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Clinical significance of neurobiochemical profiles in the lumbar cerebrospinal fluid of Alzheimer's disease patients

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Summary. Immunoreactivities of total apolipoprotein E (ApoE-IR), amyloid β peptide$_{(1-42)}$ (Aβ42-IR), interleukin-6 (IL-6-IR), substance P (SPIR) and total τ protein (TTIR) were measured in lumbar cerebrospinal fluid samples of patients with Alzheimer's disease (AD), non-Alzheimer's dementias (NAD), neurological disorders without cognitive impairment (OND) and controls without central nervous system disease using sensitive and specific enzyme immunoassay methods. TTIR was highly significantly increased ($P < 0{,}001$) and Aβ42-IR was significantly decreased ($P < 0{,}001$ vs. OND/CO, $P < 0{,}03$ vs. NAD) in the AD cohort compared with the other diagnostic groups. Significant increases in AD were also found for ApoE-IR ($P < 0{,}001$) and IL-6 ($P < 0{,}03$), but there was a considerable overlap between groups. In the total AD cohort, SPIR was not significantly changed, but AD patients with late disease onset (>65 years) showed significantly higher values than both early onset patients (<65 years) and controls ($P < 0{,}05$). Discriminant function analysis showed that Aβ42-IR (cut-off value 375 pg/ml) and TTIR (cut-off value 440 pg/ml) levels contributed most to the group classification of patients. At 85% sensitivity for AD and 100% specificity for controls, the combined evaluation of Aβ42-IR and TTIR in this cross-sectional study resulted in a graph separating AD from non-AD patients with increased specificity of 91% and 75% for AD versus OND and NAD, respectively.

Keywords: Cerebrospinal fluid, neurochemical profiles, Alzheimer's disease, apolipoprotein E, amyloid β peptide, interleukin-6, substance P, τ protein.

Introduction

Alzheimer's disease (AD) is the major cause of dementia in the elderly with a relative incidence of 70–80% (Jellinger, 1999). The clinical diagnosis of AD largely depends on the exclusion of other dementing disorders. Although necropsy confirmed diagnostic accuracy rates for AD are 63–96%, the negative predictive value of "probable" AD using established clinical criteria (McKhann et al., 1984) is only 33–53% (Nagy et al., 1998). Thus, the

unequivocal diagnosis of AD rests on (semiquantitative) assessment of its characteristic neuropathological lesions in the limbic and cerebral cortices, neurofibrillary tangles (NFT) and amyloid-bearing neuritic plaques, respectively (Jellinger, 1998). On the other hand, as therapeutic strategies are currently emerging, the establishment of valid neurobiological intra vitam markers of the disease is mandatory non only for (early) clinical diagnosis, but also for selecting patients for treatment and monitoring therapeutic effects (Blennow and Vanmechelen, 1998).

The cerebrospinal fluid (CSF) is in direct contact with the extracellular space of the brain. Thus, neurobiochemical changes in the brain are likely to be reflected in the CSF (Blennow and Vanmechelen, 1998). During the last few years, efforts including a wide variety of substances have been made to identify neurochemical markers of AD in lumbar CSF (Bancher et al., 1998). Total τ protein, its normal form probably reflecting ongoing neuronal and axonal degeneration or damage, and in hyperphosphorylated state being the main constituent of paired helical NFT filaments (Goedert, 1993), and the longer amyloid β peptide with 42 amino acids (Aβ42), major component of senile plaques (Dickson, 1997), respectively, seem to be the most promising ex vivo markers of the disease (Galasko, 1998). Levels of several other relevant markers, such as apolipoprotein E, interleukin-6 and substance P, have also been measured, but the results were inconclusive.

Apolipoprotein E (ApoE) is a glycoprotein containing 299 amino acids (molecular weight 34kDa) present in the brain, where it is synthesised by astrocytes and oligodendrocytes. As a constituent of several plasma lipoproteins, it plays a part in cholesterol and phospholipid metabolism, mainly by mediating the cellular uptake of lipid complexes through interaction with the ApoB/ApoE (low density lipoprotein (LDL)) receptor (Poirier, 1994). ApoE is present within plaques, neurofibrillary tangles, and dystrophic neurites of the brains of patients with AD (Namba et al., 1991); it binds soluble and insoluble forms of β amyloid with high avidity (Wisniewski et al., 1993), and it has been proposed that ApoE, acting as a molecular chaperone, influences conformational changes of amyloid fibrils during plaque formation (Wisniewski and Frangione, 1992). Genotyping studies have shown an increased frequency of the ApoE-ε4-allele in AD (Corder et al., 1998; St. George-Hyslop, 2000) suggesting a role for ApoE-ε4 as a susceptibility marker of the disease. On the other hand, ApoE-ε4 homozygotes can be found not only in AD, but also in many of other dementing disorders suggesting that ApoE genotyping cannot provide certainty about the presence or absence of AD, and that it should be used only as an adjunct to other diagnostic tests for AD (Arai et al., 1997; Blacker and Tanzi, 1998; Mayeux et al., 1998).

