CSF-tau, CSF-\(\text{A} \beta 1-42\), ApoE-genotype and clinical parameters in the diagnosis of Alzheimer’s disease: combination of CSF-tau and MMSE yields highest sensitivity and specificity

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Summary. This study evaluated the sensitivity and specificity of the cerebrospinal fluid (CSF) levels of tau-protein, amyloid-\(\beta\)-peptide 1-42 (\(\text{A} \beta 1-42\)), ApoE-genotype and the degree of cognitive decline as diagnostic markers for Alzheimer’s disease (AD). Data was obtained from 105 AD patients and 68 controls.

Median CSF-tau levels were increased (512 pg/ml vs. 145 pg/ml, \(p<0.001\)) and \(\text{A} \beta 1-42\)-levels were decreased (238.5 pg/ml vs. 310 pg/ml, \(p<0.001\)) in AD patients compared to controls. A weak correlation was found between CSF-\(\text{A} \beta 1-42\) and MMSE score (\(r = .245\)). Within all subjects, a correlation of CSF-\(\text{A} \beta 1-42\) (\(r = -.337\)) and CSF-tau (\(r = .384\)) with age was found. The combination of CSF-tau levels and MMSE revealed the highest sensitivity (92\%) and specificity (87\%).

In summary, CSF-tau was a useful biological marker to discriminate AD from normal aging, neurological and psychiatric disorders. CSF-\(\text{A} \beta 1-42\) showed no additional benefit in discriminating patients from controls but might be useful for tracking the severity of the disease.

Keywords: Alzheimer disease, tau-protein, amyloid-beta 1-42

Introduction

Alzheimer’s disease (AD) is the most common cause of dementia. At lifetime, diagnosis of AD is based on clinical criteria, definite diagnosis can only be
confirmed by neuropathological examination after death (McKhann et al., 1984; Mendez et al., 1992). Diagnosing AD is especially difficult at the beginning of the disease since early symptoms are often unspecific and can as well be present in a variety of other disorders. Previous studies have shown that clinical diagnosis of AD can reach an accuracy of 85–90% if made by specialized centers (Klatka et al., 1996; Tierney et al., 1988; Kosunen et al., 1996; Jellinger et al., 1998). Nevertheless, at least 10 to 15% of patients are falsely diagnosed. Therefore, biological markers are required to confirm the diagnosis and to accelerate treatment of AD (e.g. acetylcholine esterase inhibitors).

According to the Consensus Report of the Working Group on “Molecular and Biochemical Markers of Alzheimer’s Disease”, the ideal diagnostic biomarker for AD should not only be able to detect a fundamental feature of Alzheimer’s neuropathology but should also detect AD early in its course and distinguish it from other dementias (Working group on molecular and biochemical markers of Alzheimer’s disease, 1998). Further, it should be reliable and validated in neuropathologically confirmed cases. So far, no biomarker for AD has yet fully met the proposed criteria. Especially two potential biomarkers in the CSF, tau-protein and amyloid β protein 1-42 (Aß1-42) have been under thorough investigation. Both reflect a central pathogenetic process of disease. Tau protein is a microtubule-associated protein, abundant in neurons and a major component of paired helical filaments, one of the pathological hallmarks of AD (Ishiguro et al., 1999; Blennow et al., 1995). Aß1-42 is deposited in senile plaques, another central pathological feature of AD (Nitsch et al., 1995).

Several groups have found elevated CSF tau levels (Blennow et al., 1995; Vigo-Pelfrey et al., 1995; Motter et al., 1995; Munroe et al., 1995; Arai et al. 1995; Sjoegren et al., 2001; Galasko et al., 1998) and reduced CSF Aß1-42 levels (Motter et al., 1995; Galasko et al., 1998; Andresen et al., 1999; Tamaoka et al., 1997; Mehta et al., 2000) in AD patients compared to age-matched controls. Measurement of tau protein alone yielded a sensitivity between 57% and 89% (Andreasen et al., 1999; Galasko et al., 1998; Hulstaert et al., 1999; Kurz et al., 1998; Sjoegren et al., 2000). For CSF Aß1-42, sensitivity ranged from 64% to 96% (Galasko et al., 1998; Hulstaert et al., 1999; Sjoegren et al., 2000; Andreasen et al., 2002). The combination of both measurements led to an increase in sensitivity and specificity in the diagnosis of AD (Galasko et al., 1998; Hulstaert et al., 1999; Sjoegren et al., 2000; Andreasen et al., 2001).

