Cerebrospinal Fluid Protein Biomarkers for Alzheimer's Disease

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Summary: The introduction of acetylcholine esterase (AChE) inhibitors as a symptomatic treatment of Alzheimer's disease (AD) has made patients seek medical advice at an earlier stage of the disease. This has highlighted the importance of diagnostic markers for early AD. However, there is no clinical method to determine which of the patients with mild cognitive impairment (MCI) will progress to AD with dementia, and which have a benign form of MCI without progression. In this paper, the performance of cerebrospinal fluid (CSF) protein biomarkers for AD is reviewed. The diagnostic performance of the three biomarkers, total tau, phospho-tau, and the 42 amino acid form of β-amyloid have been evaluated in numerous studies and their ability to identify incipient AD in MCI cases has also been studied. Some candidate AD biomarkers including ubiquitin, neurofilament proteins, growth-associated protein 43 (neuromodulin), and neuronal thread protein (AD7c) show interesting results but have been less extensively studied. It is concluded that CSF biomarkers may have clinical utility in the differentiation between AD and several important differential diagnoses, including normal aging, depression, alcohol dementia, and Parkinson's disease, and also in the identification of Creutzfeldt-Jakob disease in cases with rapidly progressive dementia. Early diagnosis of AD is not only of importance to be able to initiate symptomatic treatment with AChE inhibitors, but will be the basis for initiation of treatment with drugs aimed at slowing down or arresting the degenerative process, such as γ-secretase inhibitors, if these prove to affect AD pathology and to have a clinical effect. **Key Words:** Alzheimer's disease, cerebrospinal fluid, biomarker, tau, phosphorylated tau, β-amyloid.

ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is the most common form of dementia. There are exceptional cases with the familial (autosomal dominant) form of AD, but the large majority of patients have the sporadic form of the disease.[1] The characteristic findings at the microscopic level are degeneration of the neurons and their synapses together with extensive amounts of senile plaques and neurofibrillary tangles.[2]

During the preclinical phase of AD the neuronal degeneration proceeds and the amount of plaques and tangles increase and at a certain threshold the first symptoms, most often impairment of episodic memory, appear. This preclinical period probably starts 20-30 years before the first clinical symptoms appear.[3]

According to current diagnostic criteria,[4] AD cannot be diagnosed clinically before the disease has progressed so far that dementia is present. This means that the symptoms must be severe enough to significantly interfere with work and social activities or relations. In recent years, however, the clinical phase of AD with mild memory impairment but without overt dementia, called mild cognitive impairment (MCI),[5] has attained increased attention in the medical community.

To make a diagnosis of MCI, memory disturbances should be "verified" by objective measures adjusted for age and education.[5] However, like dementia, MCI may be caused by several different disorders. Many MCI patients have incipient AD, i.e., they have early AD pathology and will progress to AD with dementia, whereas other MCI cases have a "benign" form of MCI as part of the normal aging process.[6] Furthermore, in some MCI cases, cerebrovascular pathology (e.g., infarcts, white-matter lesions) may contribute to the symptoms.[6]

THE IMPORTANCE OF DIAGNOSTIC MARKERS FOR AD AND MCI

The introduction of acetylcholine esterase (AChE) inhibitors as symptomatic treatment has highlighted the importance of diagnostic markers for AD. The awareness

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in the population of the availability of drug treatment has also made patients seek medical advice at an earlier stage of the disease, making the percentage of MCI cases at dementia clinics increase. This has increased the diagnostic challenge for physicians, because the characteristic clinical picture of AD with slowly progressive memory disturbances combined with parietal lobe symptoms[7] has not yet developed in MCI cases. Accordingly, there is no clinical method to determine which MCI cases will progress to AD with dementia except for a very long clinical follow-up period.

Thus, new diagnostic tools to aid the diagnosis of early AD and to identify incipient AD in MCI cases would be of great importance. Such diagnostic markers would be of even higher significance if new drugs such as β-sheet breakers and β-secretase and γ-secretase inhibitors, with promise of disease-arresting effects, will prove to have clinical effect. Such drugs will probably be more effective in the earlier stages of the disease before neurodegeneration is too severe and widespread.

THE BIOLOGICAL AND PATHOGENIC BASIS FOR AD BIOMARKERS IN CSF

The cerebrospinal fluid (CSF) is in direct contact with the extracellular space of the brain. Therefore, biochemical changes in the brain are reflected in the CSF. Since AD pathology is restricted to the brain, CSF has been the focus in research on diagnostic biomarkers for AD.

CSF biomarkers for AD should reflect the central pathogenic processes in the brain, i.e., the synaptic and axonal degeneration, the aggregation of β-amyloid (Aβ) with subsequent deposition in plaques, and the hyperphosphorylation and ubiquitination of tau with subsequent formation of tangles.

