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Amyloid-beta and tau proteins as biochemical markers of Alzheimer's disease

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Review

Abstract. With the development of new therapeutic strategies, and the concept of mild cognitive impairment (MCI) as an early stage of Alzheimer's disease (AD), there is an increasing need for an early and accurate diagnosis of sporadic AD. Therefore, biological markers allowing a positive diagnosis early in the course of the disease are highly desirable. The most extensively evaluated markers of sporadic AD are amyloid-β proteins and levels of both total and phosphorylated microtubule-associated protein tau. In this study, we review the currently available data on the aforementioned markers assessed in the cerebrospinal fluid or plasma, alone and in combinations, focusing on their clinical applicability including sensitivity in the diagnosis of AD and mild cognitive impairment, specificity in discriminating AD from other dementias and correlations with the disease progression and apolipoprotein E genotype. We also analyze advantages and potential drawbacks of using biomarkers in the laboratory diagnosis of AD.

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INTRODUCTION

The prevalence of AD in the population of developed countries has been estimated at 5-11% of those aged 65 years or over, rising to as much as 50% among those aged over 85 years (Evans et al. 1989). AD is thus the most frequent cause of dementia. With the expectation of the development of new therapeutic strategies to treat AD, there is an increasing need for an early diagnosis. Diagnosis of sporadic AD is based on clinical exclusion criteria (McKhann et al. 1984), but the necessary diagnostic work-up is time-consuming, expensive, and, at best, results in a diagnosis of "probable AD" as defined in either DSM-IV or ICD-10 sets of criteria. In reference centres where skilled physicians are available, a diagnostic accuracy of maximally 65-90% is obtained (Andreasen et al. 1999b). Most studies evaluating accuracy rates are based on follow-up periods of several years so that a much lower diagnostic accuracy can be expected in the earliest stages of the disease. Diagnosis only becomes definite following brain biopsy or *post-mortem* neuropathological examination of the brain. Therefore, identification and simple detection of a disease-specific marker that would permit diagnosis of AD at an early stage of the disease is highly desirable.

According to the Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group (1998), an ideal diagnostic marker should also enable predictive testing, monitoring of disease progression and determination of the effects of treatment using novel therapeutic compounds. A diagnostic marker of AD should reflect a central pathogenic process of the disorder, like degeneration of neurons and synapses or the development of typical lesions as neuritic plaques and neurofibrillary tangles (NFT). Markers should be validated in neuropathologically-confirmed AD cases and should have a sensitivity of at least 80% for detecting AD and a specificity of at least 80% for distinguishing other dementias. Moreover, the biological marker should be present in body fluids that are easily accessible like urine, blood or cerebrospinal fluid (CSF). As almost all AD patients suffer from the sporadic form and diagnosis is often difficult, especially in early stages of the disease when symptoms can be vague, our review gives an overview of the most extensively evaluated markers that can be helpful for diagnosing sporadic AD – microtubule-associated protein tau (MAP-tau) and amyloid-β protein (Aβ).

PATHOBIOLOGY OF AD – THE ROLE OF Aβ AND MAP-TAU

(see also: Gómez-Ramos et al. 2004 and Żekanowski et al. 2004 in this issue)

Metabolism of Aβ

Aβ is a product of a proteolytic cleavage of a larger precursor protein – amyloid-β protein precursor (APP). The *APP* gene is located on chromosome 21 in humans (Goldgaber et al. 1987). The Aβ domain of APP is partly embedded in the cellular membrane (Selkoe 1994). Aβ is metabolized along two pathways. In the first, *APP* is cleaved within the Aβ domain by a protease referred to as α-secretase (Seubert et al. 1993). As a result, α-secretase-cleaved soluble APP (α-sAPP) is released, along with peptide C83 comprising an 83 amino acid residue long C-terminal endodomain. Importantly, cleavage of APP by α-secretase precludes the generation of Aβ, which is why it is sometimes called "the non-amyloidogenic pathway". The C83 C-terminal fragment (CTF) of APP is then subject to cleavage by γ-secretase releasing a shorter peptide called p3, with a putative activity of a transcription regulator analogous to the Notch signaling pathway (Dewachter and Van Leuwen 2002). In the second pathway, APP is cleaved at the N-terminus of the Aβ domain by another protease, β-secretase (Vassar et al. 1999). This cleavage results in the release of β-secretase-cleaved soluble APP (β-sAPP) and a 99 amino acid CTF of APP (C93). In the second step, C93 is cleaved by γ-secretase to form free Aβ. Due to the pivotal role Aβ plays in the formation of neuritic plaques, the β-secretase metabolic route is often called "the amyloidogenic pathway".

According to the popular, although still controversial "amyloid cascade hypothesis", mismetabolism of APP and Aβ, resulting in subsequent aggregation of Aβ into highly insoluble fibrils which are the main constituents of neuritic plaques, initiates a complex cascade of biochemical and cellular changes that lead to neurodegeneration that culminates in cognitive impairment (Joachim and Selkoe 1992). Extracellular neuritic plaques together with intracellular NFTs are major neuropathological hallmarks of AD. Of the different forms of Aβ, the one containing 42 amino acids ($A\beta_{42}$) aggregates more rapidly and predominates in amyloid plaques, although the 40 amino acid isoform ($A\beta_{40}$) is present in higher concentrations in the brain (Iwatsubo et al. 1994). The deposition of Aβ is a spe-

cific, early event in the development of AD and it precedes NFTs and clinical dementia. The highly ordered, insoluble fibrillar Aβ is exceedingly cytotoxic, acting as a proinflammatory agent, inducing oxidative stress, and contributing to neuronal loss (Butterfield 2002, Weldon et al. 1998). Recent data suggest that soluble, pre-fibrillar aggregates of Aβ, Aβ oligomers, may be even more effective in mediating neuronal injury (Walsh et al. 2002). $A\beta_{42}$ is more neurotoxic than $A\beta_{40}$ and is more likely to generate H_2O_2 than $A\beta_{40}$ (Huang et al. 1999, Pogocki 2003), which may be central to its observed cytotoxicity. Increased levels of $A\beta_{42}$ are suggested to be an initiating factor for both sporadic and familial forms of AD (Scheuner et al. 1996).