Apolipoprotein E (ApoE) genotype is an important risk factor for AD. ApoEε4 increases the risk for the development of AD whereas ApoEε2 is supposed to have a protecting effect. Previous studies have found differing results on the effect of ApoE genotype on CSF-tau and CSF-Aß42-levels (Mottre et al., 1995; Arai et al., 1995; Galasko et al., 1998; Hulstaert et al., 1999).

The aim of this study was to further evaluate the diagnostic potential of CSF tau, CSF Aß1-42 levels and ApoE genotype to get a combination of clinical and biological markers with the highest sensitivity and specificity to detect AD and discriminate it from other dementing disorders, neurological and psychiatric disorders and normal controls. Further, the association with clinical parameters in the diagnostic process of dementia was investigated.
Patients and methods

Patients

173 patients aged 17-87 years (median age 69 (17–87)) were investigated. 122 patients came consecutively for diagnostic evaluation of cognitive impairment to the Memory Clinic at the Department of Psychiatry whereas 51 patients came consecutively for diagnostic evaluation of neurological disorders to the Department of Neurology.

Clinical evaluation included detailed medical history, psychiatric, somatic and neurological status, a neuropsychological test battery, routine blood tests (incl. vitamin B12, folate acid, thyroid hormones), routine CSF tests, an electroencephalogram, a computed tomographic scan or magnetic resonance imaging and in some cases a positron emission tomographic scan. Mini-mental-Status examination was used for staging severity of cognitive impairment (Folstein et al., 1975). The examination took place prior to the start of any treatment affecting the central nervous system (e.g. acetylcholinesterase inhibitors, antidepressants or antipsychotic drugs).

Clinical diagnosis was based on the results of these diagnostic tests according to clinical standards and current research criteria. Dementia was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria. AD was diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984) and vascular dementia according to National Institute of Neurological and Communicative Disorders and Stroke-Association Internationale pour la Recherche et l’Enseignement en Neuroscience (NINCDS-ARIEN) criteria (Roman et al., 1993). Further, suspected frontotemporal dementia and dementia with Lewy bodies were diagnosed according to current research criteria (McKeith et al., 1996; Neary et al., 1998).

Of the 173 patients, 105 patients had AD. Within this group, there were 95 patients with probable AD and 10 patients with mixed forms of dementia. These mixed forms of dementia fulfilled the NINCDS-ADRDA criteria, presented the typical clinical pattern and course of AD, but had additional signs of a minor cerebrovascular disease in the computed tomographic scan. The Non-AD group consisted of 4 patients with vascular dementia (VD), 8 patients with other forms of dementia (OD), which included 2 patients with frontal lobe dementia, 4 patients with Lewy body dementia, one patient with dementia and Parkinson’s disease and one with dementia due to alcohol abuse, 39 patients with neurological disorders (i.e. epilepsy, multiple sclerosis, Parkinson’s disease, amyotrophic lateral sclerosis, focal pareses and infectious diseases) (NDI) and 8 patients with psychiatric disorders (5 patients with depression and 3 patients with other psychiatric diseases like psychosomatic or psychotic disorders) (PDI) and 9 patients without any disease at all (NC).

Biochemical analyses

Lumbar puncture was performed in a standardized way using atraumatic needles with patients lying on their back for two hours before puncture. CSF samples were immediately deep frozen on dry ice.

CSF-Aβ1-42 levels were measured in 150 patients at the Institute for Medical Biochemistry and Molecular Biology, Department of Molecular Cell Biology, University Hospital Hamburg, 27 patients were missing at random. CSF-Aβ1-42 was determined using a commercially available sandwich ELISA-test (Biosource International, Camarillo, CA, U.S.A.). CSF-tau levels were determined in all patients using the INNOTEST hTau-Antigen sandwich ELISA (Innogenetics, Ghent, Belgium) at the Max-Planck-Institute for Structural Molecular Biology, Hamburg. This test measures total tau (phosphorylated and unphosphorylated tau).

The apolipoprotein (Apo) E genotype was determined using the restriction isotyping method as described elsewhere (Hixon et al., 1990).

Statistical analyses

Univariate Data are presented as medians (range) for continuous data, and as frequencies for categorial variables. Correlations are determined according to Spearman’s Rho, for groupwise
comparisons Fisher’s Exact Test (categorial variables) and Mann-Whitney-U-Test (continuous variables) were used. All resulting p-values should be interpreted descriptively according to the principles of an explorative data analysis. Univariate diagnostic value was assessed using empirical ROC-Methods. To assess the combined diagnostic value of several variables a regression tree model was performed using S-Plus 4.5. All univariate analyses were done with SPSS 10.0.

