Preclinical amyloid pathology biomarker positivity: effects on tau pathology and neurodegeneration

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INTRODUCTION

The pathological hallmarks of Alzheimer’s disease (AD), the most common cause of dementia, are the aggregation and deposition of β-amyloid (Aβ) peptides into plaques, hyperphosphorylation and aggregation of tau protein with formation of tangles along with atrophy due to neurodegeneration.1 Although still controversial, biomarkers reflecting the accumulation of Aβ deposition in the brain are believed to be the earliest detectable sign of AD in healthy elderly2,3 and studies both in autosomal dominant AD and late-onset AD suggest that tangle formation occurs after deposition of Aβ in brain.2,4 Three core cerebrospinal fluid (CSF) biomarkers, reflecting the key characteristics of AD pathology, are included in the diagnostic criteria.5 The presence of brain amyloid pathology is reflected by a decrease in CSF Aβ42 levels,6,7 whereas high levels of tau correlate with greater intensity of neuronal degeneration and high levels of phosphorylated tau correlate with neurofilibrillary tangle load in the brain.8

The concordance between amyloid PET images and CSF Aβ42 is above 90%.9-11 Recent failures in clinical trials, where patients who already have cognitive symptoms or dementia have been included, suggest that we need to treat AD at the prodromal or even preclinical phase of the disease. Brain autopsy studies, and more recent biomarker studies, indicate that the pathology is initiated at least 10–20 years before clinical symptoms.2,12-19 This knowledge provides a window of opportunity to initiate treatment to prevent the disease. However, this emphasizes the necessity for biomarkers that identify individuals at risk for developing AD later in life. Further, we need to gain knowledge on the development and progression of concomitant pathology.

Although presence of amyloid pathology is part of the diagnostic criteria,20 amyloid pathology is not specific for AD. Plaque pathology may be present in individuals with Parkinson’s disease21 in patients with both familial22 and iatrogenic23 Creutzfeldt-Jakob disease, and in cases with traumatic brain injury.24 We also know that around 30% of healthy elderly individuals have amyloid pathology.25-27 These data are cross-sectional and longitudinal studies are scarce. However, one longitudinal study indicates that around 20% of healthy elderly with amyloid pathology remain cognitively healthy with a follow-up of 8 years.28 In addition, other common dementias can overlap with AD both in terms of symptoms and CSF profile, and mixed pathologies are common.29 Genetic and in vitro studies have indicated that inflammation30 and synaptic function31,32 may be linked to Aβ production, aggregation and clearance, as well as Aβ toxicity. Previous biomarker studies support that CSF proteins may reflect such mechanisms.33-36 The mechanistic and pathological similarities across neurodegenerative disorders further highlight

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Brain autopsy and biomarker studies indicate that the pathology of Alzheimer’s disease (AD) is initiated at least 10–20 years before clinical symptoms. This provides a window of opportunity to initiate preventive treatment. However, this emphasizes the necessity for biomarkers that identify individuals at risk for developing AD later in life. In this cross-sectional study, originating from three epidemiologic studies in Sweden (n = 1428), the objective was to examine whether amyloid pathology, as determined by low cerebrospinal fluid (CSF) concentration of the 42 amino acid form of β-amyloid (Aβ42), is associated with biomarker evidence of other pathological changes in cognitively healthy elderly. A total of 129 patients were included and CSF levels of Aβ42, total tau, tau phosphorylated at threonine 181 (p-tau), neurogranin, VILIP-1, VEGF, FABP3, Aβ40, neurofilament light, MBP, orexin A, BDNF and YKL-40 were measured. Among these healthy elderly, 35.6% (N = 46) had CSF Aβ42 levels below 530 pg ml^-1. These individuals displayed significantly higher CSF concentrations of t-tau (P < 0.001), p-tau (181) (P < 0.001), neurogranin (P = 0.009) and FABP3 (P = 0.044) compared with amyloid-negative individuals. Our study indicates that there is a subpopulation among healthy older individuals who have amyloid pathology along with signs of ongoing neuronal and synaptic degeneration, as well as tangle pathology. Previous studies have demonstrated that increase in CSF tau and p-tau is a specific sign of AD progression that occurs downstream of the deposition of Aβ. On the basis of this, our data suggest that these subjects are at risk for developing AD. We also confirm the association between APOE ε4 and amyloid pathology in healthy older individuals.
the need for both cross-sectional studies comparing individuals with and without AD pathology and longitudinal studies starting when individuals are in the preclinical stage.