Metabolism of MAP-tau

MAP-tau is a normal human brain phosphoprotein located in axons where it binds to microtubules, thus promoting microtubule assembly and stability. In AD brain, due to abnormal phosphorylation and conformational changes, MAP-tau tends to aggregate, with the formation of paired helical filaments (PHFs) (Grundke-Iqbal et al. 1986). PHFs are the main constituent of larger pathological structures known as NFTs. As a result of NFT formation, MAP-tau is no longer available for cytoskeletal stabilization. The disorganization of the neuronal skeleton contributes to neuronal malfunction, neuronal cell death, and eventually dementia (Fillit and Refolo 2002). Hyperphosphorylation of MAP-tau is believed to be one of the mechanisms that triggers NFT formation (Brion et al. 2001). Two enzymes preferentially implicated in MAP-tau phosphorylation are the glycogen synthase kinase 3 (GSK-3) and cyclin-dependent kinase 5 (cdk5) (Lau et al. 2002).

Aβ AS A BIOCHEMICAL MARKER OF AD

Aβ is generated continuously as a soluble protein resulting from the normal constitutive APP metabolism (Haass et al. 1992) and is secreted into the extracellular space allowing its detection in the CSF and plasma (Seubert et al. 2002).

Results of early studies that could not discriminate between $A\beta_{42}$ and $A\beta_{40}$ provided no clear data on the CSF Aβ levels in AD patients, revealing either slight increase (Nakamura et al. 1994), no change (Southwick et al. 1996) or marginal decrease with a substantial overlap between the AD and control groups (Pirttila et al. 1998). For the first time, a selective reduction of CSF $A\beta_{42}$ was shown by Motter et al. (1995). The statistically significant decrease of $A\beta_{42}$ concentrations in the CSF of AD patients was later confirmed in several studies (summarized in Table I). An irrefutable explanation of this finding does not exist. There are several hypotheses, the most plausible of which comprise an increased deposition of Aβ within plaques resulting in the lower levels remaining to diffuse into the CSF; decreased clearance of Aβ from the brain; and disturbance in the metabolism of APP and Aβ leading to a neuronal dysfunction which, brings on the CSF $A\beta_{42}$ levels reduction. The first hypothesis has been challenged since the finding of reduced CSF $A\beta_{42}$ in Lewy body dementia (DLB), in a proportion of patients with frontotemporal dementia (FTD), and in vascular dementia (VaD) (Andreasen et al. 2001, Gomez-Tortosa et al. 2003, Hulstaert et al. 1999, Kanemaru et al. 2000, Nagga et al. 2002, Riemenschneider et al. 2002c, Sjogren et al. 2000a), and Creutzfeldt-Jakob disease (CJD), where typically there are no Aβ positive amyloid plaques (Kapaki et al. 2001, Otto et al. 2000). Again, the stability of $A\beta_{42}$ concentrations in the CSF after acute stroke (Hesse et al. 2000) suggests that $A\beta_{42}$, unlike CSF MAP-tau, is not a direct marker of neuronal damage. Additionally, methodological difficulties may confound measurements, specifically the inability to quantify large Aβ aggregates found in the CSF in AD in the same way as Aβ monomers (Pitschke et al. 1998).

Only one study found increased CSF $A\beta_{42}$ levels in AD patients as well as in depressed individuals (Jensen et al. 1999). Due to methodological differences, these results have been discordant with all the other available data.

A decrease of the CSF $A\beta_{42}$ has also been observed in patients with mild cognitive impairment (MCI), a state believed by some researchers to be an early stage of Alzheimer's dementia (Andreasen et al. 1999c, 2003, Riemenschneider et al. 2002a). Moreover, the value of the low CSF $A\beta_{42}$ as a tool for both separating MCI patients from cognitively healthy controls and for predicting conversion from MCI to AD has also been relatively high (70-80%), suggesting that low levels of the CSF $A\beta_{42}$ might be useful in the earliest stages of the disease, i.e., before the diagnosis of clinical dementia is established. This consistently replicated conclusion is, however, attenuated by another paper that reports comparable CSF $A\beta_{42}$ levels in MCI subjects and controls, with a decline after progression from MCI to AD (Maruyama et al. 2001).

Table I

Summary of 29 studies comparing cerebrospinal fluid (CSF) levels of $A\beta_{42}$ in patients with Alzheimer's disease (AD) and controls

Study	AD	AD $A\beta_{42}$	C	C $A\beta_{42}$	Sn	Sp	*P*	Eff
Motter et al. 1995	37	383 (76)	20	632 (156)	100	80	<0.001	2.26
Ida et al. 1996	39		11		64	91		
Tamaoka et al. 1997	20	738 (374)	34	1450 (743)			<0.001	1.12
Galasko et al. 1998	82	833 (379)	60	1485 (473)	78	83	<0.001	1.55
Kanai et al. 1998	93	495 (164)	54	1090 (405)	94	47	<0.001	2.27
Shoji et al. 1998	55		34					
Vanderstichele et al. 1998	81		51		81	80		
Andreasen et al. 1999a	53	709 (304)	21	1678 (436)	92	90	<0.001	2.80
Hulstaert et al. 1999	150	522 (197)	100	874 (293)	78	81	0.003	1.68
Jensen et al. 1999	80	536 (284)	24	333 (135)	n.g.	n.g.	<0.001	- 0.79
Fukuyama et al. 2000	23	331 (188)	13	626 (909)			0.27	0.56
Kanemaru et al. 2000	24	284 (92)	19	714 (188)	96	95	<0.001	3.02
Mehta et al. 2000	36	60 (78)	29	147 (188)	n.g.	n.g.	<0.001	0.63
Otto et al. 2000	14	361 (153)	20	903 (163)	93	95	<0.001	3.41
Riemenschneider et al. 2000	75	455 (210)	30	916 (160)			<0.001	2.35
Shoji et al. 2000	157		88		81	87		
Sjogren et al. 2000a	60	381 (127)	32	772 (244)	93	85	<0.001	2.22
Tapiola et al. 2000a	80		39		69	85		
Andreasen et al. 2001	105	523 (180)	18	897 (242)	Com	Com	<0.001	1.97
Csernansky et al. 2002	32	1777 (1055)	10	2400 (1030)	n.g.	n.g.	0.11	0.60
Mulder et al. 2002	20	480 (104)	20	1040 (213)	100	95	<0.001	3.34
Nagga et al. 2002	50	431 <239-997>	27	800 <401-1079>	n.g.	n.g.	<0.001	n.g.
Riemenschneider et al. 2002c*	74	394 {326-504}	40	1076 {941-1231}	89	95	<0.001	n.g.
Sjogren et al. 2002	19	411 (99)	17	853 (161)			<0.001	3.35
Gomez-Tortosa et al. 2003	33	348 (309)	46	462 (352)	69	44	0.25	0.34
Kapaki et al. 2003a *	49	362 {309-445}	49	738 {506-860}	82	80	<0.001	n.g.
Maddalena et al. 2003	51	420 (190)	31	730 (220)	78	90	<0.001	1.54
Rosso et al. 2003*	18	280 {222-312}	13	547 {421-625}	83	92	<0.001	n.g.
Skoog et al. 2003	12	389 (161)	28	657 (320)	n.g.	n.g.	0.002	0.94
Sunderland et al. 2003	131	183 (121)	72	491 (245)	Com	Com	<0.001	1.76