Increasing evidence suggests that some restricted immune-related processes are involved in the pathogenesis of AD (Gahtan and Overmier, 1999). The amyloid plaques of AD brains are associated with reactive microglial cells and astrocytes, which are the sources of locally produced cytokines, complement components, and proteolytic enzymes (Mc Geer et al., 1994). Among others, the cytokine interleukin-6, the main inductor of acute phase protein synthesis, has been detected in AD senile plaques (Bauer et al., 1991;

Wood et al., 1993), and it has been shown that interleukin-6 is present in early stages of plaque formation and can not be detected in plaques of patients without dementia (Hüll et al., 1995). Moreover, transgenic mice bearing an additional IL-6 gene were found to display severe neurodegeneration (Campbell et al., 1993).

Several neurotransmitter systems have been reported to be involved in AD. The degeneration within neurochemical systems, although not evenly distributed, shows a ranked order of severity, the cholinergic system being mostly affected, followed by the serotonergic, noradrenergic, peptidergic and dopaminergic systems (Gsell et al., 1997). The undecapeptide substance P (SP) is a member of a family of structurally related peptides called the tachykinins. Beside its action as a neurotransmitter, it is a major mediator of neurogenic inflammation and immunomodulatory activities within the nervous system and has been implicated in the pathophysiology of several nervous system disorders (Malek-Ahmadi, 1992). In AD, cortical SP concentrations were reported to be reduced, unchanged, increased or decreased only in the putamen (Valenti, 1996). It has been suggested that SP plays a protective role by counteracting the neurotoxic effects of amyloid β peptide (Aβ) in the brain in vivo, tachykinin antagonists being able to induce similar effects to those caused by Aβ (Yankner et al., 1990; Kowall et al., 1991).

Since measurements of single biomarkers probably will not efficiently separate AD from both control subjects, neurological disorders without dementia and non-Alzheimer's dementias, we were interested, in addition to routine CSF parameters such as cell count and protein ratios, to measure five neurobiologically relevant substances in CSF samples of different diagnostic groups and to identify neurochemical profiles which could improve neurochemical CSF diagnostics of AD.

Patients and methods

170 residual lumbar CSF samples archived for research purposes and stored frozen at −80°C until analysis were enrolled in the study. Samples contaminated with blood were excluded. All samples were tested for cell count, levels of albumin and IgG were measured in the CSF samples and the corresponding serum specimens using routine procedures, and the ratios Q_{Alb} ($[Alb_{CSF}]/[Alb_{Serum}]$) and Q_{IgG} ($[IgG_{CSF}]/[IgG_{Serum}]$) (Reiber et al., 1987) were calculated.

The whole patient group was subdivided as follows:

- 27 patients with probable AD according to the NINCDS-ADRDA criteria (McKhann et al., 1984); eleven patients had early onset (<65 years), 16 patients late onset (>65 years) of the disease. Age at onset of dementia, defined as the earliest age at which behavioral changes were noticed, was determined from the medical records; in addition, a mini mental state examination (MMSE) (Folstein et al., 1975) was performed;
- 24 patients with non-AD dementias (NAD; Parkinson's disease with dementia [four], vascular dementia [five], diffuse Lewy body disease [two], progressive supranuclear palsy [one], multisystem degeneration [two], Pick's disease [one], Huntington's disease [one], normal pressure hydrocephalus [eight];
- 70 patients with various infectious, immunological, neurodegenerative, neoplastic and vascular central nervous system (CNS) diseases without cognitive impairment (OND);

- 49 patients without CNS disease (CO), in whom lumbar puncture was performed for exclusion purposes.

Sensitive and specific enzyme linked immuno-absorbent sandwich assays (ELISA) were used to measure total apolipoprotein E-immunoreactivity (ApoE-IR) (Carlsson et al., 1991; Rösler et al., 1996a), amyloid β peptide$_{(1-42)}$-immunoreactivity (Aβ42-IR; Innogenetics, Belgium), interleukin-6-immunoreactivity (IL-6-IR; R & D Systems, U.S.A.), substance P-immunoreactivity (SPIR; Cayman Chemical, U.S.A.) and total amount of normal and hyperphosphorylated τ protein-immunoreactivity (TTIR; Innogenetics, Belgium). Detection ranges of the assays were as follows: ApoE-IR 0,2–28,5 µg/ml, Aβ42-IR 75–2,000 pg/ml, IL-6-IR 0,094–10 pg/ml, SPIR 7,8–1,000 pg/ml and TTIR 75–1,200 pg/ml. Samples with concentrations above or below this range were diluted or lyophilized adequately. Percentage coefficients of variances ranged from 3% to 8% (intra-assay) and 3% to 10% (inter-assay). Statistical analysis was performed using a commercially available software package (StatSoft, U.S.A., 1995). Pairs of groups were compared using the Wilcoxon two sample test. Correlations were calculated using the Spearman's rank correlation coefficient (R). Discriminant function analysis with backward stepwise procedure was done to test which variables contribute most to the group classification of patients. In this computational model, the program first includes all variables and then, at each step, eliminates the variable that contributes least to the prediction of group membership. As a result, only those variables contributing the most to the discrimination of groups are selected.

