Cerebrospinal Fluid Biomarkers in the Evaluation of Alzheimer Disease

Alzheimer disease (AD),¹ the most prevalent form of dementia in older adults, is a neurodegenerative disorder characterized by cerebral amyloid angiopathy, extracellular accumulation of amyloid β-protein (Aβ) in senile plaques, and intracellular accumulation of hyperphosphorylated τ proteins in neurofibrillary tangles in cortical and limbic brain regions. As life expectancy increases, the number of people suffering from dementia will grow considerably, causing an increasing burden on society, economy, and healthcare across the world.

Until recently, a definitive diagnosis of AD could be made only after a postmortem examination of the patient. In living patients only a “probable AD” diagnosis is possible and is made on the basis of clinical features, the results of neurological and neuropsychological testing, and the exclusion of other dementias, in particular frontotemporal lobe degeneration, dementia with Lewy bodies, and vascular dementia. Accurate and early diagnosis is essential for appropriate support and treatment of dementia patients, because drugs are available for the symptomatic treatment of AD, and treatment of dementia patients, because drugs are available for the symptomatic treatment of AD, and early diagnosis is essential for appropriate support and treatment of dementia patients, because drugs are available for the symptomatic treatment of AD, and drugs that may slow or halt the progression of the disease are being developed, such as β-sheet breakers, inhibitors of the enzymes that produce Aβ from its precursor proteins (β- and γ-secretase), and immunotherapy targeted at Aβ (1).

The clinical diagnostic criteria currently used for AD, the NINCDS–ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association) criteria (2), were initially developed for research purposes. Results of several clinicopathological studies showed sensitivity of these criteria for AD to be as high as 93%, but their specificity was drastically lower (3). Clearly, the identification of AD biomarkers is an important step in improving the diagnostic accuracy for this disease (4).

Analyses of various brain-specific proteins in cerebrospinal fluid (CSF), biomarkers with chemical composition that closely matches that of the brain, have already been shown to reach levels of diagnostic sensitivity and specificity that meet previously defined criteria (5), i.e., each above 80%. These biomarkers comprise the 42–amino acid isoform of Aβ (Aβ₄₂), total τ protein (t-τ), and hyperphosphorylated τ protein (p-τ), or combinations thereof. The typical pattern observed in AD patients is a decreased concentration of CSF Aβ₄₂ (i.e., <500 ng/L), and an increased concentration of t-τ (>350 ng/L) or p-τ (>85 ng/L). The combination of CSF t-τ and Aβ₄₂ concentrations yielded a sensitivity of 81% to 94% and a specificity of 79% to 95% for differentiating between AD patients and controls (6). The reference values for these biomarkers were established mostly with the (imperfect) clinical diagnoses as the gold standard. In the more challenging process of distinguishing AD from other dementia disorders, the diagnostic performance of the CSF tests has been less optimal.

For these reasons, many researchers continue to search for the “Holy Grail,” a specific and unique biomarker for AD. In this issue of Clinical Chemistry, Lee et al. (7) report the results of their search for a new AD biomarker. They quantified the levels of a brain injury marker, visinin-like protein 1 (VLP-1, also abbreviated as VLIP-1 or VSNL-1), in CSF of AD patients and age-matched controls. VLP-1 belongs to the family of neuronal calcium sensor proteins involved in calcium-dependent signal transduction mechanisms in neurons. VLP-1 increases neuronal cyclic adenosine monophosphate levels by inducing protein kinase A. VLP-1 is expressed in neurons (8) and its immunoreactivity is decreased in brains of AD patients compared to controls (9). Remarkably, VLP-1 expression is associated with neurofibrillary tangles in AD brains (10).