(AD) number of study participants in the AD group; (AD $A\beta_{42}$) mean CSF $A\beta_{42}$ in the AD group (Standard Deviation) (pg/ml); (C) number of study participants in the control group; (C $A\beta_{42}$) mean CSF $A\beta_{42}$ in the control group (Standard Deviation) (pg/ml); (Sn) calculated sensitivity (%); (Sp) calculated specificity (%); (P) P value; (Eff) effect size; (n.g.) not given/impossible to calculate; (*) median concentration {25-75 percentile}; (**) median concentration <range>; (Com) sensitivity and specificity values only for the combined assessment of $A\beta_{42}$ and total-MAP-tau (Table IV)

In one study, CSF $A\beta_{42}$ levels were examined in elderly non-demented individuals in relation to a later development of dementia (Skoog et al. 2003). It appeared that those in the lower 50th percentile of CSF $A\beta_{42}$ had a higher incidence of dementia than those in the higher 50th percentile, with an odds ratio of 8.2. Furthermore, none of those in the highest 33rd percentile of CSF $A\beta_{42}$ developed dementia.

The correlation of the CSF $A\beta_{42}$ concentrations with the disease severity has yielded controversial results. In the majority of papers, CSF $A\beta_{42}$ levels appeared to be stable for each patient and were not correlated with either severity or rate of dementia progression (Andreasen et al. 1999a, Csernansky et al. 2002, Gomez-Tortosa et al. 2003, Hulstaert et al. 1999, Kanai et al. 1998, Kapaki et al. 2003a, Mehta et al. 2000,

Mulder et al. 2002, Rosso et al. 2003, Sunderland et al. 2003, Tapiola et al. 2000a). In others, such a correlation has been confirmed (Galasko et al. 1998, Samuels et al. 1999, Skoog et al. 2003, Tapiola et al. 2000b), although sometimes very weak. Some investigators have reported such an association only for apolipoprotein E (ApoE) 3 homozygotes (Riemenschneider et al. 2002b). Ine another recent paper (Wahlund and Blennow 2003), the CSF $A\beta_{42}$ levels have been found to correlate with a lower brain volume and enlarged ventricles (as assessed by magnetic resonance imaging) in subjects with AD and progressive MCI. This correlation is unlikely to have been due to a dilution effect in a larger CSF volume, thus suggesting that the CSF $A\beta_{42}$ may reflect the stage of the disease, with decreasing levels in the CSF as the disease progresses. Another controversial issue is the hypothetical correlation between CSF $A\beta_{42}$ levels and *ApoE* genotype. *ApoE* ε4 allele is a major genetic risk factor for sporadic AD, increasing the risk 3-8 times in a dose-dependent manner. Again, the results have been contradictory. Several authors failed to find any direct association between these two factors (Andreasen et al. 1999a, Mehta et al. 2000, Motter et al. 1995, Nagga et al. 2002, Sjogren et al. 2000a, Skoog et al. 2003), while others reported lower concentrations of $A\beta_{42}$ in the CSF of AD patients harboring *ApoE* ε4 allele(s) (Andreasen et al. 2001, Galasko et al. 1998, Hulstaert et al. 1999, Riemenschneider et al. 2000, 2002b, Tapiola et al. 2000a).

The use of a low CSF $A\beta_{42}$ as a diagnostic marker of AD is greatly limited by its poor specificity since decreased CSF $A\beta_{42}$ levels can also be found even in major depression (Hock et al. 1998), not to mention other dementias including DLB, CJD, VaD, and a proportion of FTD cases (Andreasen et al. 2001, Gomez-Tortosa et al. 2003, Hulstaert et al. 1999, Kanemaru et al. 2000, Kapaki et al. 2001, Nagga et al. 2002, Otto et al. 2000, Riemenschneider et al. 2002c, Sjogren et al. 2000a). Moreover, there is a substantial overlap in CSF $A\beta_{42}$ concentrations between AD subjects and controls. Several studies have shown that there is no change in the CSF level of $A\beta_{40}$ in AD (Ida et al. 1996, Kanai et al. 1998, Mehta et al. 2000, Shoji et al. 1998, Tamaoka et al. 1997). Others have reported that a value of CSF Aβ ratio ($A\beta_{40}/A\beta_{42}$) provides a better discrimination between AD patients and controls or other dementias (Kanai et al. 1998, Shoji et al. 1998). $A\beta_{42}$ is physiologically lowered in midlife (30-59 years of age) which may overlap with pathologic decrease in early-onset AD (Shoji 2002). However, even in those studies the sensitivity and specificity of Aβ ratio have been too low to enable its use as a unique marker of AD.

CSF Aβ aggregates have been proposed as a candidate marker for AD (Pitschke et al. 1998). Aβ oligomers have been detected in the CSF of AD patients, but not in controls. Unfortunately, the need for highly specialized equipment will hamper the application of this measurement in clinical practice.