Results

Clinical data and results of lumbar CSF measurements of routine parameters and single markers are given in Table 1 and in Fig. 1a–1e. Significant intercorrelations of substances are listed in Table 2. Our findings on routine neurochemical measures showed total cell count to be in the normal range in AD CSF. AD, NAD and CO showed no significant differences of albumin ratios. Although IgG ratios of AD patients were significantly higher ($P < 0,05$) than those of controls, the values were in the normal range in both groups. Young (<55 years) and elderly (>55 years) control persons did not differ significantly regarding the five neurochemical parameters investigated. Neither level correlated significantly with MMSE scores and disease duration in AD. There was a highly significant ($P < 0,001$) increase in TTIR and a significant ($P < 0,001$ vs. OND/CO, $P < 0,03$ vs. NAD) decrease of Aβ42-IR in the AD group compared with the other groups. There were highly significant increases ($P < 0,001$) of ApoE-IR in the AD group compared with NAD, OND and CO, but considerable overlap between groups. IL-6-IR was significantly increased in the AD group ($P < 0,03$) and in the NAD and OND groups ($P < 0,03$) compared with controls. SPIR in the total AD group was not significantly different from the other groups, but patients with late onset (>65 years) AD had significantly ($P < 0,05$) higher SPIR values than both early onset patients (<65 years) and controls, whereas ApoE-IR, Aβ42-IR, IL-6-IR and TTIR did not differ significantly in patients with early and late onset AD.

At 100% specificity for CO, the cut-off values were set at 440 pg/ml for TTIR and at 375 pg/ml for Aβ42-IR, respectively. As a single measurement, TTIR showed sensitivity of 89% for AD and specificity for OND and NAD of 74 and 67%, Aβ42-IR showing sensitivity of 78% for AD and specificity for OND and NAD of 85% and 58%, respectively.

Table 1. Clinical and neurochemical CSF data of patients with "probable" Alzheimer's disease (*tAD* total group, *eAD* early onset, *lAD* late onset), non-Alzheimer dementias (NAD), other neurological disorders without cognitive impairment (OND), and control persons (*tCO* total group, *COY* young controls, *COE* elderly controls) (mean ± S.E.M.)

	tAD	eAD	lAD	NAD	OND	tCO	COY	COE
n (M/F)	27 (9/18)	11 (5/6)	16 (4/12)	24 (13/11)	70 (43/27)	49 (27/22)	32 (20/12)	17 (7/10)
Age (ys.)	68.7 ± 2.1^{a4,b4}	57.9 ± 2.2	76.1 ± 1.2	73.0 ± 1.8^{a4,b4}	53.6 ± 2.0^{a2}	45.2 ± 2.1	37.0 ± 1.9	60.7 ± 1.3
CSF cell count (/µl)	1.1 ± 0.1^{b4}	1.3 ± 0.2	1.0 ± 0.2	1.6 ± 0.3^{b3}	164.2 ± 92.9^{a4}	1.6 ± 0.2	1.4 ± 0.2	2.0 ± 0.4
Q_{Alb} (CSF/Serum) ($\times 10^{-3}$)	6.6 ± 0.7^{b2}	6.2 ± 0.6	6.9 ± 1.1	6.6 ± 0.6^{b2}	10.9 ± 1.2^{a4}	5.2 ± 0.2	5.1 ± 0.3	5.4 ± 0.4
Q_{IgG} (CSF/Serum) ($\times 10^{-3}$)	3.5 ± 0.4^{a1,b3}	3.2 ± 0.3	3.7 ± 0.6	3.4 ± 0.4^{b3}	6.3 ± 0.8^{a4}	2.5 ± 0.1	2.4 ± 0.2	2.7 ± 0.2
MMSE (points)	14.3 ± 1.4	14.0 ± 2.5	14.5 ± 1.6	–	–	–	–	–
Disease duration (ys.)	3.8 ± 0.4	4.1 ± 0.7	3.5 ± 0.5	2.8 ± 0.4	–	–	–	–
ApoE-IR (µg/ml)	14.0 ± 1.4^{a4,b4,c4} (22)	12.0 ± 1.0 (8)	15.1 ± 2.1 (14)	7.9 ± 0.7	9.2 ± 0.4	8.8 ± 0.4	8.9 ± 0.5	8.6 ± 0.8
Aβ42-IR (pg/ml)	326.0 ± 22.7^{a4,b4,c2}	298.8 ± 41.9	344.7 ± 25.4	448.9 ± 39.2^{a4}	573.1 ± 24.0^{a3}	684.7 ± 21.5	698.6 ± 24.9	658.4 ± 41.0
IL-6-IR (pg/ml)	3.5 ± 0.3^{a2}	3.2 ± 0.7	3.8 ± 0.4	5.7 ± 1.0^{a2}	5.0 ± 0.5^{a2} (69)	3.0 ± 0.4	3.0 ± 0.5	3.1 ± 0.7
SPIR (pg/ml)	37.3 ± 2.8 (22)	29.3 ± 1.6 (8)	41.9 ± 3.8*^{,a1} (14)	35.8 ± 2.9 (21)	34.5 ± 1.3^{a2} (68)	31.5 ± 1.6 (42)	31.7 ± 2.1 (28)	31.1 ± 2.4 (14)
TTIR (pg/ml)	760.5 ± 78.3^{a4,b4,c4}	738.1 ± 101.3	775.9 ± 114.8	461.7 ± 66.4^{a4,b1}	390.8 ± 40.5^{a3} (68)	237.5 ± 11.5	244.8 ± 14.2	223.8 ± 19.6

