Diagnosing Alzheimer’s disease

A new generation of test systems from EUROIMMUN

- Available assays: ELISA for the detection of beta-amyloid (Aβ\textsubscript{1-42} / Aβ\textsubscript{1-40}), total tau and P-tau; microarray for the molecular genetic detection of the APOE genotype
- Efficient ELISA processing owing to uniform protocols
- Convenient automation solutions for all ELISAs
- Improved diagnostics due to amyloid quotient determination
Alzheimer’s disease

Alzheimer’s disease is with 60 to 70% the most common cause of dementia in old age. It is characterised by progressive and irreversible deterioration of cognitive abilities. The disease generally starts with mild symptoms and ends with severe damage of the brain.

Pathology

In the brain of a patient with Alzheimer’s disease, protein deposits form within and outside the nerve cells, which lead to destruction of the nerve cells.

**Tau**

The tau protein is expressed in neurons in order to stabilise the microtubuli of the cytoskeleton. The erroneous phosphorylation of this protein leads to the formation of aggregates which accumulate as so-called neurofibrillary tangles in the nerve cells. Consequently, the axonal transport is disturbed.

**Beta-amyloid**

The processing of the neuronal, membrane-based amyloid precursor protein (APP) leads to the formation of different isoforms of the peptide beta-amyloid (including $\text{A}\beta_{1-42}$ and $\text{A}\beta_{1-40}$). In Alzheimer’s disease, the breakdown of these peptides is disturbed. The $\text{A}\beta_{1-42}$ isoform aggregates and forms plaques outside of the neurons.

Diagnosis

Suspected diagnosis is based primarily on the identification of clinical symptoms. To support the finding, imaging techniques are applied. Especially in early and pre-symptomatic stages of Alzheimer’s disease, the clinical diagnosis is unreliable and requires additional measurable biomarkers. For diagnosis, all available diagnostic information is compiled and then observed and evaluated.

**Clinical symptoms**

The clinical signs of Alzheimer’s disease may differ a lot. Common symptoms are, amongst other things, memory loss that disrupts daily life, problems understanding visual and spatial relationships, trouble in finding words, withdrawal from social activities and changes in personality, up to depression.

**Imaging techniques**

If symptoms are present, structural imaging e.g. using MRT or CT should be performed in order to identify typical atrophy patterns and exclude other causes for the cognitive impairment. Moreover, PET imaging can help detect and quantify amyloid deposits in the brain.

**Biomarkers**

**Beta-amyloid**: The CSF of persons who are developing Alzheimer’s exhibits significantly decreased concentrations of the $\text{A}\beta_{1-42}$ isoform or a decreased ratio of $\text{A}\beta_{1-42}$ to $\text{A}\beta_{1-40}$ even before the onset of cognitive changes.

**Tau**: The concentrations of unphosphorylated (total tau) and phosphorylated tau (P-tau) in the CSF of patients increase with progressing neurodegeneration and cognitive impairment.

**APOE**: The molecular genetic determination of the APOE alleles ε2, ε3 and ε4 can support the diagnosis. The gene codes for the lipoprotein ApoE, which plays a role in the breakdown of beta-amyloid. Carriers of an APOE-ε4 allele have an increased risk of developing Alzheimer’s disease.
**Improved early and differential diagnosis by amyloid quotients**

**Amyloid concentration and quotient determination**

Determination of the $A\beta_{1-42}/A\beta_{1-40}$ quotient can improve the efficiency of early diagnosis. $A\beta_{1-40}$ is a measure of the individual amyloid expression and remains unchanged by Alzheimer’s disease. The case study shows the CSF results from a patient with a high basal expression of beta amyloids. If only $A\beta_{1-40}$ is observed, the patient cannot be clearly identified. This is only enabled by quotient formation.

Studies have shown that diagnoses based on the $A\beta_{1-42}/A\beta_{1-40}$ quotient correlate better with amyloid-PET results than diagnoses based solely on the $A\beta_{1-42}$ concentration (93 % vs. 83 % agreement).


**Differential diagnostics**

The figure shows that the determination of the amyloid quotient is also helpful in the clinically difficult differentiation between Alzheimer’s and vascular dementia. The cut-off for the $A\beta_{1-42}/A\beta_{1-40}$ quotient is 0.1 (for the EUROIMMUN ELISA):

- Quotient <0.1: Abnormal $A\beta$ value, decreased $A\beta_{1-42}$ concentration
- Quotient >0.1: normal $A\beta$ value

**Influence of external factors on $A\beta_{1-42}$**

The determination of the $A\beta_{1-42}$ concentration in CSF is affected in laboratory practice by different external factors. Amongst other things, material and volume of the reaction vessels and the number of freeze/thaw cycles can have a significant influence on the amount of beta-amyloid. The isoforms $A\beta_{1-42}$ and $A\beta_{1-40}$ are subject to these influences (e.g. adsorption by polypropylene vessels) to the same extent. Also due to this reason, the determination of the $A\beta_{1-42}/A\beta_{1-40}$ quotient is the more suitable analysis method, since it is more resistant to changes by external influencing factors.

In a study, the influence of different parameters on the concentration of $A\beta_{1-42}$ and on the $A\beta_{1-42}/A\beta_{1-40}$ quotient was investigated. The change in the concentration or the quotient respectively resulting from the variation of a parameter was calculated. If, for example, the CSF samples are collected in a polypropylene vessel, the yield of $A\beta_{1-42}$ is 11 % lower compared to the use of a low-binding vessel. By using the amyloid quotient, the influence of the reaction vessel can be reduced to 4%.

Alzheimer’s diagnostics from EUROIMMUN – convenient and precise

EUROIMMUN offers ELISAs for the detection of beta-amyloid isoforms, total tau and phosphorylated tau (P-tau) for comprehensive diagnostics of Alzheimer’s disease. Furthermore, a microarray is available for the molecular genetic detection of the APOE alleles ε2, ε3 and ε4. The tests meet the high requirements of modern laboratory routine:

- Parallel performance of beta-amyloid and tau ELISAs enabled by the use of identical incubation protocols
- ELISA results within 4 hours
- Completely automatable processing of ELISA test systems

NEW pTau(181) ELISA

In a study with 110 clinically characterised samples (61 Alzheimer’s patients, 49 healthy persons) the EUROIMMUN pTau(181) ELISA was compared with the INNOTEST Phospho-Tau (181P) ELISA. The EUROIMMUN assay showed a sensitivity of 93% and a specificity of 84%. The INNOTEST ELISA achieved a sensitivity of 67% and a specificity of 92% (cut-off: 61 pg/ml). In a ROC analysis with a defined specificity of 92%, the EUROIMMUN ELISA exceeded the test from INNOTEST with a sensitivity of 87% vs 67%.

Automation

The EUROIMMUN ELISAs can be processed fully automatically with the EUROIMMUN Analyzers I and I-2P or the EUROLabWorkstation ELISA.

EURO Lab Workstation ELISA

EUROIMMUN test systems:

<table>
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<th>Test</th>
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