Within the framework of the population-based H70-studies in Gothenburg the aim of this study was to examine if amyloid pathology, as determined by low CSF concentration of Aβ42, is associated with biomarker evidence of other pathological changes, such as neurodegeneration, inflammation and lipid homeostasis, in cognitively healthy elderly. We have analyzed several CSF biomarkers reflecting the core pathological hallmarks of AD along with biomarkers reflecting the above-suggested pathology. Healthy elderly individuals were classified into those with (CSF Aβ42 ≤530 pg ml⁻¹) and without (CSF Aβ42 >530 pg ml⁻¹) amyloid plaque pathology.37

**MATERIALS AND METHODS**

**Participants**

This analysis originates from three epidemiologic studies in Gothenburg, Sweden, the Prospective Population Study of Women (PPSW) and the Gerontological and Geriatric Population Studies (H70), which have been described previously,38-41 and the H85-study. The participants were sampled from the Swedish Population Register on the basis of their birth date and were born in 1914, 1918, 1922, 1923, 1924 and 1930. Both persons living in private households and in residential care were included. In the PPSW/H70-study, 1409 individuals were eligible in 2009–2010 and 857 agreed to participate (response rate 61%). Among these, 88 (10.3%) consented to a lumbar puncture (LP). The H85 study is a population study of 85-year olds born on specific dates in 1923–1924. There were 944 individuals eligible in 2008–2010, and 571 agreed to participate (response rate 61%). Among these, 62 (10.9%) consented to an LP. Overall, among the 150 with an LP, 16 were excluded due to dementia and 5 due to incomplete biomarker information, leaving 129 for the present study. These 129 participants are defined as cognitively healthy elderly as they do not fulfill the criteria for dementia and they have no previous history of memory complaints. Demographic data are shown in Table 1.

The studies were approved by the Regional Ethical Review Board in Gothenburg, and informed consent was obtained from all participants and/or their relatives in cases of dementia.

**Study procedures**

The clinical examination, performed at the inclusion of the population study, was conducted at an outpatient department or in the participant’s home and included comprehensive social, functional, physical, neuropsychiatric and neuropsychological examinations, as well as close informant interviews.

**Neuropsychiatric examinations and interviews**

Semistructured neuropsychiatric examinations were performed by trained psychiatric research nurses. These examinations included ratings of common symptoms and signs of dementia (for example, assessments of memory, orientation, general knowledge, apraxia, visuospatial function, understanding proverbs, following commands, naming ability and language) and have been described in detail previously.42,43 Cognitive function was also measured with the Mini Mental State Examination (MMSE).44

The psychiatric nurses who performed the examinations were supervised and trained by psychiatrists. Inter-rater reliability between psychiatrists and nurses was studied in 50 individuals who had dual ratings by either psychiatric research nurses or psychiatrists. Inter-rater agreement for the symptoms and signs used to diagnose dementia was between good and excellent (kappa values between 0.74 and 1.00).45 Close informant interviews were also performed. The interviews were semistructured and comprised questions about changes in behavior and intellectual function, psychiatric symptoms and activities of daily living, and, in cases of dementia, age of onset and disease course.

**Diagnoses**

Dementia was diagnosed by geriatric psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R).46 based on symptoms rated during the neuropsychiatric examinations and information from the close informant interviews, as described previously.42,43 Participants with dementia at baseline were excluded from further analysis.

**Genotyping**

Blood samples were collected and the single-nucleotide polymorphisms (SNPs) rs7412 and rs429358, in APOE (gene map locus 19q13.2) were genotyped with KASPar PCR SNP genotyping system (LGC Genomics, Hoddesdon, Herts, UK). Genotype data for these two SNPs were used to unambiguously define e2, e3 and e4 alleles.

