarkers.

|       | Tau                     | Aβ42                     | Aβ40                     | Aβ42/Aβ40                |
|-------|-------------------------|--------------------------|--------------------------|--------------------------|
|       | Neurogranin | YKL-40      | Neurogranin | YKL-40      | Neurogranin | YKL-40      | Neurogranin | YKL-40      | Neurogranin | YKL-40      | Neurogranin | YKL-40      |
| Controls | 0.706*** | 0.358**     | 0.197       | 0.053       | 0.590*** | 0.308*       | 0.343*       | 0.251      |
| MCI-AD      | 0.708*** | 0.592***   | 0.242       | 0.012       | 0.646*** | 0.509**       | 0.530**       | 0.630***   |
| AD          | 0.719*** | 0.554***   | 0.257*      | 0.246*      | 0.625*** | 0.446***      | 0.365***      | 0.182      |

Data are derived from linear regression models adjusting age and gender. CSF, cerebrospinal fluid; AD, Alzheimer’s disease; MCI-AD, mild cognitive impairment that subsequently converted to AD. Significant results are shown in bold; *P ≤ 0.5; **P ≤ 0.01; and ***P ≤ 0.001.
monitor different pathophysiological pathways driving the disease. In this study, we assessed the diagnostic accuracy of neurogranin and YKL-40, which are considered promising biomarkers of synaptic dysfunction and neuroinflammation, two pathogenic mechanisms implicated in AD.33,34 The study included patients with AD, VaD, DLB/PDD, FTD and sMCI as well as MCI patients who later progressed to AD and healthy controls. This allowed us for the first time simultaneous measurements of CSF levels neurogranin and YKL-40 in prodromal AD, AD dementia and most non-AD dementias in a relatively large sample, thus reducing the bias associated with analytical variability. We initially compared CSF level of neurogranin and YKL-40 in different diagnostic groups. In agreement with existing data, we observed increased CSF levels of neurogranin and YKL-40 in AD patients compared with healthy controls.19,35,36 However, in the present study, neurogranin levels were not significantly increased in MCI patients who subsequently developed AD dementia (MCI-AD), which contrasts several previous papers that found increased CSF neurogranin also in this early phase of AD.10,13,37 This discrepancy in the results could be due to the relatively small number of patients in the MCI-AD group in our study. However, it could be noted that even though the MCI-AD group was not very large in the present study, the levels of both Aβ42 and tau were significantly different between sMCI and MCI-AD, which was not the case for neurogranin. At the same time, we found that neurogranin and YKL-40 were increased in AD compared with non-AD dementias including DLB/PDD and VaD. These results suggested that neurogranin and YKL-40 might improve differentiation between AD dementia and other non-AD dementias. However, to be considered for clinical applications a new CSF AD biomarker should show better diagnostic performance than CSF Aβ42 and tau, the two biomarkers that already have been incorporated in the diagnostic framework of AD proposed by the International Working Group (IWG) for New Research Criteria for the Diagnosis of AD and by the US National Institute on Aging–Alzheimer’s Association (NIA-AA).38 Recent evidence suggests that the ratios of CSF Aβ42 to Aβ40 or tau have a greater diagnostic accuracy in AD39,40 and show improved concordance with amyloid positron emission tomography.

### Table 3. ROC analysis of the CSF biomarkers.

|                  | AUC, 95% CI | AUC difference versus Aβ42/ Aβ40 (P-value) | AUC difference versus Aβ42/tau (P-value) |
|------------------|------------|-------------------------------------------|-----------------------------------------|
| **AD versus non-AD dementias** |            |                                           |                                        |
| Neurogranin      | 0.761, 0.688–0.834 |                                           |                                        |
| YKL-40           | 0.604, 0.521–0.687 |                                           |                                        |
| Aβ42/neurogranin | 0.849, 0.792–0.906 | –0.32 (0.130)                              | −0.046 (0.023)                          |
| Aβ42/YKL-40      | 0.785, 0.721–0.848 | –0.096 (<0.001)                            | −0.110 (<0.001)                         |
| Aβ42             | 0.755, 0.686–0.824 |                                           |                                        |
| Tau              | 0.858, 0.805–0.912 |                                           |                                        |
| Aβ42/Aβ40        | 0.881, 0.833–0.930 |                                           |                                        |
| Aβ42/tau         | 0.895, 0.848–0.942 |                                           |                                        |
| **AD versus MCI-AD** |            |                                           |                                        |
| Neurogranin      | 0.538, 0.423–0.652 |                                           |                                        |
| YKL-40           | 0.609, 0.500–0.719 |                                           |                                        |
| Aβ42/neurogranin | 0.642, 0.532–0.752 | –0.001 (0.980)                             | −0.128 (0.001)                          |
| Aβ42/YKL-40      | 0.725, 0.628–0.824 | 0.082 (0.136)                              | −0.045 (0.224)                          |
| Aβ42             | 0.720, 0.620–0.821 |                                           |                                        |
| Tau              | 0.650, 0.543–0.757 |                                           |                                        |
| Aβ42/Aβ40        | 0.643, 0.533–0.753 |                                           |                                        |
| Aβ42/tau         | 0.770, 0.676–0.864 |                                           |                                        |
| **sMCI versus MCI-AD** |            |                                           |                                        |
| Neurogranin      | 0.593, 0.471–0.715 |                                           |                                        |
| YKL-40           | 0.689, 0.579–0.799 |                                           |                                        |
| Aβ42/neurogranin | 0.746, 0.643–0.848 | −0.099 (0.008)                             | −0.101 (0.003)                          |
| Aβ42/YKL-40      | 0.823, 0.737–0.909 | −0.022 (0.492)                             | −0.024 (0.363)                          |
| Aβ42             | 0.774, 0.682–0.866 |                                           |                                        |
| Tau              | 0.791, 0.698–0.883 |                                           |                                        |
| Aβ42/Aβ40        | 0.845, 0.767–0.923 |                                           |                                        |
| Aβ42/tau         | 0.847, 0.767–0.927 |                                           |                                        |