If different from the first line, n is given in parentheses. ^{a1} $P < 0.05$ vs. tCO; ^{a2} $P < 0.03$ vs. tCO; ^{a3} $P < 0.01$ vs. tCO; ^{a4} $P < 0.001$ vs. tCO. ^{b1} $P < 0.05$ vs. OND; ^{b2} $P < 0.03$ vs. OND; ^{b3} $P < 0.01$ vs. OND; ^{b4} $P < 0.001$ vs. OND. ^{c2} $P < 0.03$ vs. NAD; ^{c4} $P < 0.001$ vs. NAD. * $P < 0.05$ vs. eAD

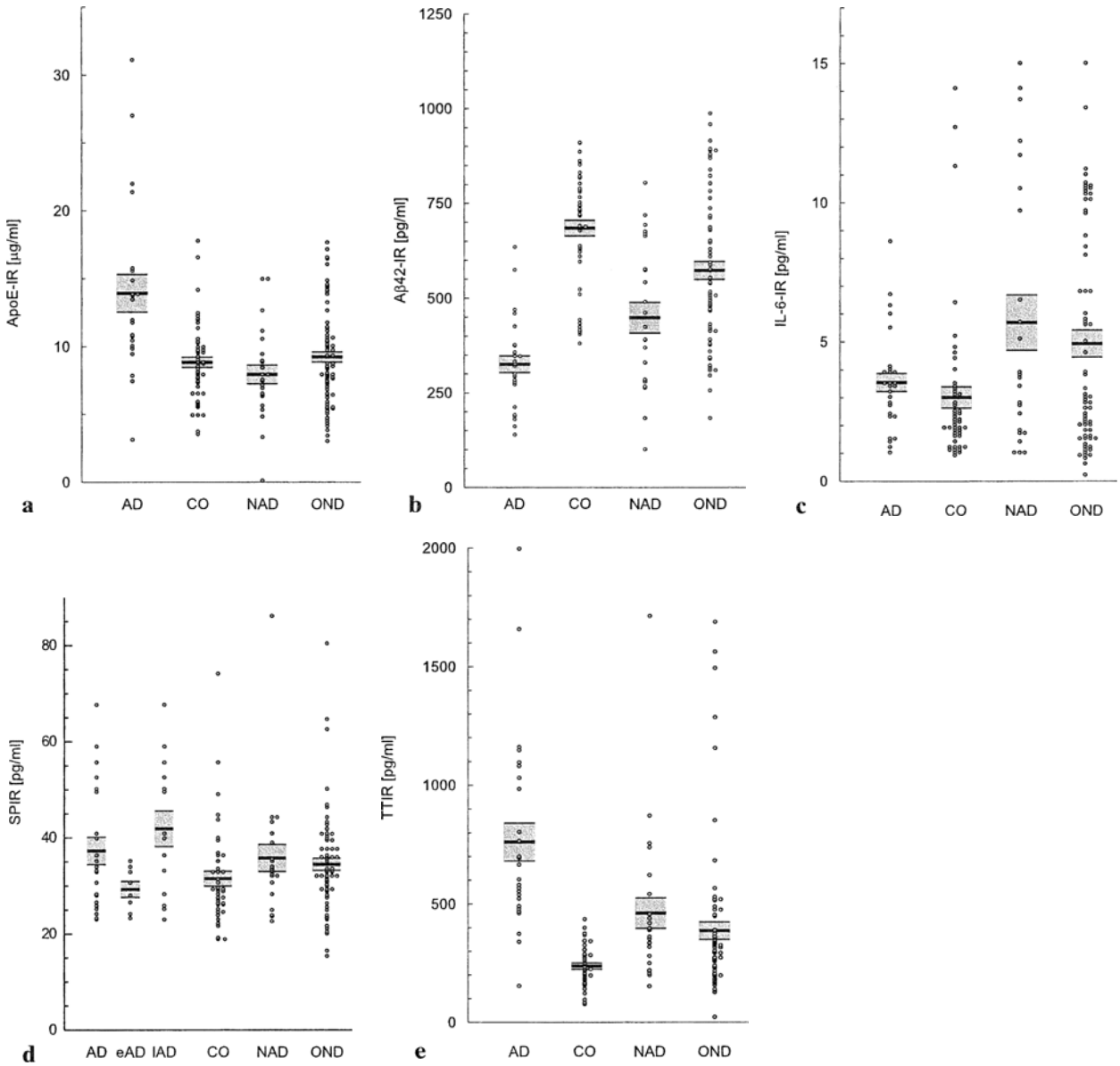

Fig. 1. Apolipoprotein E-immunoreactivity (ApoE-IR) (**a**), amyloid β peptide$_{(1-42)}$-immunoreactivity (Aβ42-IR) (**b**), interleukin-6-immunoreactivity (IL-6-IR) (**c**), substance P-immunoreactivity (SPIR) (**d**) and total τ protein-immunoreactivity (TTIR) (**e**) in the lumbar cerebrospinal fluid of patients with Alzheimer's disease (AD), non-Alzheimer's dementias (NAD), other neurological disorders without cognitive impairment (OND), and controls (CO)

Discriminant function analysis revealed that TTIR and Aβ42-IR contributed most to the group classification of patients, followed by ApoE-IR and IL-6-IR, SPIR being least important to predict a patient's group classification. For combined evaluation of TTIR and Aβ42-IR, sensitivity for AD and specificity for AD versus CO were set at 85% and 100%, respectively.

Table 2. Neurochemical parameters (*ApoE* apolipoprotein E, *Aβ42* amyloid β peptide$_{(1-42)}$, *IL-6* interleukin-6, *SP* substance P, *TT* total τ protein, *IR* immunoreactivity): significant correlations in the cerebrospinal fluid of patients with Alzheimer's disease (AD), non-Alzheimer dementias (NAD), other neurological disorders (OND), and controls (CO)