Studies evaluating Aβ levels in plasma are even more scarce and conflicting. In both Down's syndrome (Schupf et al. 2001) (with neuropathological changes virtually identical to these seen in AD) and familial AD (Kosaka et al. 1997, Scheuner et al. 1996), increased plasma $A\beta_{42}$ has been reported. Results of plasma Aβ levels in sporadic AD have been much more contradictory. Both an increase (although with a large overlap with non-AD cases) (Kuo et al. 1999, Matsubara et al. 1999) and lack of changes (Ida et al. 1996, Mehta et al. 2000, Tamaoka et al. 1996, Vanderstichele et al. 2000) in $A\beta_{42}$ plasma levels of AD patients compared to age-matched controls have been reported. In another study, healthy individuals with plasma $A\beta_{42}$ concentrations in the upper two quartiles had a three to four fold increased risk of developing AD compared to subjects from the lowest quartile (Mayeux et al. 1999). However, although the mean values differed, there was an almost complete overlap in plasma $A\beta_{42}$ concentrations between the groups. In another most recent paper age, but not a diagnosis, appeared to be the only variable correlated with plasma $A\beta_{40}$ and $A\beta_{42}$ levels, with higher values in older patients regardless of a diagnostic category (AD, MCI, Parkinson's disease - PD), duration or severity of the disease, or *ApoE* genotype (Fukumoto et al. 2003). As there is evidence that plasma Aβ levels are, at least in part, genetically determined (Ertekin-Taner et al. 2001), the influence of genetic factors other than *ApoE* cannot be ruled out. No correlation between plasma $A\beta_{42}$ and medication use, including statins, estrogen, acetylcholinesterase inhibitors, non-steroid anti-inflammatory drugs, and antioxidants has been reported (Basun et al. 2002, Fukumoto et al. 2003, Tokuda et al. 2001). The above data are concordant with the preliminary results of our longitudinal study on plasma $A\beta_{42}$ (Sobów and Kłoszewska 2003). We have observed similar plasma $A\beta_{42}$ in mid-life and in AD, while in the carefully diagnosed MCI patients of the pure amnestic type (excluding subjects with deficits in multiple cognitive domains or single, other than memory domain) the concentration has been significantly increased as com-

pared to both healthy controls and sporadic AD patients. Whether this elevation is a predictor of future development of AD needs to be established as planned in a longitudinal follow-up. Unfortunately, due to substantial variations in $A\beta_{42}$ levels among participants, this measurement could be useful as a marker in individual patients only (if repeated longitudinally measurements are available) rather than for population screening purposes. Another possible explanation of the lack of change in plasma Aβ in AD is a hypothetical peripheral origin of a major part of Aβ in the blood. Potential sources of plasma $A\beta_{42}$ are skeletal muscle (Kuo et al. 2000) and platelets (Li et al. 1998). However, contrary to previous hypotheses, plasma $A\beta_{40}$ and $A\beta_{42}$ concentrations are not related to platelet activation (Olsson et al. 2003), thus rigorous sample techniques to prevent platelet stimulation are not necessary for plasma $A\beta_{42}$ determination.

MICROTUBULE-ASSOCIATED PROTEIN TAU (MAP-TAU) AS A BIOCHEMICAL MARKER OF AD

Both MAP-tau and phosphorylated MAP-tau can be detected in the CSF. Widespread accumulation of MAP-tau in the AD brain is considered responsible for the increase in the CSF MAP-tau. Several studies have found increased CSF MAP-tau levels in AD (summarized in Table II), with a reported sensitivity of at least 80% for most studies. CSF MAP-tau concentration has been suggested to reflect: (i) neuronal and axonal degeneration or damage (Blennow et al. 1995); this suggestion is supported by the observations of increased CSF MAP-tau both after acute stroke, with a positive correlation between CSF MAP-tau level and infarct size (Hesse et al. 2000), and after severe traumatic brain injury (Franz et al. 2003); furthermore, the degree of increase in CSF MAP-tau is higher in conditions with more extensive and/or rapid neuronal degeneration, e.g., CJD (Kapaki et al. 2001, Riemenschneider et al. 2003, Van Everbroeck et al. 2003); (ii) formation of NFT; this hypothesis has been strengthened by the discovery of a positive correlation between the cerebral load of NFTs assessed post mortem and CSF MAP-tau concentrations (Tapiola et al. 1997), suggesting that CSF MAP-tau level may be a good reflection of the degree of neurofibrillary degeneration in the brain.

As can be seen in Table II, data from studies on CSF MAP-tau are even more unequivocal than on CSF $A\beta_{42}$. Despite differences in baseline levels and the degree of CSF MAP-tau alterations, the pattern of change is uniform with a reasonable sensitivity and specificity reported in the majority of papers. An increase in CSF-MAP-tau has been repeatedly observed not only in incipient AD (MMSE >24 points) (Schonknecht et al. 2003b), but also in the early stage of MCI (Andreasen et al. 1999c, 2003, Maruyama et al. 2001, Riemenschneider et al. 2002a), with no exceptions to this pattern. CSF MAP-tau concentrations have been sufficient to discriminate patients who progressed from MCI to AD or had a progressive MCI from those with stable MCI (Riemenschneider et al. 2002a). Moreover, CSF MAP-tau levels was the only variable that predicted progression of cognitive decline (Riemenschneider et al. 2002a). The sensitivity for the prediction of the MCI-AD progression by analysis of the CSF MAP-tau reached 80% (Andreasen et al. 2003).

There are conflicting reports on the matter of stability of CSF MAP-tau levels over time. The majority of studies show that the CSF MAP-tau concentrations are stable over time, with no correlation between the CSF MAP-tau and age, severity or duration of dementia, and with low inter-individual variations on repeated sampling (Andreasen et al. 1999b, 2001, Csernansky et al. 2002, Gomez-Tortosa et al. 2003, Hampel et al. 1999, 2001, Kahle et al. 2000, Kapaki et al. 2003a, Mulder et al. 2002, Rosso et al. 2003, Shoji et al. 2002, Tapiola et al. 2000b), suggesting feasibility for an early diagnosis, rather than for monitoring disease progression. Occasionally, a progression is seen only in AD patients harboring an *ApoE* ε4 allele (Blomberg et al. 1996). There are of course a few exceptions (Kanai et al. 1998, Schonknecht et al. 2003b, Sunderland et al. 2003); moreover, a confirmed correlation between CSF MAP-tau and cerebral load of NFTs (Tapiola et al. 1997) attenuates consistency of these observations, implying that CSF MAP-tau concentrations may increase with increasing NFT burden and thus with disease progression. In another recent paper (Wahlund and Blennow 2003), the CSF MAP-tau concentrations, unlike $A\beta_{42}$, correlated positively with MRI-evaluated annual change in ventricular volume, suggesting that CSF MAP-tau may reflect the intensity of the disease process, or a more rapid progression of the disease.