Significant results are shown in bold. ROC, receiver operating characteristic; CSF, cerebrospinal fluid; AUC, area under the curve; AD, Alzheimer’s disease; MCI-AD, mild cognitive impairment that subsequently converted to AD; sMCI, stable mild cognitive impairment.
(PET) imaging. Therefore, we next evaluated neurogranin and YKL-40 as biomarkers of AD in comparison with the Aβ42/β40 and Aβ42/tau ratios. Using ROC curve analysis we found that similar to Aβ and tau, the Aβ42/neurogranin and Aβ42/YKL-40 ratio performed better than neurogranin and YKL-40 alone. However, neither the Aβ42/neurogranin ratio nor the Aβ42/YKL-40 ratio was more accurate than the Aβ42/Aβ40 and Aβ42/tau ratios when differentiating AD from non-AD dementias or cognitively sMCI from MCI that converted to AD. This is in agreement with two earlier reports showing that the YKL-40/Aβ42 ratio is comparable to but not better than the tau/Aβ42 ratio for predicting cognitive decline in healthy individuals and conversion of MCI to AD. One potential explanation for our findings could be that changes in CSF levels of neurogranin and YKL-40 are closely related to amyloid and/or tau pathology. In fact, our study as well as several other studies demonstrated that CSF neurogranin and YKL-40 correlate strongly with tau levels in patients with AD dementia, prodromal AD and control subjects. On the other hand, CSF total tau and phosphorylated tau also correlate tightly in AD and control populations, see for example, Blennow et al., but not in patients with stroke or Creutzfeldt-Jakob disease who show a very marked increase in CSF (PET) imaging. Therefore, we next evaluated neurogranin and YKL-40 as biomarkers of AD in comparison with the Aβ42/β40 and Aβ42/tau ratios. Using ROC curve analysis we found that similar to Aβ and tau, the Aβ42/neurogranin and Aβ42/YKL-40 ratio performed better than neurogranin and YKL-40 alone. However, neither the Aβ42/neurogranin ratio nor the Aβ42/YKL-40 ratio was more accurate than the Aβ42/Aβ40 and Aβ42/tau ratios when differentiating AD from non-AD dementias or cognitively sMCI from MCI that converted to AD. This is in agreement with two earlier reports showing that the YKL-40/Aβ42 ratio is comparable to but not better than the tau/Aβ42 ratio for predicting cognitive decline in healthy individuals and conversion of MCI to AD.

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In conclusion, our study demonstrates that CSF neurogranin and YKL-40 do not provide any added clinical diagnostic value to already existing AD biomarkers during prodromal and dementia stages. However, longitudinal studies with repeated CSF measurements over time are warranted to determine the usefulness of the different biomarkers to measure the disease progression during different stages of AD. It is very likely that neurogranin will be a marker that can monitor the effects of new disease-modifying therapies on synaptic integrity, while YKL-40 might be used to investigate the effects of novel drugs affecting neuroinflammation.

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Author Contributions

O. H. designed the project. O. H., H. Z. and K. B. supervised the study. S. J., J. H., and O. H. performed acquisition, analysis and interpretation of data. S. J. and O. H. drafted the paper. A. S. and M. L. W. acquired the FTD data. All authors critically revised the manuscript for intellectual content.

Conflict of Interest

Drs Janelidze, Hertze, Landqvist Waldö, Santillo, Hansson report no disclosures. Drs Blennow and Zetterberg are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. KB has served at Advisory Boards for IBL International, Roche Diagnostics, Eli Lilly and Amgen, and as a consultant for Novartis and Alzheon.

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