Alzheimer CSF biomarkers in routine clinical setting


Objectives – Our work was aimed to evaluate Alzheimer's disease diagnosis improvement using cerebrospinal fluid biomarkers (CSF) in neurological daily practice. Materials and Methods – For this purpose, 150 patients clinically and neurochemically classified as having AD or cognitive impairment with or without other dementia type were included in the study. The following CSF peptides were studied, blindly to the clinical diagnosis: beta-amyloid₁₋₄₂ peptide (Ab₁₋₄₂), Tau (T-tau), threonine-181 hyperphosphorylated tau protein (P-tau₁₈₁), and beta-amyloid₁₋₄₀ peptide (Ab₁₋₄₀). From these measurements, Innotest® Amyloid Tau Index (IATI) was calculated for each patient. Results – This assessment allowed to separate 83 biochemical profiles of AD and 67 non-Alzheimer's disease (non-AD), both AD and non-AD categories match with clinical data amounting to 73% and 90%, respectively. Among mild cognitive impairment (MCI) patients, CSF biomarkers led to discriminate those who are likely to be AD. We devoted a special section to Ab₁₋₄₀ which is not a routine parameter but can help to confirm a pathological amyloid process as Ab₁₋₄₂/Ab₁₋₄₀ ratio underlining the real decline of the Ab₁₋₄₂. Conclusions – The interest of biomarkers and their ability to solve awkward cases were carefully noticed all the more when a discrepancy between clinical and CSF biological data was involved. The final proposed algorithm allowed to identify pathogenic forms of AD according to the prevailing role of hyperphosphorylated tau or amyloid beta peptide.

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Key words: Alzheimer's disease; Ab₁₋₄₂ peptide; Ab₁₋₄₀ peptide; T-tau; P-tau₁₈₁; Innotest® Amyloid Tau Index

M. Rigaud, ASTRALAB, 7-11 Avenue de Lattre de Tassigny, 87000 Limoges, France
Tel.: 00 33 5 55 30 29 30
Fax: 00 33 5 55 48 85 01
e-mail: rigaud.michel@yahoo.fr.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia increasing twofold every 5 years after 65 years, finally rising to 40% after 90 years (1).

Alzheimer's disease (AD) is a progressive, neurodegenerative disease anatomically defined by abnormal clumps, tangled fiber bundles involving deposition of amyloid beta peptide and intraneuronal lesions with neurofibrillary degeneration owing to the accumulation of hyperphosphorylated tau (1). Increasing age and genetic background are known risk factors for AD. The e4 allele of Apolipoprotein E (APOE) is the major genetic factor associated with early onset (2). A large majority of patients are not eligible for systematic APOE genotyping, this test therefore being of limited use in daily practice. Till now, diagnosis criteria (DMS IV and NINCDS-ADRDA) (3) are primarily exclusion criteria, poorly specific of the pathology, and lacking sensitivity in early stages.

In addition to memory impairment, new diagnosis criteria have been added: Magnetic Resonance Imaging (MRI) showing hippocampal or temporal pole atrophy, abnormal levels of CSF biomarkers, particular aspect of functional neuroimaging, identified gene mutation in familial cases (4–6). Since 2008, such recommendations are advocated by H.A.S (Haute Autorité de Santé, French Health Authority).

It is admitted that, before dementia stage, there is a progressive cognitive decline reported as heterogeneous concept of MCI (mild cognitive impair-
Cerebrospinal fluid biomarkers (CSF) biomarkers quantification has shown the benefits of Aβ sensitivity and specificity of methods. In AD, numerous data have been reported on the CSF biomarkers as witnesses of the pathological process. Patients with AD present with a characteristic pattern: decreased beta-amyloid1–42 peptide (Aβ1–42), increased total tau (T-tau), and threonine-181 hyperphosphorylated tau protein (P-tau181) (8–10). Performances obtained by different laboratories dealing with assessment of CSF biomarkers are reported in Table 1 (11–21). The pitfall concerning biomarkers value differences needs to be underlined, and besides a uniform standardization of pre- and analytical procedures, multicentre comparison is required to assess sensitivity and specificity of methods.