Reports on the effect of the *ApoE* genotype on CSF MAP-tau concentration are contradictory. No correlation between these two parameters has been found in some studies (Andreasen et al. 2001, Arai et al. 1995,

Table II

Summary of 46 studies comparing cerebrospinal fluid (CSF) levels of MAP-tau in patients with Alzheimer's disease (AD) and controls

Study	AD	AD Tau	C	C Tau	Sn	Sp	*P*	Eff
Vandermeeren et al. 1993	27	10.9 (4.9)	51	0.1 (0.5)	81	96	<0.001	3.73
Arai et al. 1995	70	77 (46)	19	9 (5)	99	100	<0.001	1.68
Blennow et al. 1995	44	524 (280)	31	185 (50)	84	97	<0.001	1.56
Mori et al. 1995	14	820 (90)	36	380 (120)	100	94	<0.001	3.91
Munroe et al. 1995	24	1430 (739)	14	816 (355)			0.002	0.98
Motter et al. 1995	37	407 (241)	20	212 (102)	58	95	<0.001	0.96
Skoog et al. 1995	11	254 (113)	36	171 (78)			0.04	0.95
Tato et al. 1995	23	279 (100)	23	26 (11)			<0.001	3.56
Vigo-Pelfrey et al. 1995	71	361 (166)	26	190 (80)	39		<0.001	1.15
Arai et al. 1997	17	95 (44)	15	19 (15)			<0.001	2.28
Golombowski et al. 1997	19	53 (39)	12	31 (17)			0.04	0.66
Andreasen et al. 1998	43	796 (382)	18	190 (57)	95	94	<0.001	1.87
Arai et al. 1998	69	90 (45)	17	20 (13)	99	100	<0.001	1.69
Galasko et al. 1998	82	663 (481)	60	387 (167)	57	83	<0.001	0.72
Kanai et al. 1998	93	489 (298)	41	217 (128)	63	75	<0.001	1.05
Kurz et al. 1998	40	697 (447)	36	169 (64)	89	97	<0.001	1.62
Mecocci et al. 1998	29	436 (360)	23	212 (200)			0.007	0.75
Nishimura et al. 1998	163	426 (234)	65	188 (103)	66	83	<0.001	1.16
Shoji et al. 1998	55	467 (285)	34	218 (139)			<0.001	1.03
Andreasen et al. 1999b	274	690 (341)	65	227 (101)	93	86	<0.001	1.49
Burger nee Buch et al. 1999	38	580 (370)	28	273 (203)	84	62	<0.001	0.99
Green et al. 1999	17	802 (381)	9	198 (49)			<0.001	1.93
Hampel et al. 1999	25	566 (329)	19	245 (154)	80	85	<0.001	1.20
Hulstaert et al. 1999 *	150	425 {274-713}	100	195 {121-294}	79	70	<0.001	n.g.
Molina et al. 1999	83	522 (290)	8	216 (150)			<0.001	1.09
Kahle et al. 2000	30	840 (560)	16	340 (230)	63	89	<0.001	1.06
Kanemaru et al. 2000	24	460 (301)	19	115 (76)	87	95	<0.001	1.50
Sjogren et al. 2000a	60	743 (503)	32	307 (168)	85	95	<0.001	1.04
Sjogren et al. 2000b	42	725 (356)	18	375 (176)	65	85	<0.01	1.11
Andreasen et al. 2001	105	759 (417)	18	264 (102)	95	94	<0.001	1.27
Hampel et al. 2001	17	496 (205)	12	312 (98)	n.g.	n.g.	0.004	1.09
Itoh et al. 2001	236	450 (252)	95	149 (107)	77	78	<0.001	1.37
Rosler et al. 2001	27	761 (407)	17	224 (81)			<0.001	1.66
Csernansky et al. 2002	32	1260 (460)	10	800 (260)	72	80	<0.001	1.09
Buerger et al. 2002b	80	n.g.	21	n.g.	81	90	<0.001	n.g.
Mulder et al. 2002	20	618 (292)	20	277 (136)	90	90	<0.001	1.65
Nagga et al. 2002 **	50	596 <144-1415>	27	292 <128-833>	n.g.	n.g.	<0.001	n.g.
Riemenschneider et al. 2002c *	74	540 {373-869}	40	152 {104-190}	95	98	<0.001	n.g.
Hu et al. 2002	52	486 (168)	56	215 (77)	n.g.	n.g.	<0.001	2.10
Shoji et al. 2002	366	482 (271)	113	186 (107)	68	94	<0.001	1.22
Sjogren et al. 2002	19	919 (349)	17	342 (116)			<0.001	2.17
Gomez-Tortosa et al. 2003	33	500 (399)	46	200 (243)	73	80	<0.001	0.95
Kapaki et al. 2003a *	49	504 {348-854}	49	140 {110-223}	88	96	<0.001	n.g.
Rosso et al. 2003 *	18	479 {360-698}	13	171 {117-310}	94	77	<0.001	n.g.
Schonknecht et al. 2003b **	43	578 <180-1200>	16	254 <80-385>	63	100	<0.05	n.g.
Sunderland et al. 2003	131	587 (365)	72	224 (156)	Com	Com	<0.001	1.18

(AD) number of study participants in the AD group; (AD Tau) mean CSF MAP-tau in the AD group (Standard Deviation) (pg/ml); (C) number of study participants in the control group; (C Tau) mean CSF MAP-tau in the control group (Standard Deviation) (pg/ml); (Sn) calculated sensitivity (%); (Sp) calculated specificity (%); (P) P value; (Eff) effect size; (n.g.) not given/impossible to calculate; (*) median concentration {25-75 percentile}; (**) median <range>; (Com) sensitivity and specificity values only for the combined assessment of $A\beta_{42}$ and total-MAP-tau (Table IV)

Hulstaert et al. 1999, Motter et al. 1995, Nagga et al. 2002). However, other papers confirm the existence of such an association (Molina et al. 1999, Tapiola et al. 2000a). In one study, CSF MAP-tau levels have been elevated only in patients with one ε4 allele, but not in homozygous patients (Galasko et al. 1998).