The three biomarkers, total tau (T-tau), phospho-tau (P-tau), and the 42 amino acid form of β-amyloid (Aβ42), have been evaluated in numerous studies. Furthermore, their performance in the identification of incipient AD in MCI cases has also been examined. Lastly, there have been some longitudinal studies, also some performed in clinical practice. Therefore, these biomarkers are reviewed separately.

Some candidate AD protein biomarkers, including ubiquitin, neurofilament (NF) proteins, growth-associated protein 43 (GAP43) (neuromodulin) and neuronal thread protein (NTP) (AD7c), have been less extensively studied. These biomarkers are reviewed at the end of this paper.

TAU AND β-AMYLOID AS BIOMARKERS FOR AD

Tau protein

Tau is a microtubule-associated protein, primarily located in the neuronal axons. Because of alternative splicing of tau mRNA, there are six isoforms with 352–441 amino acids and with molecular weights between 50 and 65 kDa.[8] By binding to tubulin in the axonal microtubules, tau promotes microtubule assembly and stability,[8,9] which is important for axonal function and transport. Tau is a phosphoprotein, with more than 30 potential phosphorylation sites.[9,10] The tangles in AD are made up of an abnormally hyperphosphorylated form of tau.[11] Because of the hyperphosphorylation, tau also loses its ability to bind to the microtubules and to stimulate their assembly.[12]

CSF T-tau. As shown in Table 1, four different ELISA methods for quantification of T-tau have been published.[13–16] A moderate to marked increase in CSF T-tau in AD has consistently been found in numerous publications.[17]

The first paper on CSF T-tau as a protein biomarker for AD used an ELISA method with a polyclonal reporter antibody and found a very marked increase in CSF T-tau in AD.[13] Subsequent studies used ELISA methods[14–16] based on monoclonal antibodies that detect all isoforms of tau independently of phosphorylation state, and found increases in CSF T-tau of approximately 300% and 200%.[17]

The CSF level of T-tau probably reflects the intensity of the neuronal damage and degeneration. This assumption is based on the findings that in acute conditions such as stroke, there is a marked transient increase in CSF T-tau that shows a correlation with computerized tomography measurements of infarct size.[18] Furthermore, the highest increase in CSF T-tau is found in disorders with very intense neuronal degeneration, such as Creutzfeldt-Jakob disease (CJD),[19] whereas a moderate to marked increase is found in AD with less intense degeneration,[20] and normal levels are found in patients with depression, with limited or no degeneration.[14]

CSF P-tau. At least 30 phosphorylation sites have been identified on tau protein.[9] Most of these are Ser-Pro or Thr-Pro motives and are localized outside the microtubule-binding domains.[9] Hyperphosphorylation of tau is found during neuronal development and in several neurodegenerative disorders.[9,10] There is no consensus whether there are any phosphorylation sites that are specific for AD and thus not found in other tauopathies.

As shown in Table 1, six different ELISA methods have been developed for quantification of tau phosphorylated at different epitopes, including threonine 181 + 231,[14] threonine 181,[21] threonine 231 + serine 235,[22] serine 199,[22] threonine 231,[23] and serine 396 + 404.[24] A moderate to marked increase in CSF P-tau has been found using all of these different ELISA methods (Table 1).

The CSF level of P-tau probably reflects the phosphorylation state of tau. This hypothesis comes from indirect evidence such as the finding that there is no change in CSF P-tau after acute stroke,[25] although there is a marked increase in T-tau.[18] Furthermore, CSF P-tau lev-

TABLE 1. *Immunoassays for Cerebrospinal Fluid Total Tau, Phospho-Tau and β-Amyloid (Aβ42)*