**CSF sampling and biomarker analyses**

All CSF samples were collected by LP in the L3/L4 or the L4/L5 interspace in the morning. The first 12 ml of CSF were collected in a polypropylene tube and immediately transported to the local laboratory for centrifugation at 1800 g in 20 °C for 10 min. The supernatant was gently mixed to avoid possible gradient effects, aliquoted in polypropylene tubes and stored at −70 °C.

The CSF total tau and tau phosphorylated at threonine 181 (p-tau) were determined using a sandwich enzyme-linked immunosorbent assay (ELISA; INNOTEST, Fujirebio, Ghent, Belgium) httAg and PHOSPHO, TAU (181P); Innogenetics, as previously described.47,48 CSF Aβ42 was measured using a sandwich ELISA (INNOTEST β-amyloid1-42), specifically constructed to measure Aβ starting at amino acid 1 and ending at amino acid 42.49 Neurogranin and Aβ40 were measured on the Meso Scale Discovery (MSD) platform using an internally developed assay50 or the V-PLEX (Meso Scale Diagnostics, Rockland, MD, USA) Aβ Peptide Panel 1 (40B) Kit, respectively. CSF levels of neurofilament light (NFL) were measured using the ELISA-kit from UmanDiagostics (NF-light Umeå, Sweden).51 CSF levels of YKL-40 were determined using a sandwich ELISA (R&D Systems, Minneapolis, MN, USA)52 and levels of myelin basic protein (MBP) and brain-derived neuritic factor (BDNF) were analyzed by ELISA (Active MBP, Diagnostic Systems Laboratories, Webster, TX, USA;52 BDNF Emax Immunoassay System, Promega, Madison, WI, USA), while the CSF levels of vascular endothelial growth factor (VEGF) and heart type fatty acid binding protein 3 (FABP3) were analyzed on the MSD platform (PLEX Plus Human VEGF Kit and Human FABP3 Kit, Meso Scale Diagnostics). CSF orexin A was analyzed by an in-house RIA.53 Finally, visinin-like protein 1 (VILIP-1) was analyzed using a commercially available ELISA (VILIP-1 Human ELISA, BioVendor R&D, Kassel, Germany) according to manufacturer’s instructions but with minor modifications, the calibration curve ranged from 1.25-0.02 ng ml⁻¹ and samples were analyzed in a twofold dilution. All assays have been validated using CSF samples following a detailed protocol including precision and accuracy. CSF samples in the present study underwent a single freeze thaw cycle prior to analyses and all samples were analyzed in duplicate with a coefficient of variability (CV) acceptance criteria of < 20%. Values had to pass quality control criteria, internal control samples for approval of individual plates, and kit quality controls within the expected range as defined by the Clinical Neurochemistry lab or the manufacturer.

**Classification**

The 129 cognitively healthy elderly individuals were classified as having brain amyloid pathology, defined as CSF Aβ42 levels (≤530 pg ml⁻¹), or...
not (>530 pg ml⁻¹) where CSF Aβ42 was measured using ELISA (INNOTEST β-amyloid1-42). This cutoff was previously defined in a longitudinal study at the Sahlgrenska University Hospital Laboratory, to classify all subjects according to presence of biochemical evidence of AD pathology.37

Statistical analyses

Statistical analyses were performed using PASW Statistics for Windows (Version 18.0.; SPSS Chicago, IL, USA). Associations between each biomarker with age, gender, MMSE and APOE ε4 carrier status were tested using multivariate linear regression models. Differences in biomarker levels between the two categories of CSF Aβ42 were tested with Mann–Whitney U-tests, while differences in the distribution of APOE ε4 carriers between the two CSF Aβ42 categories were tested with Fisher’s exact test. Interaction effects between age group and categories of CSF Aβ42 were examined with general linear models. All tests were two-sided and statistical significance was defined as P-values < 0.05.