The investigation of the concentration of VLP-1 in CSF reported in this issue by Lee et al. was based on findings they reported in a previous issue of this journal (11). VLP-1 appeared to be a protein that was relatively brain specific; its concentration was increased in plasma of stroke patients and in CSF in a rat model for stroke, suggesting that VLP-1 is a marker for (rapid) neuronal cell injury. In the present study, CSF VLP-1 concentrations were 50% higher in AD patients than in the control population. An interesting aspect of the studies of Lee and colleagues is that their original approach to find novel markers of brain injury, i.e., mRNA profiling and selection for products that were highly enriched in brain tissue (11), resulted in the identification of VLP-1, which was not picked up by comparable

¹ Nonstandard abbreviations: AD, Alzheimer disease; Aβ, amyloid β-protein; CSF, cerebrospinal fluid; Aβ₄₂, 42–amino acid isoform of Aβ; t-τ, total τ protein; p-τ, hyperphosphorylated τ protein; VLP-1, visinin-like protein-1; MMSE, Mini Mental Status Examination.
fishing expeditions using a proteomics approach with human CSF from AD patients [for an overview see (6)]. Although relatively small patient cohorts were used in the study by Lee et al. (33 patients with AD, 24 controls), a significant and strong correlation was observed with CSF t-τ and p-τ. Despite this association, mean VLP-1 concentrations in AD patients were not increased to the same degree as were t-τ concentrations, which on average approached a 300% increase (12). Furthermore, the correlation between VLP-1 and p-τ was even stronger. These findings are in line with an in vitro study that demonstrated, by an indirect method, that transfection of PC12 cells with VLP-1 induced τ phosphorylation (10).

Interestingly, VLP-1 concentrations in AD patients with an apolipoprotein E (APOE) e4/e4 genotype were approximately double those in e3/e3 carriers. Although the current study includes a relatively small patient series and the results await confirmation in larger cohorts and from independent studies, this association of VLP-1 with the APOE genotype seems to be remarkably different from the association of the APOE genotype with t-τ concentrations. Several clinical studies showed an equivocal relationship between CSF t-τ and the APOE genotype, with positive, negative, and neutral results being reported [for an overview, see (13)]. In a recent series of autopsy-confirmed diagnoses of 50 AD patients, such a correlation was not observed (14). This finding suggests that the release of t-τ and VLP-1 in the extracellular space and CSF follow different pathophysiological mechanisms and indicates unique specificity of VLP-1 as a neuronal injury marker different from that of t-τ and neurofilament proteins.

Another remarkable finding by Lee et al. is the correlation between Mini Mental Status Examination (MMSE) scores as a marker for disease severity and CSF VLP-1 concentrations. Many reported studies have found no correlation of CSF Aβ42 and t-τ with MMSE score [summarized in (13) and confirmed in the small cohort described by Lee et al. (7)]. VLP-1 is negatively correlated to MMSE scores, suggesting VLP-1 may also have a role as a biomarker of disease severity, and role in monitoring disease activity (loss of neurons and cognition per period of time) can also be envisioned. The findings of MMSE correlation with VLP-1, however, should be confirmed in wider ranges of MMSE values and larger groups. The observed correlation may also implicate a limitation of the current findings: Whereas a correlation with MMSE does not seem to exist for Aβ42, t-τ, and p-τ concentrations, these 3 biomarkers appear to be very useful in the identification of incipient AD in patients with mild cognitive impairment (15). Given the association of VLP-1 concentrations with MMSE scores, CSF VLP-1 concentrations may be less aberrant in patients with mild cognitive impairment, who typically have MMSE scores above 23 and below 28, than in patients with AD. Although more studies are needed, these findings suggest that VLP-1 may be of limited usefulness as a biomarker for early AD.

Finally, as Lee et al. point out in their report, the real clinical challenge is not the differentiation of patients with AD from controls but of patients with AD from patients with other types of dementia, including vascular dementia, dementia with Lewy bodies, or frontotemporal lobe degeneration, and also in patients whose dementia is attributable to treatable disorders such as vitamin deficiencies, depression, alcohol abuse, and normal-pressure hydrocephalus. Whether the CSF concentrations of VLP-1 can be used to differentiate AD from other dementia disorders remains to be determined, particularly given the assumption of Lee and coworkers that VLP-1 is a neuronal injury marker, because neuronal cell loss is a characteristic of these other dementia syndromes as well.

In conclusion, with this study the authors have gone one step further in the identification of a possible new biomarker for AD. Before this biomarker reaches the clinical application stage, however, additional case-control studies that include other dementia syndromes are needed, followed by prospective clinical validation studies. With the increasing clinician awareness that CSF biomarkers have additional value in the diagnostic work-up of dementia patients and that CSF analysis appears likely to gain a position in the diagnostic (research) criteria for AD (4), this study will motivate other researchers in their quest to find specific biomarkers for dementia syndromes.

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References


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