Type of Method	Ref.	Specificity	Antibodies	Mean Change (% of controls)
Total tau				
ELISA	13	Total tau	AT120 + PAb	Increase
ELISA	14	Total tau	AT120 + HT7/BT2	Increase (mean ≈ 300%)[17]
ELISA	15	Total tau	16B5 + 16G7	Increase (mean ≈ 200%)[17]
Microsphere ELISA	16	Total tau	PAb-ht2 + MAbs F-F11/F-H5	Increase (216%)
Phospho-tau				
ELISA	14	P-Thr181 + P-Thr231	AT180/AT270 + HT7/AT120	Increase (348%)
ELISA	22	P-Thr231 + P-Ser235	MAb anti-tau + anti-PT231PS235	Increase (no mean level given)
ELISA	22	P-Ser199	MAb anti-tau + anti-PS199	Increase (≈ 2–300%)[86,93]
ELISA	23	P-Thr231	Tau1/CP27 + CP9	Increase
ELISA	21	P-Thr181	HT7 + AT270	Increase (≈ 2–300%)[26,93]
ELISA	24	P-Ser396 + P-Ser404	PAb92e + PHF-1	Increase (346%)
β-amyloid				
ELISA	39	Aβ 1–42	BAN-50 + BC-05	Decrease (mean ≈ 50%)[17]
ELISA	41	Aβ 1–42	21F12 + 3D6	Decrease (mean ≈ 50%)[17]
ELISA	35	Aβ X-42	266 + PAb 277-2	Decrease (mean ≈ 50%)[17]
ELISA	39	Aβ X-42	BNT-77 + BC-05	Decrease (mean ≈ 50%)[17]
ELISA	46	Aβ X-42	WO-2 + G2-11	Increase (161%)
ELISA	42	Aβ X-42	6E10 + PAb164	Decrease (32%)
ELISA	43	Aβ X-42	4G8 + PAb44-344	Decrease (37%)
Western blot	44	Aβ X-42	G2-11	Decrease (43%)
Immunoprecipitation + Western blot	45	Aβ X-42	G2-11	Decrease (39%)
Urea SDS-PAGE + Western blot	47	Aβ 1-42	n.a.	Decrease (50%)
SELDI-TOF	48	Aβ 1-42	n.a.	Decrease (71%)

Ref. = reference number; PAb = polyclonal antibody; MAb = monoclonal antibody; Thr = threonine; Ser = serine; n.a. = not applicable; SELDI-TOF = surface-enhanced laser desorption ionization time-of-flight masspectrometry.

els are normal or only mildly increased in CJD despite a very marked increase in T-tau.[26] These data suggest that P-tau in CSF is not simply a marker for neuronal degeneration or damage but that it specifically reflects the phosphorylation state of tau and thus possibly the formation of tangles in AD brain.

β-amyloid

The main protein component of plaques is β-amyloid (Aβ),[27] which is generated by proteolytic cleavage of its precursor, the amyloid precursor protein (APP).[28,29]

Total Aβ. After it was found that Aβ is generated as a soluble protein during normal cellular metabolism and is secreted to CSF,[30] studies examining CSF Aβ as a candidate biomarker for AD were published. However, these initial reports on Aβ in CSF used ELISA methods for "total Aβ" that did not discriminate between different Aβ isoforms. Although some studies found a slight decrease in the CSF level of total Aβ in AD,[31–33] there was a large overlap between AD patients and controls, and in other studies no change in CSF total Aβ in AD was found in AD.[34–36]

Aβ42. There are several both N-terminally and C-terminally truncated forms of Aβ. The two major C-terminal variants of Aβ consist of a shorter form ending at Val-40 (Aβ40), and a longer form ending at Ala-42 (Aβ42). Aβ42 was found to aggregate more rapidly than Aβ40,[37] and to be the initial and predominating form of Aβ deposited in diffuse plaques.[38–40] These data made it logical to set focus on immunoassays specific for Aβ42.

As shown in Table 1, 11 different methods[35,39,41–48] have been developed for quantification of Aβ42 in CSF. A moderate to marked decrease in CSF Aβ42 in AD to about 50% of control levels has been found using the majority of these methods (Table 1).[35,39,41–45,47,48] An increase in CSF Aβ42 in AD was found in one study.[46] This finding may be attributable to methodological differences (e.g., assay specificity for aggregated or truncated Aβ variants). Alternatively, it may be due to differences in patient and control materials; in that study, increased CSF-Aβ42 was also found in depression,[46] whereas other studies have found normal CSF Aβ42 levels in depression.[49,50]

The reduced CSF level of Aβ42 in AD is often hypothesized to be caused by deposition of Aβ42 in plaques, with lower levels diffusing to CSF. However, some studies have also found a marked reduction in CSF

TABLE 2. *Performance of CSF Total Tau as a Biomarker for Alzheimer's Disease*

Study Number	Number of AD Cases	AD Sensitivity	Number of Controls	Controls Specificity	Comment	Ref.
1	70	100	19	100		62
2	44	84	31	97		14
3	43	95	18	94	Community-based study	63
4	69	89	17	100		64
5	93	40	41	100	Multicenter study	56
6	40	89	36	97		65
7	163	66	65	83	Multicenter study	66
8	150	79	100	70	Multicenter study	67
9	81	90	15	67		41
10	407	93	93	86	Community-based study	20
11	60	79	32	82		50
12	54	87	15	93		68
13	47	77	12	92		69
14	41	85	17	95		70
15	80	81.3	21	91		71
16	52	79	56	100		24
17	74	95	40	98		72
18	366	59	316	97	Multicenter study	73
19	49	88	49	96	3-year follow-up	74
20	39	72	12	93		75
Total	2022	81.4	1005	91.5		

Ref. = reference number.

