

New development

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.

DIAGNOSIS OF DISEASE

During patient consultations, clinical examination, MRI and neuropsychology provide a number of clues allowing the diagnosis of Alzheimer's disease. Nevertheless, diagnosis remains uncertain in the numerous atypical forms of the disease and for neurodegenerative diseases still in the early stages.

Specific and sensitive biomarkers for cognitive disorders associated with Alzheimer's type dementia assist clinicians with the diagnosis of such atypical forms of dementia and help rule out potentially curable diseases such as encephalopathies.

- 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 A β 42 forming senile plaques.
- Such lesions are evident on immunohistochemical study of brain sections.

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 2-ml Aliquots of CSF in suitable propylene tubes

Sample tubes and the decanting tubes can be obtained from our laboratory upon request:

+33.1.34.40.20.20

or

www.lab-cerba.com

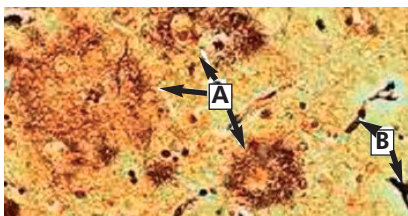
(rubrique: analyses informations / consommables)

STORAGE: FROZEN

METHOD: ELISA

RUN FREQUENCY: Twice montly

Immunohistochemistry of cortical lesions (neuronal and synaptic loss) in Alzheimer's disease



A : Senile plaque after immunohistochemistry of peptide A β 42

B : Neurofibrillary degeneration demonstrated by immunohistochemistry of Tau protein.

Note severity of lesions: all brown structures are pathological.

- 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 A β 42.

VALUE OF CSF BIOMARKERS: TAU PROTEIN, PHOSPHO-TAU PROTEIN, β -AMYLOIDE (peptide A β 42)

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 A β 42 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.

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)

Pathologie	Tau	Ph-Tau	Aβ42-amyloid
Normal senescence	Normal (< 500 pg/ml)	Normal (< 60 pg/ml)	Normal (> 500 pg/ml)
Alzheimer's disease	moderate ↗	moderate ↗	↓↓↓
Depression	N	N	N
Parkinson's disease	N	N	N
Alcoholic dementia	N	N	N
Frontotemporal dementia	N or slight ↗	N or slight ↘	N or slight ↘
Lewy body dementia	N or slight ↗	N	slight to moderate ↘
Creutzfeld-Jakob disease	↗↗↗	N or slight ↗	↘
Stroke	transient ↗↗	unchanged	unchanged
Vascular dementia	Divergent data	N	N or slight ↘

Total Tau protein assay is specific for neuronal death (intraneuronal 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

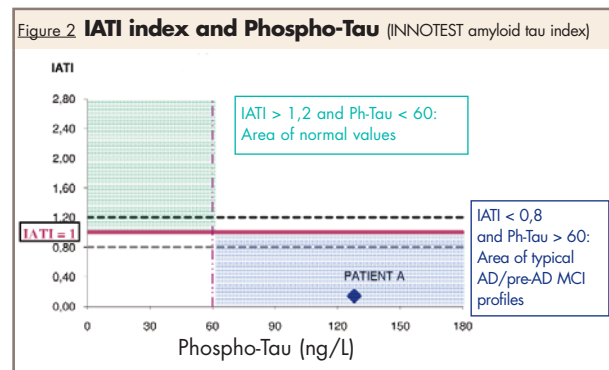
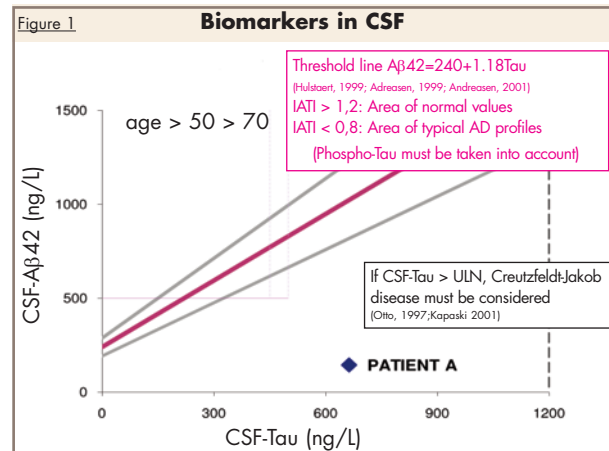
The reference values for Tau, phospho-Tau and Aβ42 have been determined in the literature, thus providing quantitative values for each of these proteins.

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 allow 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.

Bouwman S.H. et coll. CSF biomarkers and medial temporal lobe atrophy predict dementia in mild cognitive impairment. Neurobiol Aging. 2007 Jul;28(7):1070-4.
 Sjögren M et coll. Tau and Abeta42 in cerebrospinal fluid from healthy adults 21-93 years of age: establishment of reference values. Clin Chem. 2001 Oct;47(10):1776-81.
 Blennow K et coll. CSF markers for incipient Alzheimer's disease. Lancet Neurol. 2003 Oct;2(10):605-13. Review.

Blennow K et coll. CSF total tau, Abeta42 and phosphorylated tau protein as biomarkers for Alzheimer's disease. Mol Neurobiol. 2001 Aug-Dec;24(1-3):87-97. Review.
 Dubois B et coll. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol. 2007 Aug;6(8):734-46. Review.