CSF MAP-tau has been shown to have a high sensitivity and specificity to differentiate AD patients from normal ageing and depression (Andreasen et al. 1999b). Furthermore, no increase in CSF MAP-tau has been observed in patients with schizophrenia irrespective of age (Schonknecht et al. 2003a). CSF MAP-tau measurements may be particularly useful for differentiating AD from CJD, as the concentration of MAP-tau in the CSF of CJD patients is many times higher than in any other neurodegenerative disorder, including AD (Kapaki et al. 2001, Otto et al. 2002, Riemenschneider et al. 2003, Van Everbroeck et al. 2003). In all of these reports, both sensitivity and specificity of CSF MAP-tau measurements in the diagnosis of CJD reached or even exceeded 90%, which is similar to the sensitivity and specificity of 14-3-3 protein in the CSF for the detection of CJD. CSF MAP-tau evaluation is effective to distinguish between AD and α-synucleinopathies (DLB, PD dementia); in the latter, CSF MAP-tau levels have consistently been reported to be similar to control groups, thus significantly lower than in AD subjects (Andreasen et al. 2001, Gomez-Tortosa et al. 2003, Kanemaru et al. 2000, Kapaki et al. 2003a, Molina et al. 1999). Moreover, the concentrations of this biomarker have been shown not to be affected by psychotropic medications use (neuroleptics, antidepressants) (Schonknecht et al. 2003b). However, there are several factors limiting the use of CSF MAP-tau as a single diagnostic marker of AD. Firstly, its specificity towards FTD and VaD is uncertain. In FTD, elevated CSF MAP-tau has been observed in some studies (Green et al. 1999, Molina et al. 1999, Riemenschneider et al. 2002c, Rosso et al. 2003, Shoji et al. 2002), while not in the others (Hulstaert et al. 1999, Motter et al. 1995, Sjogren et al. 2000a,b, Vigo-Pelfrey et al. 1995). Even though the elevation is usually lower than in AD, a considerable overlap between AD and FTD subjects as well as controls does not permit discrimination. The case is the same for VaD, with some papers reporting high CSF MAP-tau in the majority of VaD patients (Andreasen et al. 1998, 2001, Blennow et al. 1995, Hu et al. 2002, Nagga et al. 2002), while only in occasional cases in other studies (Arai et al. 1998, Hulstaert et al. 1999, Mecocci et al. 1998, Schonknecht et al. 2003b). Furthermore, CSF levels of MAP-tau overlap between AD patients and controls.

PHOSPHORYLATED MAP-TAU (P-MAP-TAU) AS A BIOCHEMICAL MARKER OF AD

A significant elevation of P-MAP-tau in the CSF of AD subjects has consistently been observed in several studies (summarized in Table III). Several phosphorylated epitopes of MAP-tau have been subject of research as potential biomarkers of AD. So far, these comprise threonine 181, serine 199, threonine 231, threonine 235, serine 396, serine 404, and combination thereof.

Contrary to total-MAP-tau, P-MAP-tau is not merely a marker for neuronal damage (P-MAP-tau does not change after acute stroke (Hesse et al. 2001)), but rather is a reflection of hyperphosphorylation of tau and possibly formation of NFT. As a result, an increase in specificity towards AD compared with total-MAP-tau measurements has been repeatedly observed.

CSF P-MAP-tau has been reported to be normal (concentrations similar to control groups) in DLB (Buerger et al. 2002b, Itoh et al. 2001, Parnetti et al. 2001), FTD (Buerger et al. 2002b, Rosso et al. 2003) or VaD (Buerger et al. 2002b, Hu et al. 2002, Itoh et al. 2001, Nagga et al. 2002, Schonknecht et al. 2003b). Despite some exceptions in the latter two groups showing CSF P-MAP-tau levels between those found in AD and controls (Blennow et al. 1995, Itoh et al. 2001), or even below those in the control group for FTD patients (Kohnken et al. 2000, Sjogren et al. 2001, Vanmechelen et al. 2000), diagnostic accuracy of this measurement seems higher than $A\beta_{42}$ or total-MAP-tau alone. The observation of improved specificity of CSF P-MAP-tau assessment is underscored by a recent paper showing decreased P-MAP-tau levels in subjects with CJD compared to AD, despite a huge elevation of total-MAP-tau in CJD (Riemenschneider et al. 2003). Clinical applicability of CSF P-MAP-tau measurements is even greater, taking into consideration a very accurate discrimination between geriatric major depression and AD. P-MAP-tau yielded a specificity of 85% and a sensitivity of 92% correctly allocating 87% of subjects, whereas clinical symptoms can overlap considerably (Buerger et al. 2003). Moreover, the levels of this biomarker have not been affected by psychotropic medication (neither neuroleptics nor antidepressants) which underlines their importance in clinical practice (Schonknecht et al. 2003b).

Table III

Summary of 13 studies comparing cerebrospinal fluid (CSF) levels of phosphorylated MAP-tau (P-MAP-tau) in patients with Alzheimer's disease (AD) and controls

Study	Epi	AD	AD P-tau	C	C P-tau	Sn	Sp	*P*	Eff
Blennow et al. 1995	Thr 181/231	44	2230 (930)	31	640 (230)	88	97		2.18
Ishiguro et al. 1999	Thr 181/231	36		20		53	100		
	Thr 231/235	36		20		94	80		
Kohnken et al. 2000	Ser 199	27		31		85	97		
Vanmechelen et al. 2000 / /Sjogren et al. 2001 #	Thr 181	41	23,1 (10,1)	17	15,9 (5,7)				0.79
Hampel et al. 2001 ^	Thr 231	12	53,3 (25.5)	12	2.2 (11.7)	n.g.	n.g.	<0.001	2.58
Itoh et al. 2001 #	Ser 199	236	1900 (900)	95	600 (400)	85	85	<0.001	1.65
Buerger et al. 2002b	Thr 231	82	58 (29)	21	2 (9)	100	90	<0.001	2.13
Hu et al. 2002	Ser 396/404	52	187 (84)	56	54 (33)	84	95	<0.001	2.11
Nagga et al. 2002 # **	Thr 181	50	19.4 <9.4-35.8>	27	14.9 <6.8-25.1>	n.g.	n.g.	<0.005	n.g.
Maddalena et al. 2003	Thr 181	51	52 (19)	31	27 (10)	84	84	<0.001	1.54
Riemenschneider et al. 2003 # *	Thr 181	42	13,5 {10.2-21.8}	43	3.6 {2.9-4.7}	79	93	<0.001	n.g.
Rosso et al. 2003 *	Thr 181	18	80 {54-101}	13	31 {21-42}	89	77	<0.001	n.g.
Schonknecht et al. 2003b **	Thr 181	43	73 <41-172>	16	51.5 <28-69>	71	94	<0.05	n.g.

(Epi) epitope; (AD) number of study participants in the AD group; (AD P- tau) mean CSF P-MAP-tau in the AD group (Standard Deviation) (pg/ml); (C) number of study participants in the control group; (C P-tau) mean CSF P-MAP-tau in the control group (Standard Deviation) (pg/ml); (Sn) calculated sensitivity (%); (Sp) calculated specificity (%); (P) P value; (Eff) effect size; (n.g.) not given/impossible to calculate; (#) pM/ml; (^) AU; (*) median concentration {25-75 percentile}; (**) median concentration <range>

An increase in CSF-P-MAP-tau levels has furthermore been reported in both patients with incipient AD (Schonknecht et al. 2003b) and in MCI subjects (Andreasen et al. 2003, Arai et al. 2000, Buerger et al. 2002a), with a positive correlation between the extent of this increase and both cognitive impairment progression rate and the risk of conversion into AD (such correlations have not been observed for CSF-total-MAP-tau levels in the same patients) (Buerger et al. 2002a). CSF P-MAP-tau discriminated MCI subjects with worsening cognitive functions during follow-up, from healthy controls with a sensitivity of 82% and specificity of 87% (Buerger et al. 2002a). Some authors report a lower, 70% sensitivity of such measurement (Andreasen et al. 2003). These data support the view that AD CSF biomarkers are positive even before the onset of clinical dementia.