Results

The aim was to find markers that discriminate best between AD and Non-AD-patients. Therefore, we combined all patients with vascular dementias, other dementias, neurological disorders, psychiatric disorders and normal controls to one control-group, named Non-AD-group. Table 1 shows gender distribution, median age, age of onset, duration of disease, MMSE-values, CSF-tau and Aβ1-42-levels and ApoE ε4 frequency for both diagnostic groups.

There were marked differences between the two groups concerning median age (AD- vs. Non-AD-group: 72 vs. 58 years, Mann-Whitney-U-test: p < 0.001), gender (31% vs. 54% male, Fisher’s exact test: p < 0.01) and median MMSE-values (19 vs. 28, Mann-Whitney-U-test: p < 0.001).

CSF-tau levels had a wide range from 18 pg/ml to 1861 pg/ml in total. There was a marked increase in median CSF-tau in the AD-group (512 pg/ml) in comparison to the Non-AD-group (145 pg/ml, Mann-Whitney-U-test: p < 0.001). Median CSF-Aβ42 were markedly decreased in the AD-group (239 pg/ml) when compared to the Non-AD-group (310 pg/ml, Mann-Whitney-U-Test: p < 0.001).

There was a negative correlation between CSF-levels of tau and Aβ1-42 (Spearman’s Rho: r = –.466, AD-group: r = –.307, Non-AD-group: r = –.109) and a weak positive correlation for CSF-tau with age (r = .384) in all patients (AD-group: r = .169, Non-AD-group: r = .479). In contrast, CSF-tau correlated negatively with MMSE-scores in all patients (r = –.542, AD-group: r = –.100, Non-AD-group: r = –.361).

CSF-Aβ1-42 showed a negative correlation with age (r = –.337, AD-group: r = –.024, Non-AD-group: r = –.389) and a positive correlation with MMSE in all patients (r = .402, AD-group: r = .245, Non-AD-group: r = .125).

68% of patients in the AD-group had one or more ApoEε4-alleles whereas only 31% of patients in the Non-AD-group had at least one ApoEε4-allele (Fisher’s exact test: p < 0.001). There was a significant increase of CSF-tau levels and a significant decrease of CSF-Aβ1-42-levels with rising numbers of ApoEε4-alleles (Kruskal-Wallis-Test: p < 0.001, see Table 2).

For sensitivity and specificity CSF-tau, CSF-Aβ1-42 and MMSE-scores were calculated separately first. By equally weighing false-positive and false-negative classifications, the optimal cut-off-level for tau was established at 279.5 pg/ml. Using this cut-off, sensitivity for diagnosis of AD was 0.84 (approximate 95%-confidence interval (CI): 0.80–0.87) with a specificity for diagnosis of Non-AD of 0.79 (CI: 0.75–0.84) With an optimal cut-off level for Aβ1-42 at 293.5 pg/ml sensitivity was 0.91 (CI: 0.88–0.94) and specificity was 0.56 (CI: 0.50–0.62). With a cut-off MMSE of 25.5 sensitivity was 0.93 (CI: 0.92–0.97) and specificity was 0.75 (CI: 0.67–0.78).
Table 1. Clinical and biochemical variables of the diagnostic groups

<table>
<thead>
<tr>
<th></th>
<th>Male (%)</th>
<th>Age (yrs)</th>
<th>Age of onset (yrs, n = 118)</th>
<th>Duration of disease (mths, n = 118)</th>
<th>MMSE</th>
<th>CSF-Tau (pg/ml, n = 150)</th>
<th>CSF-Aβ42 (pg/ml, n = 150)</th>
<th>ApoE4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer group</td>
<td>32** (30.5%)</td>
<td>72* (49–87)</td>
<td>68 (46–84)</td>
<td>35 (6–144)</td>
<td>19* (0–27)</td>
<td>512* (48–1579)</td>
<td>238.5* (115–366)</td>
<td>71* (67.6%)</td>
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<tr>
<td>(n = 105)</td>
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<tr>
<td>Non-Alzheimer group</td>
<td>37** (54.4%)</td>
<td>58* (17–83)</td>
<td>64 (51–76)</td>
<td>35 (2–120)</td>
<td>28* (7–30)</td>
<td>145* (18–1861)</td>
<td>310* (130–743)</td>
<td>21* (30.9%)</td>
</tr>
<tr>
<td>(n = 68)</td>
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<tr>
<td>Total (n = 173)</td>
<td>69 (39.9%)</td>
<td>69 (17–87)</td>
<td>67 (46–84)</td>
<td>35 (2–144)</td>
<td>22 (0–30)</td>
<td>336 (18–1861)</td>
<td>257.5 (115–743)</td>
<td>92 (53.2%)</td>
</tr>
</tbody>
</table>