Cerebrospinal fluid biomarkers (CSF) biomarkers quantification has shown the benefits of Aβ1–42, T-tau and P-tau181 measurement for diagnosis of Alzheimer-type dementia in clinical routine (10). Changes of these specific proteins, amyloid, and tau in CSF are connected with the amount of brain damage (19, 22, 23). P-tau181 and T-tau increase is associated with neocortical neurofibrillary pathology (23); Aβ1–42 reduction is correlated to the density of neuritic plaques (19). There is a link between functional imaging, cortical amyloid labeling by PIB (C Pittsburgh Compound B, radio tracer), and Aβ1–42 decrease (24). Evaluation of cerebral metabolism by PET or SPECT showed that the increase of total tau and P-tau181 in Alzheimer patients’ CSF was linked to hypometabolism in temporal regions (25).

### Table 1 Concentrations of Aβ1–42, T-tau, and P-tau181 in CSF obtained from literature studies

<table>
<thead>
<tr>
<th>References</th>
<th>No of AD patients</th>
<th>Aβ1–42</th>
<th>T-tau</th>
<th>P-tau181</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hulstaert et al. (11)</td>
<td>150</td>
<td>556*</td>
<td>239*</td>
<td>ND</td>
</tr>
<tr>
<td>Andreassen et al. (12)</td>
<td>208</td>
<td>523 ± 180</td>
<td>759 ± 417</td>
<td>ND</td>
</tr>
<tr>
<td>Hampel et al. (13)</td>
<td>93</td>
<td>545 ± 230</td>
<td>725 ± 266</td>
<td>ND</td>
</tr>
<tr>
<td>Ibach et al. (14)</td>
<td>76</td>
<td>463 ± 218</td>
<td>615 ± 362</td>
<td>ND</td>
</tr>
<tr>
<td>Ibach et al. (14)</td>
<td>76</td>
<td>530*</td>
<td>348*</td>
<td>65*</td>
</tr>
<tr>
<td>Vanderstichele et al. (15)</td>
<td>94</td>
<td>378 (285–484)</td>
<td>610 (416–868)</td>
<td>84 (54–106)</td>
</tr>
<tr>
<td>Parnetti et al. (16)</td>
<td>100</td>
<td>411 ± 148</td>
<td>628 ± 314</td>
<td>136 ± 59</td>
</tr>
<tr>
<td>Lunari and Parnetti (17)</td>
<td>18</td>
<td>402 ± 195</td>
<td>402 ± 318</td>
<td>91 ± 31</td>
</tr>
<tr>
<td>Henneman et al. (18)</td>
<td>31</td>
<td>474 ± 113</td>
<td>811 ± 486</td>
<td>92 ± 38</td>
</tr>
<tr>
<td>Tapiola et al. (19)</td>
<td>123</td>
<td>515*</td>
<td>350*</td>
<td>52.5*</td>
</tr>
<tr>
<td>Wallin et al. (20)</td>
<td>151</td>
<td>398 ± 97</td>
<td>620 ± 349</td>
<td>77 ± 33</td>
</tr>
<tr>
<td>Kester et al. (21)</td>
<td>47</td>
<td>474 (335–602)</td>
<td>482 (393–853)</td>
<td>77 (50–103)</td>
</tr>
</tbody>
</table>

These concentrations (mean ± SD or *cut-off values) were determined using Inogenetics assays. Results are expressed in ng/l.

Wiltfang et al. (26) proposed Aβ1–40 peptide measurement as a tool to overcome inter-individual variability, then checking the value of Aβ1–42/Aβ1–40 ratio. Aβ1–40, the predominant form of soluble Aβ peptides in brain and CSF, is not a routine parameter. We evaluated it in a forward-looking purpose. This analysis enabled to assess more precisely amyloid disease profile for patients whose initial CSF biomarker profiles are not discriminating.