As with total-MAP-tau, P-MAP-tau concentrations have been shown to remain stable, irrespective of age or dementia severity as assessed with MMSE (Buerger et al. 2002a,b, Itoh et al. 2001, Maddalena et al. 2003, Rosso et al. 2003), with one exception (Hampel et al. 2001) reporting a longitudinal decrease of P-MAP-tau in AD subjects correlated with MMSE score at baseline. As for total-MAP-tau, P-MAP-tau CSF concentrations have been observed to correlate with MRI-evaluated annual change in ventricular volume (Wahlund and Blennow 2003). Higher CSF levels of these biomarkers may thus reflect a more aggressive disease, with a more rapid progression. In another paper, after adjustment for the increased ventricular volume assessed with MRI, the CSF P(231)-MAP-tau load, contrary to total MAP-tau, has been shown to increase in patients with MCI (de Leon et al. 2002). *ApoE* ε4 allele burden has not influenced P-MAP-tau CSF levels (Itoh et al. 2001, Maddalena et al. 2003, Nagga et al. 2002).

Up till now, neither total- nor P-MAP tau concentrations have been reported to have been measured in plasma or urine.

COMBINATIONS OF DIFFERENT MARKERS

Discriminative power can be improved by combining different markers. The most widely evaluated combina-

tion is of CSF $A\beta_{42}$ and CSF total-MAP-tau levels (sensitivity and specificity figures summarized in Table IV). The sensitivity and specificity of such assessment usually exceeds 80% when healthy controls are the comparison group. However, specificity of the combined measurement is lower when comparing AD with other dementias. The sensitivity and specificity of CSF total-MAP-tau and $A\beta_{42}$ simultaneous measurements are high enough for discriminating AD and normal ageing when combined with the assessment of cognitive functions. There are several methods of increasing the diagnostic potential of the combination of these markers. These include using optimized cut-off points, scatterplots of tau against $A\beta_{42}$ (Galasko et al. 1998, Motter et al. 1995, Rosler et al. 2001), the discrimination line ($A\beta_{42}$ = 240 + 1.18 × tau) (Andreasen et al. 2001, Hulstaert et al. 1999), and the "AD index" = tau × $A\beta_{40}/A\beta_{42}$ (Kanai et al. 1998, Shoji et al. 1998).

According to several studies, the total-MAP-tau/$A\beta_{42}$ ratio has proved to be better than any of the candidate markers alone (Gomez-Tortosa et al. 2003, Kapaki et al. 2003a) and better than the combined assessment with the use of the discrimination line (Kapaki et al. 2003a). This quotient's superiority was particularly evident in the discrimination of AD from non-Alzheimer dementias (Kapaki et al. 2003a) (Table IV). However, total-MAP-tau alone provided a better distinction between AD and DLB subjects than the total-MAP-tau/$A\beta_{42}$ ratio (Gomez-Tortosa et al. 2003). In another study, using the ratio of CSF total-MAP-tau/Aβ total improved sensitivity and specificity of the separation of AD subjects and controls, contrary to the total-MAP-tau/$A\beta_{42}$ quotient which did not improve group discrimination (Csernansky et al. 2002).

The CSF $A\beta_{42}$ – total-MAP-tau combination is also useful for identifying subjects with the highest risk of conversion from MCI to AD (Andreasen et al. 1999c, Riemenschneider et al. 2002a). Application of both markers for this purpose has yielded a sensitivity and specificity of 90% (Riemenschneider et al. 2002a).

With the increasing popularity of the CSF P-MAP-tau assessments, new possible combinations have emerged. P-MAP-tau/total MAP-tau ratio has permitted discrimination between subjects with CJD and all other neurodegenerative disorders including patients with AD and FTD with no overlap (Riemenschneider et al. 2003). However, the calculation of this ratio has not added any value to either of these markers alone in discriminating AD from controls and FTD (Rosso et al. 2003). In another recent paper, the ratio of CSF P-MAP-tau to $A\beta_{42}$ has allowed for better discrimination of AD patients from healthy controls, non-Alzheimer dementias, and other neurological disorders than any of these markers alone (Maddalena et al. 2003).

Other evaluated combination comprises a 2-step algorithm using CSF $A\beta_{42}$, total-MAP-tau and F2-isoprostanes, *in vivo* biomarkers of oxidative damage (Montine et al. 2001). Addition of F2-iPs has significantly improved diagnostic specificity towards AD – from 50% to 89% – while lowering sensitivity by only 10%, from 95 to 85%. A simultaneous assessment of total-MAP-tau and neuronal thread protein (AD7C-NTP), a protein that may be involved in neuritic sprouting, resulted in a small increase in specificity (from 89 to 93%), whereas sensitivity remained unchanged (63%) compared to total-MAP-tau alone (Kahle et al. 2000).

CLINICAL APPLICABILITY OF THE BIOMARKERS STUDIES IN AD

As a result of the use of widely accepted criteria and better understanding of the clinical picture of different dementias, the diagnostic accuracy of Alzheimer's disease on clinical grounds has increased in the last decade, amounting to as high as 80-90% (Klatka et al. 1996). Given this, one may argue that CSF biomarkers probably do not offer a significant improvement to the accuracy of diagnosis. However, the rates of clinical accuracy are usually much lower outside specialized memory clinics or research centers. In the primary care settings, especially in the presence of confounding factors and in the earliest stages of the disease when symptoms can be vague, diagnostic uncertainty increases substantially. It is there, where biomarkers are required most, especially with the rapid expansion of AD therapeutics' research and with some compounds promising to modify the course of the disease on the horizon.