Aβ42 in disorders without β-amyloid plaques, such as CJD,[51] amyotrophic lateral sclerosis,[52] and multiple system atrophy.[53] These findings make a direct correlation questionable between low CSF Aβ42 and deposition of Aβ in plaque. In contrast, a recent autopsy study found strong correlations between low Aβ42 in ventricular CSF and high numbers of plaques in the neocortex and hippocampus,[54] suggesting that the reduction in CSF Aβ42 in AD may at least partly be due to a sequestration of β-amyloid in plaques.

Aβ40. There is no change in CSF Aβ40 in AD.[42,55–58] As a consequence, a marked decrease in the ratio of Aβ42/Aβ40 (or increase in the ratio of Aβ40/Aβ42) in CSF has been found in AD in several papers, which is more marked than the reduction in CSF Aβ42.[56–58] Future studies are needed to determine if the CSF Aβ42/Aβ40 ratio has a larger diagnostic potential than CSF Aβ42 alone.

Other Aβ species. Using mass spectrometry, it has been found that there is a heterogeneous set of Aβ peptides in CSF.[59] Also using urea-based sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblot,[47] it is possible to separate several C-terminally truncated Aβ peptides in CSF, including A$\beta_{1\text{-}37}$, A$\beta_{1\text{-}38}$, A$\beta_{1\text{-}39}$, A$\beta_{1\text{-}40}$, and A$\beta_{1\text{-}42}$. A new finding is that the second most abundant Aβ peptide is A$\beta_{1\text{-}38}$, which is more abundant than A$\beta_{1\text{-}42}$.[47] In AD, increased CSF levels of both A$\beta_{1\text{-}40}$ and A$\beta_{1\text{-}38}$ were found, along with a decrease in A$\beta_{1\text{-}42}$.[47] Similar data has been found using surface-enhanced laser desorption/ionization (SELDI) time-of-flight (TOF) mass spectrometry.[48] Further studies on large patient and control series are needed to determine the diagnostic potential of these Aβ variants.

Using two-dimensional electrophoresis combined with Western blotting and mass spectrometry for characterization of formic acid-extracted insoluble Aβ from brain tissue,[60] it was found that N-terminally truncated Aβ42 variants are found in AD at the earliest stage. Preliminary data from our laboratory show that these N-terminally truncated Aβ42 species are found in CSF and may be of diagnostic use in early AD cases.

DIAGNOSTIC PERFORMANCE FOR TAU AND Aβ AS AD BIOMARKERS IN CSF

The diagnostic performance of T-tau, Aβ42, and P-tau has been extensively studied. In the section below, only studies presenting sensitivity and specificity figures, or in which such figures could be calculated from graphs, are reviewed.

CSF T-tau

There are numerous studies on the diagnostic performance of CSF T-tau.[61] The 20 largest studies[14,20,24,41,50,56,62–75] including more than 2000 AD patients and 1000 controls, evaluating the most commonly used ELISA method for T-tau in CSF,[14] are summarized in Table 2. The mean sensitivity to discriminate AD from nondemented aged individuals has been 81%, at a specificity level of 91% (Table 2).

TABLE 3. *Performance of CSF Phospho-Tau as a Biomarker for Alzheimer's Disease*

Study Number	P-tau Epitope	Number of AD Cases	AD Sensitivity	Number of Controls	Controls Specificity	Comment	Ref.
1	P-Thr181	80	84	40	88		92
2	P-Thr181	41	44	17	95		70
3	P-Thr181	19	58	17	94		52
4	P-Thr181	108	85	23	91	Two-center study	93
5	P-Thr181	51	84	31	84		94
6	P-Thr181	42	88	43	100		26
7	P-Thr181	18	89	13	85		95
8	P-Ser199	36	94	20	80	"Non-AD" controls	22
9	P-Ser199	236	85	95	n.g.	Multicenter	86
10	P-Ser199	108	85	23	82	Two-center study	93
11	P-Thr231	27	85	31	97	"Non-AD" controls	23
12	P-Thr231	82	100	21	91		71
13	P-Thr231	108	85	23	96	Two-center study	93
14	P-Thr181 + P-Thr231	40	88	31	97		14
15	P-Thr231 + P-Ser235	36	53	20	100	"Non-AD" controls	22
16	P-Ser396+404	52	94	56	89	Cut-off P-tau > 100 pg/mL	24
Total		1084	81.3	504	91.2		

n.g. = not given.