Our work was aimed to evaluate AD diagnosis improvement using CSF in neurological daily practice. As far as this matter is considered, as post-mortem confirmation is scarcely available, follow-up examination was carried to confirm the clinical diagnosis.

### Materials and methods

**Patients and clinical assessments**

Between October 2008 and March 2010, 150 patients were included: 61 men, 89 women with an average age of 72.2 years (44–86). Patients consulted a neurologist for cognitive disorders assessment. Patient clinical evaluation consisted of history, evolution, effects on daily life activities, clinical and neurological examination, neuropsychological rating (Mini mental state examination (MMSE), clock test, five word test of Dubois, verbal fluency), blood tests as recommended by the H.A.S, and radiological assessment with either CT scan or MRI. A complete neuropsychological check was performed: memory tests including Grober–Buschke test, executive functions tests (TMT, BREF: short frontal evaluation test, STROOP test) and, when necessary, other evaluations.

Petersen criteria were used for the diagnosis of MCI (7), NINCDS-ADRDA criteria for the diagnosis of AD (3), Neary criteria for the diagnosis of fronto-temporal dementia (27), and McKeith’s criteria for Lewy bodies dementia (28). The diagnosis of mixed dementia was established for patients with vascular risk factors, imaging showing stroke sequelae or major vascular lesions, i.e., leukopathy with cortical and/or subcortical atrophy without selective hippocampal topography. The neuropsychological assessment of these patients indicated an overall impairment of cognitive functions with executive functions, praxis, memory and phasic disorders being involved without dominant hippocampal damage.

The initial clinical diagnosis was set out without knowledge of CSF biomarker results. Patients were grouped into three clinical categories: AD, non-AD, and MCI.
Biological samples

After lumbar puncture (the patients or their caregivers gave written information consent in accordance with the Helsinki Declaration of 1975), CSF was directly collected in a polypropylene tube and sent to laboratory within 4 h, centrifuged at 2500 rpm for 15 min. Aliquots in polypropylene tubes were stored at −80°C. CSF frozen samples were analyzed after thawing.

Biochemical evaluation

All markers (Aβ1–42, T-tau, P-tau181) were assayed by ELISA following supplier recommendations (Innotest®; Innogenetics NV, Gent, Belgium). Aβ1–40 was quantified by ELISA (Human Amyloid β(1–40) (N) Assay kit-IBL-Japan handed out by Innogenetics NV, Belgium).

Aβ1–42 (29) and T-tau (19, 30) mean values, respectively, 500 and 350 ng/l have been proposed as pathological thresholds. The IATI is an index derived from the Hulstaert discrimination line: IATI = (measured Aβ1–42)/(240 + 1.18*measured Tau) (11, 12).

A control subject with normal Aβ1–42 and T-tau values has an IATI > 1. A patient with possible AD, with a lowered Aβ1–42 value and increased tau value, has an IATI < 1. A 95% sensitivity to diagnose AD patients vs normal patients/other neurological disorders has been associated with a IATI value <0.8. IATI value >1.2 allows to rule out AD with a 95% specificity and classify among ‘normal/other neurological disorders’ profiles (31, 32). P-tau181 cut-off value used in this study was 50 ng/l (19).

The CSF profile is characterized by a biochemical algorithm. If ‘IATI < 0.8 and P-tau181 > 50 ng/l’, CSF profile is considered as AD biochemical profile. When results are not included in this algorithm, the pattern is qualified as ‘other profile’. If ‘IATI > 1.2 and P-tau181 < 50 ng/l’, CSF profile is considered as normal. If ‘IATI < 0.8 or P-tau181 > 50 ng/l’, CSF profile is not considered as typical or distinctive. Both normal and non-discriminating profiles are biochemically classified as non-AD. The biochemical classification is thereafter compared to the clinical one.