	Aβ42-IR	IL-6-IR	SPIR	TTIR
ApoE-IR	CO: R = 0.33 P < 0.03		OND: R = 0.35 P < 0.004	OND: R = 0.49 P < 0.0001 CO: R = 0.40 P < 0.004
Aβ42-IR		OND: R = −0.26 P < 0.03	OND: R = 0.38 P < 0.002 CO: R = 0.51 P < 0.001	OND: R = 0.38 P < 0.002 CO: R = 0.59 P < 0.0001
IL-6-IR			AD: R = 0.53 P < 0.02 OND: R = 0.31 P < 0.02	
SPIR				NAD: R = 0.46 P < 0.04 OND: R = 0.26 P < 0.04

Numbers indicate Spearman's rank correlation coefficients (R) and corresponding P-values. All other correlations are not significant (P > 0.05)

Considering higher specificity of single TTIR measurement for NAD than Aβ42-IR, a vertical discrimination line was introduced at the TTIR cut-off value. For TTIR values >440 pg/ml, a second line with the following equation was defined: Aβ42-IR [pg/ml] = 1,01 × TTIR + 150 [pg/ml]. This graph separated area 1 (AD) from area 2 (non-AD, i.e. NAD, OND, CO) with increased specificity of 91% and 75% for AD versus OND and NAD, respectively (Fig. 2a–2c).

Discussion

To the best of our knowledge, this is the first study which includes five CSF biomarkers probably reflecting different neurobiological correlates of AD. Since it has been proposed that the use of a combination of several CSF biomarkers may increase both the sensitivity and specificity of separating AD from other dementing and non-dementing brain disorders and control persons (Blennow and Vanmechelen, 1998; Andreasen et al., 1999b; Kahle et al., 2000), we here report findings of routine neurochemical measures as well as levels of single biomarkers and evaluate the clinical significance of their combined assessment.

