The first laboratory test for Alzheimer’s disease and related degenerative diseases

According to the French Health Ministry, neurological degenerative diseases affect 1,000,000 people in France, with 165,000 new cases diagnosed annually, of which 80% are reported as Alzheimer’s disease. There is an inexorable increase in Alzheimer’s disease and related diseases with age: 1 in 4 women and 1 in 5 men are affected after the age of 85 years. These alarming figures have resulted in the introduction for the period 2008-12 of the Alzheimer plan, a specific government initiative for funding of research into therapy, improvement of patients’ daily life and slowing of the inevitable progression towards profound dementia.

- Neuropathology

At present, certain diagnosis of Alzheimer’s disease (AD) may only be made on the basis of a number of histopathological features and thus on post mortem results. These criteria consist of the coexistence within the cerebral cortex of two specific neuropathological lesions:

✓ neurofibrillary degeneration comprising TAU (tubulin-associated unit) protein.
✓ amyloid deposits, extracellular aggregates of peptide Aβ42 forming senile plaques.

Such lesions are evident on immunohistochemical study of brain sections.

- Laboratory Tests

Current laboratory tests are based on assay of proteins involved in the disease. These various pathological components were discovered during the 1980s and in 2001 the research team of Kaj Blennow suggested use of these biomarkers in the diagnosis of Alzheimer’s disease. Assays are performed on CSF, the medium most representative of cerebral biochemistry. The principal proteins found in intracellular and extracellular lesions specific to Alzheimer’s disease are total Tau protein, phosphorylated Tau protein and peptide Aβ42.

VALUE OF CSF BIOMARKERS: TAU PROTEIN, PHOSPHO-TAU PROTEIN, β-AMYLOIDE (peptide Aβ42)

A number of different processes result in the formation of fibrillary degeneration products that accumulate within nerve cells leading to neuronal death. Neuronal destruction causes release of Tau protein normally found within axons, as well as aggregation of protein Aβ42 to form so-called senile plaques. Following its release into the extracellular medium, Tau protein causes an increase in CSF of aggregated amyloid protein which does not have the same degree of immunoreactivity but rather a lower level.

Sample tubes and the decanting tubes can be obtained from our laboratory upon request:
☎ +33.1.34.40.20.20
www.lab-cerba.com
(rubrique: analyses informations /consumables)

**In Practice…**

- **SAMPLE**
  ✓ Collect 3 ml of CSF in a propylene tube (e.g. Falcon)

- **PRETREATMENT**
  (in accordance with the recommendations of the SFBC working group)
  ✓ To be performed within four hours of lumbar puncture
  ✓ Homogenise carefully to avoid any concentration gradient
  ✓ Centrifuge to eliminate cells and other insoluble matter (10 min at 4000 g and at +4 °C)
  ✓ Place 2ml Aliquots of CSF in suitable propylene tubes

**STORAGE:** FROZEN

**METHOD:** ELISA

**RUN FREQUENCY:** Twice monthly
Furthermore, Alzheimer’s disease (AD) presents a specific biochemical feature not seen in other neurodegenerative diseases, namely highly phosphorylated Tau protein (see table); assay of this specific form of the protein thus provides vital information that frequently allows differential diagnosis to be made.

### Diagnostic value of biomarkers in CSF (from K. Blennow)

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Tau</th>
<th>Ph-Tau</th>
<th>Aβ42-amyloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal senescence</td>
<td>Normal (&lt; 500 pg/ml)</td>
<td>Normal (&lt; 60 pg/ml)</td>
<td>Normal (&gt; 500 pg/ml)</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>moderate</td>
<td>moderate</td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Alcoholic dementia</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Frontotemporal dementia</td>
<td>N or slight</td>
<td>N or slight</td>
<td>N or slight</td>
</tr>
<tr>
<td>Lewy body dementia</td>
<td>N or slight</td>
<td>N</td>
<td>slight to moderate</td>
</tr>
<tr>
<td>Creutzfeld-Jakob disease</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>transient</td>
<td>unchanged</td>
<td>unchanged</td>
</tr>
<tr>
<td>Vascular dementia</td>
<td>Divergent data</td>
<td>N</td>
<td>N or slight</td>
</tr>
</tbody>
</table>

Total Tau protein assay is specific for neuronal death (intranuclear Tau protein released into the extracellular medium passes into CSF, where its concentration rises).

Peptide Aβ42 assay is specific for amyloid deposits, since peptide Aβ42 is sequestered in these aggregates and its concentration in CSF is thus reduced.

Increased phosphorylation of Tau protein is specific for Alzheimer’s disease.

#### INTERPRETATION OF RESULTS

The reference values for Tau, phospho-Tau and Aβ42 have been determined in the literature, thus providing quantitative values for each of these proteins.

The results must be interpreted together for two reasons:
- Concentrations of Tau protein in CSF increase with age whereas concentrations of peptide Aβ42 remain unchanged. However, there is no gender-related variation. Consequently, for each test a simple index that takes into account these physiological variations is calculated, i.e. the index IATI (Innotest Amyloid Tau Index), which is > 1.2 in healthy subjects (figure 1).
- Assay of phosphorylated Tau protein is essential for diagnosis of AD, and results thus also give a specific value for this protein as a function of the IATI index.

Combined use of the IATI index and of Phospho-Tau concentrations allows patients to be classified into 4 areas (see Figure 2):
- top left (green text box): Healthy patients: IATI > 1 and Ph-Tau < 60 pg/ml. The majority of stable cases of Mild Cognitive Impairment (MCI) are in this area.
- bottom right (blue text box): Alzheimer patients: IATI < 0.8 and Ph-Tau > 60 pg/ml (Patient A). Most MCI-pre-Alzheimer patients fall in this area.
- two other areas (in white): results are not sufficient to infer AD but may suggest another neurodegenerative disease. Subsequent testing may be necessary in accordance with the course of the disease (see Table).

#### CONCLUSION

These laboratory tests allow differential diagnosis to be made for various types of dementia and above all, they may be used to confirm or rule out Alzheimer’s disease in young subjects (HAS recommendation). As a result, therapy may be properly targeted and its early institution ensures efficiency.

However, collection of CSF samples is complex and requires at least day-hospital admission. Clearly, blood tests would be ideal, and the preliminary results of plasma assays for these proteins are beginning to be published. They principally involve Aβ42, which is in fact the target of treatments currently in development; however, for the moment, assay is restricted to clinical trial patients.