Statistical analysis

The distribution of parameter values between both groups (IATI < 0.8 + P-tau181 > 50 ng/l, other CSF profiles) was analyzed using a Kolmogorov–Smirnov test.

Owing to size and asymmetrical distribution of groups, a non-parametric test was conducted (Kruskal–Wallis) for group comparison.

Results

All patients were clinically classified: of the 150 patients, 97 were AD, 29 non-AD, the others 24 patients belonging to the MCI group. All CSF samples were frozen and analyzed after thawing.

The biochemical classification was then applied to the entire cohort. Results are presented in Table 2. Eighty-three patients had an AD biochemical profile (IATI < 0.8 + P-tau181 > 50 ng/l): 71 upon 97 AD, 3 upon 29 non-AD, and 9 upon 24 MCI clinical diagnoses. In the cohort, 67 patients had other biochemical profiles: 26 upon 97 AD, 26 upon 29 non-AD, and 15 upon 24 MCI patients. Relative to AD or non-AD clinical diagnoses (not including MCI), there was a mismatch for 29 cases. Concordance between first clinical feeling and CSF profile was 77% overall. In AD and non-AD groups, the match was 73% and 90%, respectively.

Using Kolmogorov–Smirnov test, a significant distribution difference between the two CSF profile groups was found for T-tau, Aβ1–42, IATI, P-tau181, Aβ42/P-tau181 ($P < 0.0001$). The comparison of age and MMSE between these two groups showed no significant difference for MMSE (data not shown).

Table 3 (upper part) shows T-tau, Aβ1–42, IATI, P-tau181, Aβ42/P-tau181 average values in patients with AD biochemical profile (IATI < 0.8 + P-tau181 > 50 ng/l) for the clinically AD patients (71/97) and for those with other CSF profile and who are non-clinically AD (26/29). The Kruskal–Wallis statistical analysis (Fig. 1) confirmed the significant difference between AD and non-AD groups for all parameters ($P < 0.0001$). Table 3 (middle part) delineates the CSF biomarker values.

### Table 2. CSF profiles and clinical categories

<table>
<thead>
<tr>
<th>Clinical profile</th>
<th>AD</th>
<th>Non-AD</th>
<th>MCI</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>73.1</td>
<td>70.7</td>
<td>69.3</td>
<td>72.04</td>
</tr>
<tr>
<td>SD</td>
<td>7.2</td>
<td>6.2</td>
<td>10.5</td>
<td>7.74</td>
</tr>
<tr>
<td><strong>MMSE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>20.7</td>
<td>22</td>
<td>27.6</td>
<td>22.14</td>
</tr>
<tr>
<td>SD</td>
<td>4.4</td>
<td>4.4</td>
<td>1.5</td>
<td>4.78</td>
</tr>
<tr>
<td>IATI &lt; 0.8 + P-tau181 &gt; 50 ng/l</td>
<td>71</td>
<td>3*</td>
<td>9</td>
<td>83</td>
</tr>
<tr>
<td>Other CSF profiles</td>
<td>26*</td>
<td>26</td>
<td>15</td>
<td>67</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>29</td>
<td>24</td>
<td>150</td>
</tr>
</tbody>
</table>

*Mismatch between CSF biochemical profiles and clinical diagnoses.
for three patients showing an AD biochemical profile despite the fact they were clinically non-AD. Table 3 (lower part) reports results concerning 26 clinically AD patients belonging to biochemical group ‘other combinations’. They are classified into three groups (A, B, and C). Statistical analysis using Kruskal–Wallis test was applied for group comparison. Group A corresponded to eight patients having low P-tau but a disrupted IATI (IATI < 0.8) owing to Aβ1–42 low value. This group was significantly different from AD group concerning T-tau and P-tau181 suggesting that these parameters were not the pathological process predominant factor. Group B gathered eight patients with positive P-tau181 (>50 ng/l) and IATI included between 0.8 and 1.2 and normal Aβ1–42 (>500 ng/l). Group C was constituted by ten patients with IATI superior to 1.2 or by those with P-tau181 level lower than 50 ng/l. Group C classified as clinical AD had biological markers significantly different (P < 0.0001) from AD group. Their biochemical data were statistically similar to those of the non-AD population.