Young (<55 years) and elderly (>55 years) control persons had similar levels of ApoE-IR, Aβ42-IR, IL-6-IR, SPIR and TTIR. This result is in accordance with most studies (Lefranc et al., 1996; Rösler et al., 1996b;

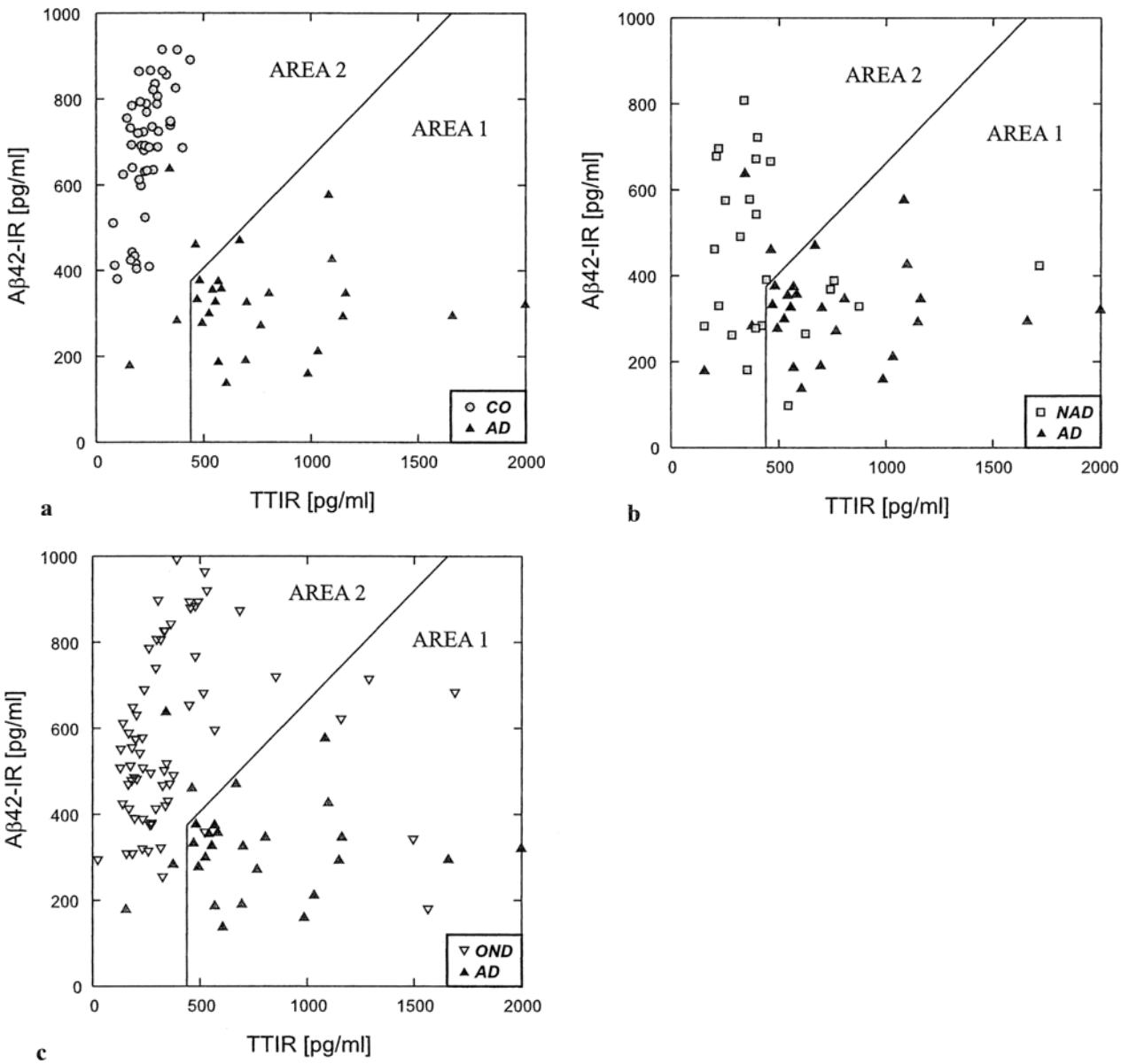

Fig. 2. Combined evaluation of amyloid β peptide$_{(1-42)}$-immunoreactivity (Aβ42-IR) and total τ protein-immunoreactivity (TTIR) in the lumbar cerebrospinal fluid of patients with Alzheimer's disease (AD), non-Alzheimer's dementias (NAD), other neurological disorders without dementia (NAD), and controls (CO). Discrimination lines separate area 1 (AD) and area 2 (non-AD, i.e. NAD, OND, CO)

Hampel et al., 1997; Green et al., 1999), but at some variance with other reports which found increasing concentrations of total β amyloid peptide (van Gool et al., 1994) or decreased SPIR in elderly compared with young controls (Cramer et al., 1985). Increasing age is an important risk factor of AD, but our present results indicate that the levels of the substances investigated are, in general, not merely markers of aging itself. Lack of correlations with MMSE scores and disease duration in our study suggests that levels may not be

altered substantially with disease progression and, thus, probably can be pathologically changed even in early disease stages (Andreasen et al., 1999b). On the other hand, some studies on TTIR reported increasing levels with advancing dementia (Hock et al., 1995; Tato et al., 1995), while others showed stable CSF tau over extended periods of time (Sunderland et al., 1999), and a recent longitudinal study detected decreasing CSF Aβ42 over time suggesting that it may be useful in monitoring the long-term progression of AD (Tapiola et al., 2000).