*p = <0.001, **p = <0.01; yrs years, mths months
In a further step it was evaluated, whether the diagnostic value could be improved by using some of the before mentioned variables simultaneously. A regression tree model including the independent variables CSF-tau, CSF-\(\beta\)-1-42, MMSE, ApoE \(\varepsilon\)4-frequency, age and sex and using Alzheimer Disease Yes/No as dichotomous response variable was fitted.

The final tree model (after pruning manually) consisted of two variables and is presented in Fig. 1.

According to the model, people with MMSE > 28 are classified as Non-AD Patients and in a second step, for patients with MMSE of 28 or less, the value of tau is used for further discrimination. CSF-tau values above 250 pg/ml then lead to classification as Alzheimer patient, for people with CSF-tau \(\leq\) 250 pg/ml a third step is necessary in which subjects with MMSE < 18 are classified as Alzheimer patients whereas subjects with MMSE between 18 and 27 are classified as Non-Alzheimer-Patients.

**Table 2.** ApoE \(\varepsilon\)4-frequency and corresponding CSF-tau and CSF-\(\beta\)-1-42-concentrations

<table>
<thead>
<tr>
<th>No</th>
<th>CSF-Tau (pg/ml)</th>
<th>(\beta)-1-42 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Apo-E 4</td>
<td>63</td>
<td>208 (18–1861)</td>
</tr>
<tr>
<td>1 Apo-E 4</td>
<td>61</td>
<td>389 (84–1579)</td>
</tr>
<tr>
<td>2 Apo-E 4</td>
<td>16</td>
<td>598 (305–1280)</td>
</tr>
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</table>

**Fig. 1.** Classification tree
The Separation Line between Alzheimer und Non-Alzheimer Patients can be seen in Fig. 2.

Using this classification, 97 of 105 AD-patients are correctly classified which corresponds to a sensitivity of 0.92 (CI: 0.87–0.97) and 58 of 68 Non-AD patients are correctly classified which equals a specificity of 0.87 (CI: 0.79–0.95).

It should be noted that adding one or more of the additional four variables (especially age) in the model did not result in better discriminative power.

Despite of small sample size in some subgroups, we also calculated sensitivity and specificity values for all diagnostic groups separately. Sensitivity was 92% for AD (N = 95) and 100% for mixed forms of dementia (N = 10). Specificity of diagnosis varied between the different diagnostic groups with 100% in the NC-group (N = 9) and PDI-group (N = 8), 86% in the NDI-group (N = 39), 75% in the VD-group (N = 4) and 67% in the OD-group (N = 8).

**Discussion**

The aim of this study was to evaluate the usefulness of clinical parameters and biochemical markers alone and in combination in the diagnosis of Alzheimer’s disease. In our study, best sensitivity and specificity is found using the combination of CSF-tau-levels and MMSE-scores. Previous studies have analyzed the usefulness of a combination of the biochemical markers CSF-tau and CSF-Aβ1-42 (Galasko et al., 1998; Hulstaert et al., 1999; Sjoegren et al., 2000; Andreasen et al., 2001; Briani et al., 2002). To our knowledge, the combination of biological and clinical parameters has never been tested before. Remarkably, in our
study, the combination of CSF-tau and MMSE discriminates better between AD and controls than a combination of CSF-tau together with CSF-Aβ1-42. Further, it is interesting that CSF-tau levels discriminate best between AD patients and controls in the MMSE range between 18 and 28 points which means that they contribute to diagnosis in the early stages of dementia. Nevertheless, overall classification would be slightly worse if one would solely look at mildly impaired patients (MMSE > 20) since most AD patients which are falsely identified as controls have an MMSE between 20 and 25.