Most of these studies are cross-sectional. There is one large longitudinal study enrolling more than 400 consecutive AD cases and finding that the sensitivity of CSF T-tau to identify AD was 93%.[20] Furthermore, few studies have examined CSF T-tau in pathologically confirmed AD cases. However, in a large study on 131 AD cases in which the diagnosis was confirmed at autopsy in 31 cases, the performance of CSF T-tau and Aβ42 was similar in both groups.[43]

Since CSF T-tau reflects neuronal and axonal degeneration, it is not specific for AD; high CSF T-tau levels will be found in all CNS disorders with neuronal degeneration or damage. The highest levels are found in acute stroke[18] and in CJD.[19] Importantly, several studies have consistently found a very marked increase in CSF T-tau in CJD.[19,26,51,76–78] The mean level of CSF T-tau in CJD is 10-50 times higher than in controls, resulting in a sensitivity close to 100% and also a specificity above 90% against other dementias such as AD.[19,26,51,76–78] This diagnostic performance is similar to that of CSF 14-3-3 protein.[77] However, since the method for 14-3-3 protein in CSF is based on SDS-PAGE and Western blot and thus gives results in the form of "14-3-3 positive" or "14-3-3 negative,"[79] the quantitative ELISA methods for T-tau may be preferable in the clinical laboratory.

In vascular dementia (VAD), high CSF T-tau has been found in some[14,20,50] but not in all[15,57,69] studies. These discrepant findings may depend on the patient characteristics and diagnostic criteria used. High T-tau in clinically diagnosed VAD cases may be caused by concomitant AD pathology in VAD cases, which is a frequent finding at autopsy[80,81] but difficult to identify clinically. A study with longitudinal magnetic resonance tomography (MRT) scans in VAD cases[20] also found that VAD cases with progressive white-matter changes have normal CSF T-tau. Furthermore, in patients with nonacute cerebrovascular disease without dementia, CSF T-tau is normal.[82,83] One way to interpret these findings is that a high CSF-tau level in a patient with clinical and brain imaging findings indicative of VAD suggests that this patient has mixed (AD/VAD) dementia.

Most studies have found normal to mildly increased CSF T-tau levels in other dementias, such as frontotemporal dementia (FTD)[14,15,21,50,52,69,70,73,82–86] and Lewy body dementia (LBD).[85–90]

Other than in aged nondemented individuals, normal CSF T-tau is found in depression, alcoholic dementia, and in chronic neurological disorders such as Parkinson's disease (PD) and progressive supranuclear palsy.[14,20,50,52,57,63,70,85,88,89,91] Thus, CSF T-tau has a clear clinical diagnostic value in the differentiation between AD and these important and often difficult differential diagnoses.

CSF P-tau

There are 13 different papers[14,22–24,26,52,70,71,86,92–95] on the diagnostic performance of the different ELISA methods for P-tau in CSF, including more than 1000 AD patients and 500 controls (Table 3). The mean sensitivity to discriminate AD from nondemented aged individuals has been 81%, at a specificity level of 91% (Table 3).

In these relatively few papers, there has been a relatively large variation in sensitivity and specificity figures of P-tau between studies using ELISA methods specific

TABLE 4. *Performance of CSF Aβ42 as a Biomarker for Alzheimer's Disease*

Study Number	Number of AD Cases	AD Sensitivity	Number of Controls	Controls Specificity	Comment	Ref.
1	81	81	51	80		41
2	53	92	21	95	Community-based sample	49
3	150	78	100	81	Multicenter study	67
4	24	96	19	95		89
5	14	93	20	95	Numbers from graph	51
6	60	93	32	85		50
7	38	76	47	85		76
8	54	89	15	67	Numbers from graph	68
9	27	78	49	100		97
10	9	55	17	94		98
11	20	100	20	95		99
12	74	89	40	95		72
13	19	100	17	94		52
14	49	82	49	80	3-year follow-up	74
15	51	78	31	90		94
16	18	89	13	85	Moderate decrease; median	95
Total	660	85.9	541	88.5		

for different phosphorylated tau epitopes (Table 3). Therefore, a study was performed to directly compare the diagnostic performance of P-Tau_{181}, P-Tau_{199}, and P-Tau_{231} in the same patient material.[93] Among cases with AD, DLB, FTD, VAD, and a group with other neurological disorders, all three P-tau assays performed equally well in the discrimination of AD from other disorders and nondemented controls.[93] Minor differences found between the assays were that group separation was maximized between AD and FTD using P-Tau_{231} and between AD and DLB using P-Tau_{181}.[93] Thus, the sensitivity for AD seems equal, whereas minor differences in the phosphorylation of specific tau epitopes between dementia disorders may be reflected in the CSF level of the corresponding P-tau variant.