AD is a diagnosis clinically established by exclusion, and measures of cell count and protein ratios are basic parameters of CSF evaluation not only in dementing illnesses. Significant pleocytosis is an unequivocal criterion to exclude the diagnosis of AD (Fishman, 1992). On the other hand, studies on protein ratios in AD have reported differing results. Whereas most investigators found no significant changes (Kay et al., 1987; Leonardi et al., 1985; Frölich et al., 1991; Mecocci et al., 1991), others reported elevated albumin ratios (Skoog et al., 1998; Hampel et al., 1999) and qualitative or quantitative intrathecal immunoglobulin synthesis, at least in subsets of AD patients (Blennow et al., 1990; Small et al., 1994; Hampel et al., 1999). Nevertheless, our findings with total cell count and protein ratios being in the normal range suggest that in particular combination of significant pleocytosis and marked elevations of the albumin ratio or intrathecal immunoglobulin synthesis makes the diagnosis of AD rather improbable and should give rise to consider other causes of dementia.

Total ApoE was significantly increased in AD patients compared with the other groups, but showed considerable overlap among groups. The results regarding total ApoE-IR in AD CSF are contradictory. With or without correlation to the ApoE genotype, unchanged (Lefranc et al., 1996; Rösler et al., 1996a; Hahne et al., 1997), decreased (Blennow et al., 1994; Hesse et al., 2000) and increased levels (Lindh et al., 1997; Merched et al., 1997; Fukuyama et al., 2000) have been reported. Most studies found no significant correlations of total ApoE-IR and the number of ApoE-ε4 alleles (Lefranc et al., 1996; Skoog et al., 1997) while Hesse et al. (2000) reported higher levels of CSF-ApoE in individuals possessing the ApoE alleles. Methodological aspects may account for differences in total levels, but can not explain differences among groups. ApoE is involved in the regeneration and maintenance of myelin and neuronal membranes in both the peripheral and central nervous system and has been linked to synaptoneogenesis and, thus, phenomena of synaptic plasticity (Poirier, 1994). As the present findings are inconclusive even in comparison to studies with similar experimental methodology (Rösler et al., 1996a), it may be speculated that ApoE elevations in single AD patients could reflect aspects of their individual re-innervation capacity. On the other hand, due to the broad overlap with other diseases, it can be assumed that CSF ApoE-IR measures alone are unlikely to provide a sensitive and specific marker for the diagnosis of AD.

Aβ42-IR was significantly decreased in AD compared with all other diagnostic groups. Measurements of β-amyloid in CSF have a potential for reflecting cerebral β-amyloid metabolism and/or deposition (Galasko, 1998). Studies which measured the total β-amyloid level in CSF found no significant

differences between AD patients and controls (Motter et al., 1995). Our results corroborate previous findings which also used ELISA procedures specifically recognising the longer amyloid β peptide ending at residue 42 (Aβ42; Motter et al., 1995; Galasko et al., 1998; Kanai et al., 1998; Shoji et al., 1998; Andreasen et al., 1999a; Hulstaert et al., 1999). It has been suggested that this decline may reflect an increased recruitment of Aβ42 from the CSF and the brain interstitial fluid to deposits in the form of plaques or decreased secretion into the CSF (Samuels et al., 1999). On the other hand, decreased CSF levels may be caused by increased deposition in cerebral vessels (Pirttilä et al., 1996). Although there was some overlap among groups, in our study even single measurement of Aβ42 reached high sensitivity for detecting AD and efficient specificity for discriminating OND and CO.

IL-6-IR showed marked overlap among groups and was elevated not only in AD, but also in OND and NAD groups versus CO. Several studies have measured CSF levels of IL-6 in Alzheimer's disease patients, but the results were ambiguous. Unchanged (Bauer et al., 1991; Hampel et al., 1997; März et al., 1997; Lanzrein et al., 1998; Engelborghs et al., 1999), increased (Blum-Degen et al., 1995) and decreased levels (Yamada et al., 1995) have been reported. Differences in sample size, patient selection or in experimental methodology may, in part, account for these differences. It has been suggested that a major part of CSF IL-6 originates from brain-derived synthesis by microglia and astrocytes (Frei et al., 1989). CSF levels may reflect its biological functions including the induction of the acute phase protein synthesis as well as the differentiation of neuronal cells and improvement of catecholaminergic and cholinergic cell survival and oligodendrocyte growth (Nijsten et al., 1987; Hama et al., 1991). Considering the broad overlap among groups, in particular NAD, its use as a sensitive and specific marker of the disease seems to be limited. As the local concentrations of cytokines in the brain tissue are different not only between controls and AD, but even within distinct areas of an individual brain analysed (Wood et al., 1993), locally restricted immunological mechanisms in AD may not be detected in CSF reliably. On the other hand, considering the lack of correlation of IL-6-IR with age, MMSE scores and disease duration in our study, it is tempting to speculate that, in single AD patients, the activity of IL-6 mediated immunological processes or the capability to support neuronal survival in the CNS could be estimated by CSF IL-6-IR measurements.