RESULTS

The subgroup without dementia who underwent LP had a tendency to more often be men (43.4% vs 33.8%, P = 0.039), had higher MMSE (range in LP subgroup 23–30, mean 28.4 vs mean 27.6; P = 0.003) and lower Brief Scale of Anxiety (BSA) score (mean 6.4 vs mean 7.7; P = 0.025) compared with those who did not agree to LP. There was no difference in education, age at examination or Montgomery–Asberg Depression Scale.

In this population-based sample, 36% of cognitively healthy individuals were biomarker positive for amyloid pathology. They had increased CSF levels of total tau (P < 0.001), P-tau (181) (P < 0.001), FABP3 (P = 0.004) and neurogranin (P = 0.009), compared with those who were biomarker negative for amyloid pathology (Figures 1a–d, Table 2). Levels of total tau, p-tau and neurogranin were significantly associated with levels of Aβ42 (P < 0.001, P < 0.001 and P = 0.005, respectively) where lower levels of Aβ42 are linked to increased levels of the biomarker. There was no difference in the CSF levels of NFL, YKL-40, MBP, VILIP-1, BDNF, Aβ40, VEGF or orexin A between the two groups. There was no association between the CSF biomarker levels and either age, gender or MMSE.

APOE4 status was significantly associated with amyloid pathology (higher levels of Aβ42 were seen in those without allele 4, (P < 0.001, Figure 2)) but no significant associations were seen with any other biomarker. After controlling for the effect of Aβ42 by stratifying by the cutoff value of 530 pg ml⁻¹ there were still no apparent associations between APOE ε4 allele and the CSF biomarkers.

Sixty-five percent of APOE ε4 carriers had CSF Aβ42 ≤ 530 pg ml⁻¹, whereas only 20% of the non-carriers had CSF Aβ42 ≤ 530 pg ml⁻¹. Among APOE ε4 non-carriers, those biomarker positive for amyloid pathology had higher CSF levels of total tau (P < 0.001), p-tau (P < 0.001), neurogranin (P = 0.005), YKL-40 (P = 0.042), FABP3 (P = 0.005) and VILIP-1 (P = 0.006) than those biomarker negative for plaque pathology (Table 3). Comparing those with and without biomarker positivity for plaque pathology among the APOE ε4 carriers only, CSF levels of Aβ40 were significantly different, with lower levels in the group biomarker positive for plaque pathology.

DISCUSSION

We found that 36% of cognitively healthy individuals with a mean age of 81.9 years had pathological CSF Aβ42, and that these individuals more often had two other ongoing neuropathological processes; tangle pathology, indicated by significantly increased CSF levels of p-tau, and neurodegeneration, indicated by significantly increased CSF levels of total tau (Figures 1a and b). Previous studies have demonstrated that increase in CSF p-tau is a specific sign of AD progression that occurs downstream of the deposition of Aβ.37 On the basis of this, our data suggest that these cognitively healthy older individuals are at risk for developing AD. Ongoing neurodegeneration is further supported by the significantly increased levels of neurogranin (Figure 1d), previously shown to be a specific and novel biomarker for synaptic degeneration in AD and MCI.33,35

In further support for an ongoing neuronal degeneration associated with amyloid pathology, is the finding of increased CSF levels of the heart type FABP (FABP3; Figure 1c). FABP3 is a cytoplasmic protein abundantly expressed in tissues with an active fatty acid metabolism, such as heart, brain and liver.56 FABPs are considered as markers for neuronal damage as levels are increased after traumatic brain injury and Creutzfeldt–Jakob disease.57 Previous studies report that CSF levels of FABP3 have a diagnostic and prognostic value for AD.58–61 In agreement with the present study, a recent study also found that CSF FABP3 levels (reflecting neurodegeneration) are influenced by amyloid pathology.62