Interestingly, the specificity of CSF P-tau to differentiate AD from other dementias seems to be higher than for T-tau and Aβ42. Normal CSF levels of P-tau are found in psychiatric disorders such as depression,[14,96] in chronic neurological disorders such as amyotrophic lateral sclerosis and PD,[14,52,70] and also in other most cases with other dementia disorders such as VAD, FTD, and LBD.[21,52,70,71,86,92,93] Furthermore, although there is a very marked increase in CSF T-tau in CJD, most patients with CJD have normal or only mildly elevated CSF P-tau.[26] In a large set of patients with CJD cases and other dementias, the ratio of P-tau to T-tau in CSF was found to discriminate CJD from other neurodegenerative disorders without any overlap.[26]

Aβ42

There are 16 studies[41,49–52,67,68,72,74,76,89,94,95,97–99] on the diagnostic performance of the most commonly used ELISA method[41] for Aβ42 in CSF (Table 4). These studies include more than 650 AD patients and 500 controls (Table 4). The mean sensitivity to discriminate between AD and normal aging is 86%, at a specificity level of 89% (Table 4).

Other than in nondemented aged individuals, normal CSF Aβ42 is found in psychiatric disorders such as depression, and in chronic neurological disorders such as PD, and progressive supranuclear palsy.[50,52,53,89] Thus, CSF Aβ42 helps in the clinical differentiation between AD and these important and often difficult differential diagnoses.

However, data on the performance of CSF Aβ42 in the discrimination between AD and other dementias and neurological disorders are relatively limited. A mild to moderate decrease in CSF Aβ42 is found in a percentage of patients with FTD and VAD.[50,52,67,72]

Combination of CSF biomarkers for AD

There are several studies in which the diagnostic potential of the combination of CSF T-tau and Aβ42 have been evaluated. For the most commonly used ELISA methods for T-tau[14] and Aβ42,[41] sensitivity and specificity figures are available from 12 studies (Table 5).[41,43,50,52,67,72,74,97,99–102] The sensitivity and specificity for the combination of CSF T-tau and Ab42 have been slightly higher (89% and 90%, respectively) than for T-tau (81% and 91%, respectively) or Ab42 (86% and 89%, respectively) alone (Tables 2, 4, and 5).

Other combinations of CSF markers have also resulted in slightly better diagnostic performance than the use of single markers. In a study on the combination of CSF P-Tau_{181} and Aβ42, the sensitivity was 86% at a specificity of 97%,[94] and in another study the combination of

TABLE 5. *Performance of the Combination of CSF Total Tau and Aβ42 as Biomarkers for Alzheimer's Disease*

Study Number	Number of AD Cases	AD Sensitivity	Number of Controls	Controls Specificity	Method for Discrimination	Comment	Ref.
1	105	94	n.a.	n.a.	Discrimination line	Community-based patient sample	101
2	16	88	15	80	Discrimination line		100
3	35	92	19	90	PCA		102
4	131	92	72	82	Quadrant with cut-offs	Includes 31 autopsy-proven AD	43
5	150	85	100	87	Discrimination line	Multicenter study	67
6	60	73	32	84	Quadrant with cut-offs		50
7	27	85	49	100	Discrimination line		97
8	19	100	17	100	Quadrant with cut-offs		52
9	49	96	49	86	T-tau/Aβ42 ratio	3-year follow-up	74
10	74	92	40	95	Discrimination line		72
11	81	74	15	93	Quadrant with cut-offs		41
12	20	100	20	95	Quadrant with cut-offs		99
Total	767	89.3	428	90.2			

PCA = principal component analysis; n.a. = not applicable.

CSF T-tau and P-tau$_{396/404}$ resulted in a sensitivity of 96% at a specificity of 100%.[24]

CSF MARKERS IN EARLY AD AND MCI

Early AD

The performance of CSF T-tau, P-tau, and Aβ42 in early AD cases with Mini-Mental State Examination (MMSE) scores above 23-25 has been examined in some studies. Also in this early phase of the disease, the sensitivity figures have been similar to those found in more advanced AD cases.[20,49,65,67,103–105]

MCI

Several studies have also evaluated the performance of CSF markers in MCI cases that developed AD during a clinical follow-up period of 1-2 years, finding sensitivity figures similar to those found in AD cases with clinical dementia.[68,100,101,106–110]