Unfortunately, there are a number of drawbacks hampering the clinical utility of the CSF AD biomarkers. The first problem is quite an extensive overlap between controls and affected participants diminishing the applicability of such assessments for diagnosing individuals. An improvement may be achieved by combining different markers into one statistical analysis. While the differences between AD subjects and healthy controls in the concentration of Aβ and tau or both species in the CSF may indeed look impressive, the interpretation of such data requires caution, as they result from contrasts of com-

Table IV

Diagnostic accuracy of the combined cerebrospinal fluid $A\beta_{42}$ and total-MAP-tau analysis in Alzheimer's disease

Study	Sensitivity (%)	Specificity (%)
Motter et al. 1995	59	100 (AD vs. C)
Galasko et al. 1998	77	93 (AD vs. C)
		65 (AD vs. NAD)
Kanai et al. 1998	71	83 (AD vs. C)
Andreasen et al. 1999c	88	80 (MCI v C)
Hulstaert et al. 1999	85	87 (AD vs. C)
		86 (AD vs. OND)
		58 (AD vs. NAD)
Tapiola et al. 2000b	50	95 (AD vs. C)
		85 (AD vs. NAD)
Andreasen et al. 2001	94 (probable AD)	100 (AD vs. PS)
	88 (possible AD)	89 (AD vs. C)
	75 (MCI)	67 (AD vs. DLB)
		48 (AD vs. VaD)
Mulder et al. 2002	95	90 (AD vs. C)
Riemenschneider et al. 2002c	92	95 (AD vs. C)
	85	85 (AD vs. FTD)
Riemenschneider et al. 2002a	90 (MCI)	90 (MCI v C)
Gomez-Tortosa et al. 2003	84 (tau/$A\beta_{42}$ ratio)	79 (AD vs. C)
		36 (AD vs. DLB)
Kapaki et al. 2003a	88	92 (AD vs. C)
		60 (AD vs. NAND)
		67 (AD vs. VaD)
	96 (tau/$A\beta_{42}$ ratio)	86 (AD vs. C)
	71	100 (AD vs. NAND)
	90	83 (AD vs. VaD)
Sunderland et al. 2003	92	82 (AD vs. C)
Maddalena et al. 2003 *	86 (P-tau/$A\beta_{42}$ ratio)	97 (AD vs. C)
	80	73 (AD vs. NAD)
	80	89 (AD vs. OND)

(AD) Alzheimer's disease; (C) controls; (OND) other neurological diseases; (NAD) non-AD dementia; (NAND) non-AD neurodegenerative dementia; (DLB) Lewy body dementia; (VaD) vascular dementia; (MCI) mild cognitive impairment; (PS) psychiatric disorders; (vs.) *versus*; (*) P-MAP-tau instead of total-MAP-tau

pletely different populations. Such artificial contrast hardly resembles everyday clinical dilemmas. A step in the right direction would be the inclusion of various dementia types, including DLB, VaD, FTD, and other disorders manifesting themselves with cognitive impairment (e.g., MCI, depression) into different comparison groups. This most often results in a substantial, sometimes even dramatic, decrease in the sensitivity and particularly specificity of the assessments. The way these numbers are obtained is another issue worthy of consideration. In the majority of studies, either the optimal cut-off values are chosen or different ratios are calculated, with the principle of maximizing the parameters. Unfortunately, large differences between studies in the concentration of biomarkers result in similar discrepancies between the chosen cut-off values. Unless the assays are standardized and international cut-off values estimated, the routine use of CSF biomarkers in clinical practice will not be possible. Another major concern of these studies is that classification is based on clinical diagnosis of dementia syndromes, which are known to suffer from misclassification in up to 15-20% of cases (Larson et al. 1996). Furthermore, this introduces

a risk that patients with other dementia disorders may have concomitant AD pathology (thus reducing specificity figures), which is impossible to exclude clinically. Indeed, such concomitant AD pathology has been found in 40-80% of clinically diagnosed patients with VaD (Kosunen et al. 1996). Moreover, even if they are cognitively healthy, age-matched control subjects may harbor presymptomatic AD lesions in the brain (Price and Morris 1999) which additionally reduces the sensitivity figures of CSF biomarkers for the diagnosis of AD.

From the more optimistic point of view, the effect of lower specificity on the clinical usefulness of the CSF biomarkers assessments should not be overestimated. Not all disorders characterized by abnormal levels of these biomarkers are important in the differential diagnosis of AD, e.g., stroke (Hesse et al. 2000), brain injury (Franz et al. 2003), or HIV dementia (Andersson et al. 1999). Instead, CSF MAP-tau and $A\beta_{42}$ may play an important role in the differentiation of AD from age-associated memory impairment, depressive impairment of cognitive functions, PD dementia or alcoholic dementia.

Another argument is that lumbar puncture is an invasive procedure, with the main complication being post-lumbar headache. The incidence of this complication is clearly age-dependent, with young age being a risk factor. In studies on patients investigated for dementia an incidence of around 4% or less has been reported (Andreasen et al. 2001, Blennow et al. 1993, Kapaki et al. 2003a), indicating that lumbar puncture in the elderly is a safe procedure that can be included in routine investigation of patients. Nonetheless, taking into consideration the psychological barriers associated with this technique, extensive research should be conducted on putative peripheral markers of AD present in easily accessible body fluids (plasma, urine).

Taking into account the complex and not yet fully understood pathobiology of AD, it is probably unrealistic to expect that measuring CSF $A\beta_{42}$ or MAP-tau alone can provide a sensitive and specific tool in the diagnosis of AD. In the future, a biochemical marker pattern reflecting the whole spectrum of abnormal proteins deposited in the brain will most likely provide a more accurate diagnosis of AD. At present, AD can be diagnosed on the basis of cumulative information gained from the clinical examination, brain-imaging techniques, and analysis of biochemical markers in the CSF (Brzyska and Elbaum 2003, Kapaki et al. 2003b, Religa and Winblad 2003).

CONCLUSIONS

The majority of studies report a statistically significant decrease in CSF $A\beta_{42}$ levels and significant increase in both total- and P-MAP-tau CSF levels in AD subjects compared with controls. In the majority of studies sensitivity and specificity figures exceed the 80% recommended in the Consensus Report on biochemical markers for AD. Because of the absence of assay standardization and the lack of comparison patient populations, the findings reported here should be treated cautiously and addressed in future prospective studies including different dementia types. At present, apart from the analysis of biochemical markers reported here, the desired diagnostic accuracy can be obtained only with both precise clinical assessment and data acquired from neuroimaging techniques. Unfortunately, CSF is not a matrix that can easily be used for diagnostic purposes, let alone for screening populations for risk factors. Thus the importance of identifying biomarkers for AD that occur peripherally, and which can be detected by analysis of plasma, urine or other easily accessible body fluids using non-invasive sampling techniques, is paramount.

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