We further showed a strong association between APOE ε4 and amyloid pathology (Figure 2). Thus, almost 70% of APOE ε4 carriers displayed amyloid pathology. This is in agreement with previous reports on the role of APOE ε4 in aggregation and clearance of Aβ.63,64 as well as with previous biomarker studies in healthy elderly.65 Several previous studies65,66 found a clear, allele-dependent, association between APOE ε4 and levels of CSF Aβ42 in older people. A recent study showed that amyloid PET-positive individuals, regardless of APOE ε4 status, have equally low CSF Aβ42, and PET-negative cases equally high CSF Aβ42, indicating that the lowering of CSF Aβ42 in APOE ε4 carriers is due to cortical Aβ deposition.67 No association between APOE ε4 and CSF total Aβ was found in agreement with previous studies.68 Further, no relationship between APOE ε4 status and any of the other CSF biomarkers could be demonstrated.

There was no difference in any CSF biomarkers between those with and without plaque pathology within the APOE ε4 carrier group, whereas CSF levels of total tau, p-tau, neurogranin, FABP3, VILIP-1 and YKL-40 were increased in those with plaque pathology in the APOE ε4 non-carrier group. These data indicate that amyloid pathology alone is driving concomitant pathology represented by neurodegeneration (VILIP-1, total tau and FABP3), synaptic degeneration (neurogranin), tangle pathology (p-tau) and inflammation (YKL-40), independently of the APOE ε4 carrier status. Longitudinal data are needed to elucidate whether these individuals have an even higher risk of developing AD, and whether the potential disease progression rate is different.

Individuals with no amyloid pathology and no APOE ε4 allele seem to present few signs of ongoing pathological processes indicating that they are at very low risk of developing AD or other neurodegenerative disorders. Long-term follow-up studies are of great importance to confirm this hypothesis. A recent study with longitudinal follow-up69 indicates that AD biomarker patterns are detected already during early middle age and that these are associated with amyloid PET positivity and cognitive decline, supporting that there is a long preclinical period where concomitant pathology is present. Although the present study is cross-sectional, our data support this conclusion.

The cutoff for classification into amyloid-positive and -negative has previously been defined in a longitudinal study at the Sahlgrenska hospital.37 Several studies have confirmed that there is a direct correlation between CSF Aβ42 levels and amyloid plaque load measured by PET in patients with AD and MCI. However, less is known about the correlation between these two readouts in healthy elderly and absence of amyloid PET in the present study is a potential weakness. However, a recent study by Sutphen et al.69 found an association between CSF Aβ42 and amyloid PET in middle-aged individuals. It is likely that this is true also in healthy older persons. It has also been suggested that CSF
Figure 1. Box plots of CSF biomarkers (a) total tau \((P < 0.001)\), (b) p-tau \((P < 0.001)\), (c) FABP3 \((P = 0.044)\) and (d) neurogranin \((P = 0.009)\) Neurogranin, comparing those with low \((< 530 \text{ pg ml}^{-1})\) and high \((> 530 \text{ pg ml}^{-1})\) CSF A\(\beta\)42 (42 amino acid form of \(\beta\)-amyloid). A\(\beta\)42, amyloid \(\beta\) 42; CSF, cerebrospinal fluid; FABP3, fatty acid binding protein-3; p-tau, tau phosphorylated at 181.

Figure 2. Box plots of CSF A\(\beta\)42 comparing \textsc{APOE} \(\varepsilon4\) carriers and \textsc{APOE} \(\varepsilon4\) non-carriers. There was a clear association between \textsc{APOE} \(\varepsilon4\) status and CSF A\(\beta\)42 \((P < 0.001)\). CSF, cerebrospinal fluid.

Table 2. Mean biomarker values in CSF by levels of Amyloid \(\beta\) (42)