Some studies have evaluated the performance of CSF biomarkers to identify MCI cases that later will progress to AD with dementia, i.e., have "preclinical" or incipient AD. In the first study using this approach, high CSF T-tau was found to discriminate MCI patients that later progressed to AD from those that did not progress, with 90% sensitivity and 100% specificity.[111] Another study found high CSF T-tau and low CSF Aβ42 in 90% of MCI cases that later progressed to AD with dementia, as compared with 10% of stable MCI cases.[109] Similarly, a marked increase in CSF P-tau was found in MCI cases that at follow-up had progressed to AD compared with stable MCI cases.[112] A recent population-based study also found that reduced CSF Aβ42 is present in asymptomatic elderly who developed dementia during a 3-year follow-up period.[113]

The fact that only ~15% of MCI cases progress to AD each year[6] makes a very extensive follow-up period (more than 5 years) needed to ascertain which MCI patients will not develop dementia, i.e., have stable MCI. This may introduce a risk that the specificity figures in these studies thus far are too low. However, although further longitudinal studies clearly are needed, data from the studies on early AD and MCI suggest that these CSF markers may be of clinical value to differentiate MCI cases that will progress to AD from benign MCI cases.

Use of AD Biomarkers in CSF in Clinical Practice

The diagnostic performance of AD biomarkers has been evaluated in clinical practice in two studies.[20,101] In these studies, the CSF markers have been evaluated on prospective patient samples from clinical practice, and ELISA assays have been run each week in clinical neurochemical routine. The diagnostic performance of CSF T-tau[20] and the combination of CSF T-tau and Aβ42[101] has been similar to that found in other studies, with a high ability to differentiate AD from normal aging, depression, and PD, but lower specificity against other dementias like VAD and LBD.

A summary of the diagnostic performance of T-tau, P-tau, and Aβ42 is given in Table 6. Details on the performance of these CSF markers in the separation between AD and nondemented aged individuals has been published previously.[61] The diagnostic performance of the CSF markers seems to be highest in the differentiation between AD and several important differential diagnoses, including normal aging, depression, alcohol dementia, and PD (Table 6). Another useful clinical application is the identification of CJD in cases with rapidly progressive dementia, in which the combination of very marked increased CSF T-tau and normal or mildly increased P-tau has high diagnostic value. Lastly, these CSF markers may be useful in identifying mixed AD/VAD dementia.

TABLE 6. *Summary of the Specificity of CSF Markers for Alzheimer's Disease*

Disorder	Total Tau	Phospho-Tau	Aβ42
Alzheimer's disease	Moderate to marked increase	Moderate to marked increase	Moderate to marked decrease
Normal aging	Normal	Normal	Normal
Depression	Normal	Normal	Normal
Alcohol dementia	Normal	Normal	Normal
Parkinson's disease	Normal	Normal	Normal
Creutzfeldt-Jakob disease	Very marked increase	Normal, but some cases with mild increase	Normal to marked decrease
Frontotemporal dementia	Normal to mild increase	Normal	Mild to moderate decrease
Lewy body dementia	Normal to mild increase	Normal to mild increase	Moderate decrease
Vascular dementia	Inconsistent data, some studies with normal and some with increased levels	Normal	Mild to moderate decrease
Acute stroke	Mild to very marked increase, depending on the infarct size	Normal	Normal
Non-acute CVD without dementia	Normal	N.E.	Normal

CVD = cerebrovascular disease; N.E. = not examined.

The lower specificity against other dementias, such as LBD and FTD, may hamper the clinical utility of these CSF markers. However, in the clinic, dementias with differing history, symptoms, and findings on brain imaging (e.g., FTD, VAD) can often be identified by means of the medical history, clinical examination, and auxiliary investigations (e.g., blood tests, SPECT, and computerized tomography or MRT). A common "final question" is whether a patient with MCI will progress to AD with dementia or not. Although more studies are needed, these CSF markers seem to be of clinical use in the identification of incipient AD in MCI cases. Early diagnosis of AD is not only of importance to be able to initiate symptomatic treatment with AChE inhibitors, but will be the basis for initiation of treatment with drugs aimed at slowing down or arresting the degenerative process, such as γ-secretase inhibitors, if these prove to affect AD pathology and to have a clinical effect.

Candidate CSF Protein Biomarkers for AD

In the following section, some promising candidate CSF protein biomarkers that have been evaluated in a few studies, often by single research groups, are reviewed.