Owing to the observed discrepancies, Aβ1–40 quantification was performed to gain additional information either through its own value or Aβ1–42/Aβ1–40 ratio when CSF profile was non-discriminant (IATI < 0.8 or P-tau181 > 50 ng/l). According to Wiltfang (26), Aβ1–40 value and Aβ1–42/Aβ1–40 ratio better correlate with the total Aβ peptides load than the patient pathological status.

Thus in group A, Aβ1–42/Aβ1–40 ratio underlined a prevailing pathological amyloid process. In group B, a significant difference with regard to AD group for Aβ1–42/Aβ1–40 ratio indicated absence of pathological amyloid process suggesting a major pathogenic role of T-tau and P-tau181 (P < 0.017 and 0.054, respectively, for T-tau and P-tau181 vs the non-AD group). Moreover for three patients of this group, Aβ1–42/Aβ1–40 ratio indicated that the initial value of Aβ1–42 could be considered low if referred to global Aβ peptides concentration. In group C, Aβ1–42/Aβ1–40 ratio was above the 0.05 cut-off value in accordance with the non-AD biochemical profile.

As far as MCI is concerned, because first clinical examination is unable to predict disease development, we classified them in a different section (Table 4). Of 24 patients clinically classified as MCI, nine presented with a characteristic AD biochemical profile. For every one of them, as regards P-tau181, Aβ1–42, IATI values, obvious differences were shown between progressive and stable disease (respectively P = 0.001, P = 0.003, P = 0.001). Furthermore, there are no significant differences, on the one hand between AD and progressive MCI and, on the other, between non-AD and stable MCI (data not shown).

**Discussion**

The purpose of this study was to appraise contribution of CSF biomarkers in daily clinical practice. For either public or liberal memory consultation centers, conclusions seemed similar. When biomarkers fit in with clinical assessment, they allow to assert more firmly the clinical impression, thus contributing to a faster and more reliable diagnosis. Numerous studies have shown the CSF biomarkers combination to be more effective than cognitive profile alone (20). Our data demonstrated the ability of the elected combination, i.e., IATI (<0.8) and P-tau181 (>50 ng/l) to conclude to Alzheimer’s pathology.
(73%), and turn it out with a 90% reliability. A more firmly set diagnosis thus allowed better coping with patient and family.

Analysis of AD and non-AD population (defined by the clinico-biochemical concordance) showed, for each of the parameters or ratio, similar

### Table 4 CSF biological values in evolutive and stable MCI

<table>
<thead>
<tr>
<th></th>
<th>T-tau ng/l</th>
<th>Aβ1–42 ng/l</th>
<th>IATI</th>
<th>P-tau181 ng/l</th>
<th>Aβ1–42/P-tau181</th>
<th>Aβ1–42/Aβ1–40</th>
<th>Aβ1–40 ng/l</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Evolutive MCI (n = 9)</strong></td>
<td>695 ± 261</td>
<td>396 ± 133</td>
<td>0.42 ± 0.12</td>
<td>114 ± 41</td>
<td>3.99 ± 2.4</td>
<td>0.0029 ± 0.010</td>
<td>15982 ± 5173</td>
</tr>
<tr>
<td><strong>Stable MCI (n = 15)</strong></td>
<td>234 ± 64</td>
<td>763 ± 233</td>
<td>1.47 ± 0.40</td>
<td>49 ± 13</td>
<td>15.84 ± 4.94</td>
<td>0.072 ± 0.018</td>
<td>10943 ± 2902</td>
</tr>
</tbody>
</table>