| CSF biomarker (pg ml\(^{-1}\)) | \(A\beta42 \leq 530 \text{ pg ml}^{-1}\) \((n = 43)\) | \(A\beta42 > 530 \text{ pg ml}^{-1}\) \((n = 86)\) |
|--------------------------------|-----------------|-----------------|
| p-Tau                         | 83.6 (25.5)     | 65.2 (19.7)*    |
| Total tau                      | 609.1 (230.4)   | 428.2 (163.6)*  |
| NFL                            | 1.847 (0.987)   | 1.940 (1.353)   |
| Neurogranin                    | 889.3 (414.5)   | 686.1 (322.8)*  |
| VILIP-1                        | 0.13 (0.06)     | 0.12 (0.05)     |
| YKL-40                         | 303.3 (92.2)    | 274.4 (89.2)    |
| FABP3                          | 7.9 (2.8)       | 7.2 (2.7)**     |
| BDNF                           | 12.593 (3876)   | 12.824 (4175)   |
| VEGF                           | 1.8 (0.5)       | 1.9 (0.5)       |
| MBP                            | 1.8 (0.5)       | 1.7 (0.5)       |
| Orexin A                       | 691.1 (159.4)   | 724.1 (189.4)   |

Abbreviations: A\(\beta\)42, amyloid \(\beta\) (42); BDNF, brain-derived neurotrophic factor; CSF, cerebrospinal fluid; FABP3, fatty acid binding protein-3; MBP, myelin basic protein; NFL, neurofilament light; p-tau, tau phosphorylated at 181; VEGF, vascular endothelial growth factor; VILIP-1, visinin-like protein 1; YKL-40, also called chitinase 3-like 1. Values are provided as mean (s.d.) in pg ml\(^{-1}\) for all CSF protein biomarkers except for VILIP-1 where levels are presented as ng ml\(^{-1}\). *P-value < 0.05 using Mann Whitney U-Test comparing those with high A\(\beta\)42 \((> 530 \text{ pg ml})\) and low CSF A\(\beta\)42 \((\leq 530 \text{ pg ml}^{-1})\).
Aβ42 is an earlier indicator of Aβ aggregation compared with PET.\textsuperscript{2,66} Our finding that 36% of healthy older persons in the population had amyloid pathology is consistent with previous findings. It is even slightly lower compared with a study where 65% of healthy elderly above 80 years were amyloid PET positive.\textsuperscript{70} The latter study support a gradual increase in number of PET-positive healthy elderly with age, 10% in the age between 50–59 years and 18% in the age between 60–69 years.

There was no increase in CSF levels of NFL, a suggested marker for subcortical pathology, among those with amyloid pathology. Previous studies indicate a positive correlation between CSF NFL and total tau in AD as well as an association between subcortical axonal degeneration and the three core biomarkers.\textsuperscript{71} However, these individuals were under clinical investigation for AD in a memory clinic, indicating that cognitive symptoms were present, which is not the case in the present study. One may speculate that changes in NFL may be a later event; however, this needs to be confirmed in a longitudinal follow-up study.

Among the strengths of this study are the representative population-based sample, the comprehensive examinations conducted by trained psychiatric nurses and the exclusion of participants with dementia. However, some limitations need to be addressed. First, the overall number of participants is relatively low. We therefore did not have the statistical power to carry out a stratified analysis regarding heterozygous and homozygous APOE ε4 status. Second, only ~15% consented to LP. This group had higher global cognitive function and is probably healthier than the general population of the same age. Finally, this is a population study focusing on Scandinavian participants aged 79–95 years at baseline, and results cannot be generalized to clinical samples to younger populations or to other ethnic groups.

Our study indicates that there is a subpopulation among healthy older individuals that have amyloid pathology have abnormal levels of the CSF biomarkers tau, p-tau, FABP3 and neurogranin. We also confirm the association between APOE ε4 and amyloid pathology in healthy elderly individuals. These findings support the notion that preclinical amyloid pathology is associated with biomarker evidence of neurodegeneration, tau pathology and synaptic dysfunction already in cognitively normal elderly.

**CONFLICT OF INTEREST**

KB and HZ are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. KB has served as a consultant for Eli Lilly, Novartis, Roche Diagnostics, and Sanofi-Aventis and at Advisory Boards for Amgen and IBL International, and given lectures for Fujirebio Europe and Lundbeck. KB’s research team has received grants for collaborative research projects from Eli Lilly and Roche Diagnostics. KH, AZ, SK and AB have nothing to disclose. As primary authors, KH and SK have had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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KB and HZ are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg.

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