Ubiquitin

Ubiquitin is a small (8.7 kDa) protein involved in the ATP-dependent degradation of proteins, in which it is covalently conjugated to lysine residues of target proteins, for which it serves as a signal for degradation of the protein by proteases.[114–116] Ubiquitin-conjugated proteins may also be stable and exist in the cell indefinitely.[116] In AD brain, the paired helical filaments (PHF) in tangles are ubiquitinated.[117,118] The level of ubiquitin in AD cerebral cortex is increased several-fold and correlates with the amount of neurofibrillary changes.[119]

Using an ELISA method based on the monoclonal antibody 5-25, raised against PHF from AD brains,[120] Mehta and co-workers reported in 1985 high levels of "paired helical filaments antigen" in CSF in AD.[121] This antibody is specific for residues 64-76 of ubiquitin[122] and recognizes this epitope on ubiquitin-conjugated proteins, including PHF in tangles.[117] This finding has been replicated in subsequent studies.[123] There is also a correlation between ubiquitin in CSF and in brain tissue.[124] Using an ELISA method based on the monoclonal antibody 1510, which is specific for free nonconjugated ubiquitin, an increase in CSF–ubiquitin was found in AD.[125]

Thus, there seems to be an increase in both free ubiquitin and of ubiquitin-conjugated proteins in CSF in AD. Further studies are on the way on the diagnostic potential of ubiquitin in CSF as a protein biomarker for AD.

NF proteins

NF proteins are structural components in the neuronal axons that are important for axonal structure and transport. Neurofilaments are composed of three subunits based on the molecular weight, termed high (NF-H), medium (NF-M), and light (NF-L).

The light subunit of the NF protein (NF-L) is mainly localized in the large myelinated axons.[126] The CSF level of NF-L correlates with the degree of white matter changes in the brain.[127] CSF-NFL is therefore suggested to be a biomarker for degeneration of large myelinated axons. Although increased CSF NF-L levels are found in AD,[102,127–129] CSF NF-L is even more increased in FTD and in VAD.[127–129] Also using another ELISA method, a

more pronounced increase in CSF NF-L was found in VAD than in AD.[130] These findings suggest that CSF NF-L may be valuable in the differentiation between AD and VAD/FTD.

NF proteins are phosphorylated, which regulates their function in axonal transport and their tendency for polymerization.[131] Using ELISA methods based on antibodies reacting with phosphorylated NF, a marked increase in phospho-NF-H/M was found in AD compared with VAD and other neurological disorders.[130] These promising findings call for further studies on the diagnostic potential of different forms of NF proteins in CSF.

GAP43 (neuromodulin)

GAP43, or neuromodulin, is a protein localized in the presynaptic terminals and axons of cortical neurons.[132–134] In AD brain, GAP43 is found in the dystrophic neurites in plaques.[135,136] GAP43 protein levels are decreased in the frontal cortex and the hippocampus in AD.[137–139]

GAP43 is secreted to the CSF at which it serves as a potential marker for synaptic degeneration or plasticity.[139] In AD, increased CSF levels of GAP43 are found, although CSF GAP43 is normal in FTD and in PD.[50,69,140] There is a clear correlation between the CSF levels of T-tau and GAP[50,69] and some data suggest that the ratio of CSF GAP43/T-tau may be of diagnostic importance.[50] However, further studies are needed on the potential of GAP43 as a diagnostic marker for AD.

NTP and AD7c protein

NTP was identified a brain protein cross-reacting with antibodies against pancreatic thread protein (PTP).[141] Both brain NTP immunoreactivity[141,142] and mRNA levels[143] are increased in AD.

In the first study on NTP in CSF, a marked increase was found in AD.[144] Based on the knowledge that the antibodies used by de la Monte and co-workers[144] were generated against human PTP[145] and that the concentration of PTP is about one million times higher in pancreatic fluid than that of NTP in CSF,[145] a study was performed to examine the origin of NTP in CSF.[146] The level of NTP was about 40 times higher in serum than in CSF and highly significant correlations were found between serum–NTP and CSF–NTP and between the CSF/serum albumin ratio (as a marker for the blood-brain barrier function) and CSF–NTP.[146] These findings suggested that NTP in CSF comes from the serum by passage over the blood-brain barrier. Indeed, increased CSF-NTP was only found in AD and VAD cases with signs of blood-brain barrier damage.[146]

These disappointing findings initiated a search for a brain-specific NTP transcript. Screening a human brain cDNA library, again with the same antibodies against PTP,[144,145] a cDNA named AD7c was isolated.[147] Despite the nucleotide sequence showing no homology to PTP, this cDNA was named AD7c-NTP.[147]

Using a new ELISA method based on antibodies generated against recombinant AD7c-NTP protein, a marked increase in CSF-AD7c-NTP was found in AD compared with controls and other neurological disorders including PD and multiple sclerosis.[147] Although initial studies reported a good separation between AD and control groups,[147,148] later studies showed a less pronounced increase, with a marked overlap between AD and both controls and patients with PD.[88] Furthermore, an evidence-based evaluation of this biomarker is rendered because, to my knowledge, the AD7c-NTP ELISA method is not commercially available; CSF samples have to be sent for analyses to the company that developed the method. Scientific studies performed independently by the research community are thus still lacking.

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