SPIR did not show significant changes in the total AD group. However, patients with late onset AD had significantly higher SPIR levels than patients with early onset of the disease and controls. Previous studies have reported decreased SPIR in the CSF of AD (Cramer et al., 1985; Martinez et al., 1993), but the changes were rather moderate and in one study only detectable when including severely dementing patients suggesting that the decrease of lumbar SPIR in AD may be a secondary and less specific change (Cramer et al., 1985). As mentioned, there is strong evidence for the involvement of immunological processes in the pathogenesis of AD. For instance, a post mortem study has detected elevated IgG synthesis rates in late onset compared with early onset

AD (Small et al., 1994). IL-6 has been shown to be present in early stages of plaque formation and to be restricted to the brains of AD patients (Hüll et al., 1995). As SP is an inductor of IL-6 expression (Gitter et al., 1994) and SPIR in our study correlated significantly with IL-6-IR in the AD group, elevated CSF SPIR possibly could indicate enhanced SP synthesis or release and, thus, explain elevated CSF-IL-6 in our study. Since the neurotoxic effects of amyloid β peptide have been reported to be blocked by substance P (Kowall et al., 1991), elevated SPIR could, hypothetically, indicate the presence of endogenous neuroprotective mechanisms modulated by SP in at least a subset of late onset AD patients.

In our study, TTIR was significantly elevated in AD compared with the other diagnostic groups. τ is a normal brain phosphoprotein which binds to microtubules in the neuronal axons, thereby promoting microtubule assembly and stability. Increased CSF-tau is believed to reflect ongoing neuronal and axonal degeneration or damage. During the past few years, an increase in CSF-(-total)τ in AD and even in patients with mild cognitive impairment later progressing to AD has been reported unequivocally (Vandermeeren et al., 1993; Hock et al., 1995; Tato et al., 1995; Rösler et al., 1996b; Galasko et al., 1998; Kanai et al., 1998; Shoji et al., 1998; Andreasen et al., 1999b; Green et al., 1999; Hulstaert et al., 1999, Ishiguro et al., 1999). Although in our study a marked increase of TTIR was also found in some cases after acute cerebral infarction and meningoencephalitis of various origin, this does not cause a major limitation of its clinical usefulness, since acute brain disorders usually are not considered in the differential diagnoses of AD. Despite some overlap between groups, single TTIR measurement reached high sensitivity for AD and efficient specificity for detecting OND and CO.

Our study shows that even single measurements of CSF Aβ42 and TTIR provide high sensitivity and specificity for detecting AD and for separating other neurological disorders without dementia and controls, but needs improvement regarding the discrimination of NAD. Statistical evaluation using discriminant function analysis revealed that TTIR and Aβ42-IR are the two variables out of five that contribute most to the group classification of patients. This finding is in accordance with recent studies proposing combined measurement of TTIR and Aβ42-IR for AD CSF diagnostics (Galasko et al., 1998; Kanai et al., 1998; Shoji et al., 1998; Hulstaert et al., 1999). At 85% sensitivity and 100% specificity for controls, our evaluation resulted in efficient specificity for separating OND and NAD, with specificity for NAD reaching 75%, thus improving recent cross-sectional results which reported, at sensitivity set at >80%, a maximum specificity of about 60% for NAD (Hulstaert et al., 1999). Interestingly, a total of eight patients with normal pressure hydrocephalus were diagnosed correctly. Although the small number of cases precludes a generalization, this result deserves further studies including more patients with secondary causes of dementia. According to current consensus criteria of the "Working Group on Molecular and Biochemical Markers of Alzheimer's Disease" (1998), an ideal biomarker for AD should have a sensitivity >80% for detecting AD and a specificity >80% for distinguishing other dementias. Our results approximate these criteria, thus arguing for the

application of combined CSF TTIR and Aβ42 measurement as a useful step in clinical routine diagnostics of dementing illnesses.

In conclusion, our results show that, regarding the selection of biomarkers presented herein, TTIR and Aβ42-IR are the most promising candidates for sensitive and specific CSF diagnostics of AD. On the other hand, ApoE-IR, IL-6-IR and SPIR may, at least in subsets of patients, be meaningful as accessory constituents of neurobiochemical CSF profiles in assessing the activity of immunological phenomena or the capacity of synaptic plasticity processes.

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