One critical point of this study is the selection of the control group. First of all, there might be concerns about the difference in median ages between AD and controls in combination with the correlation of both CSF markers with age. These findings could suggest that age plays an important role in the differentiation of our groups. However, adding age to our model did not result in better discriminative power between AD and Non-AD group. This fact should diminish concerns about interactions between age and the biological markers influencing validity of the results. Secondly, our control group consists of a broad spectrum of other dementias, psychiatric and neurological disorders and normal controls. This is very interesting from a clinical point of view because many of these diseases are quite regularly seen in larger memory clinics and it’s therefore of great clinical interest to look at the possibility of our method to distinguish different diseases from AD. Of course, no general conclusion can be drawn out of these results due to small sample size in some of the subgroups (e.g., VD, ND, PD). Thus an evaluation of the final model on an independent sample with a carefully selected control group is definitely necessary before applying it in clinical practice.

Overall, our results confirm the hypothesis that CSF-tau is a useful biological marker in the diagnosis of AD but question the role of CSF-Aβ1-42 in the diagnostic process. Interestingly, both biological markers show a correlation with MMSE in all subjects suggesting a relationship between them and cognitive decline in a broad spectrum of diseases. However, only CSF-Aβ1-42 correlates slightly with severity of dementia in AD patients. Therefore, it might serve as a marker for progression of disease as described in earlier studies as well (Kanai et al., 1998; Shoji et al., 1998; Hock et al., 1998; Riemenschneider et al., 2000, 2002).

Using the described diagnostic model, all psychiatric patients and all normal controls within the Non-AD-group are correctly identified. This is especially important within the group of psychiatric patients who had MMSE-values of 18, 22 and 26 points plus depressive disorders, because this combination is especially difficult to discriminate from dementia in the clinical practice. This result agrees with studies of Sjoegren et al. (2001) and Andreasen et al. (2001) who also correctly identified all psychiatric patients but used a combination of tau and Aβ1-42 in the CSF. Within the group of vascular dementias three of four patients were recognized correctly. Consistent with previous findings we found elevated CSF-tau levels in two of four VD-patients (Sjoegren et al., 2000; Andreasen et al., 2001; Schoenknecht et al., 2000; Hesse et al., 2000). The reason for this phenomenon is still unknown, most likely, it is due to an additional AD-pathology which is clinically disguised by the apparent vascular
findings. 40–80% of the neuropathologically examined cases which were clinically diagnosed as vascular dementia exhibit AD features (Andreasen et al., 1999; Galasko et al., 1994). In the group of patients with other forms of dementia, three of eight patients were classified falsely. One patient with Lewy-body-dementia, one patient with frontotemporal lobe dementia and one patient with dementia due to alcohol abuse had elevated CSF-tau levels. These diagnoses were made according to leading clinical symptoms. However, in these cases an underlying AD pathology can also not be excluded and might be responsible for the high CSF-tau-values. Previous studies also have reported elevated CSF-tau in patients with Lewy-body dementia (Kanemaru et al., 2000; Montine et al., 2001; Shoji et al., 2002) and frontotemporal dementia (Riemenschneider et al., 2002; Shoji et al., 2002). In the group of neurological disorders 6 of 39 patients (15.6%) were incorrectly classified. Two of these six patients had epilepsy, one amyotrophic lateral sclerosis, one ataxia, one cerebral infection and one desorientated syndrome of unknown origin. Altogether, 8% of AD patients and 13% of Non-AD patients are still falsely classified. Therefore, further markers for AD have to be established.

This discussion shows that this study and most other studies concerning biochemical markers have their limitations. In all studies, a clinical diagnosis is correlated with other potential diagnostic parameters. Nevertheless, clinical diagnosis maybe not correct since there is no neuropathological validation of diagnosis. In questionable cases it remains unclear if the clinical diagnosis is wrong or if the biochemical parameters are not good enough. Therefore, there is a need for neuropathologically confirmed studies which is particularly difficult within this patient group.

However, the results of this study present a new and promising approach in the diagnosis of AD. Whereas in previous studies it has been tried to improve accuracy of diagnosis by combining CSF-tau and CSF-Aβ1-42 levels, we combined several biological and clinical markers and found best discrimination with a combination of the biochemical marker tau and the clinical marker MMSE. We suggest that a combination of biochemical and clinical markers might improve sensitivity and specificity in the diagnosis of AD and might be especially useful in the discrimination of AD and psychiatric disorders that might mimic clinical features of AD. However, due to the limited number of patients in our study, the use of this approach has to be verified in further prospective studies with independent age-matched samples.

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References


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