Figure 1. Kruskal–Wallis statistical analysis. Determination of clinical-biochemical correlation or discrepancy. * non-AD group differs significantly from AD group ($P < 0.0001$).
values to those described in the literature. The analysis of Receiver Operating Characteristic (ROC) curves analysis (data not shown) for each parameter between the two reference groups sets up following thresholds: 474 ng/l (Sens. 95%; Spe.92%) for Aβ₁₋₄₂; 336 ng/l (Sens.75%; Spe.88%) for T-tau; and 57 ng/l (Sens.87%; Spe.69%) for P-tau₁₈₁. The respective 350 and 500 ng/l thresholds for Tau and Aβ₁₋₄₂ thus perfectly apply to the two groups. P-tau₁₈₁ thresholds usually vary between 50 and 60 ng/l (30, 32). To increase sensitivity, we chose to use the 50 ng/l limit. Likewise, the 9 threshold Aβ₁₋₄₂/P-tau₁₈₁ ratio value, first advocated by Welge et al. (33), correctly separates different groups (Sens. 98.6%; Spe.85%).

Concerning Aβ₁₋₄₀ peptide dosage, its only value was not contributive to diagnosis but enabled us to establish Aβ₁₋₄₂/Aβ₁₋₄₀ ratio. This later used with a 0.05 threshold can be of additional help to confirm the amyloid involvement in the pathogenic process (Sens. 90.5%; Spe. 80%). Owing to a distinct methodology, our threshold differs from Wiltfang’s one (26). Biochemical results, if limited to Aβ₁₋₄₂, T-tau, P-tau₁₈₁, are unable to provide every time definite endings. This lack of information can be overcome with help of the Aβ₁₋₄₂/Aβ₁₋₄₀ ratio, enabling to separate two groups of patients (A and B) depending upon etiopathogenic pathways, either Aβ amyloid or T-tau and P-tau₁₈₁. Although non-systematic, this dosage seems to be able to spare complementary imaging, thus reducing costs. Additional Aβ₁₋₄₀ measurements were performed on previously frozen aliquots. We also focused on atypical cases. Three patients clinically considered as non-AD showed a typical AD CSF profile without any MRI atrophy. Follow-up eventually brought up evidence for AD development. Numerous records have clearly shown that CSF biomarkers are susceptible to disclose an incipient AD disease (30, 34). Pure usual dementia forms are not the rule in elderly people (35). Senile amyloid patches are evidenced by post-mortem examination of patients with Parkinson’s disease (36). Thus, routine CSF biomarkers use is advisable when clinical assessment is puzzling, and facing Parkinson’s disease cognitive impairment, we were able to evidence typical AD profile in some patients.

Of 24 MCI patients 9 had an AD profile, meeting up well with hippocampus atrophy on MRI examination. Biomarkers are a convincing argument to predict disease progression from MCI to AD (20, 30). Follow-up (18 months) of these nine MCI patients confirmed the prodromal AD diagnosis established by CSF biomarkers, thus underlining their relevance in the earliest phase of the disease. Of 15 stable MCI, one of them showed hippocampus atrophy along with normal CSF biomarkers. As a clinical diagnosis mistake was turned down, further close monitoring is required.

**Conclusion**

This study confirms the paramount significance of CSF biomarkers measurements in AD diagnosis in routine practice. Pathognomonic results allow to

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![Figure 2. Biomarkers integration in daily clinical practice.](image-url)
assert the diagnosis in the possible or likely forms and, moreover, in the occurrence of associated pathologies (vascular disease, psychiatric, basal ganglia disease, and others) synonymous with a wandering clinical diagnosis. Non-specific CSF biomarkers results require further investigation as other diagnoses have to be looked for. Including CSF biomarkers into the diagnostic processes seems to be relevant as summarized in the Fig. 2 algorithm.

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Conflict of interest

The authors declare no conflict of interest.

References


