biomarkers for early-stage Alzheimer’s disease

By: Dr Jacqueline Gosink, EUROIMMUN AG, Luebeck, Germany
Alzheimer’s disease is a major global challenge of our time, with its prevalence predicted to rise dramatically as population’s age. No cure is available, and early diagnosis is crucial for managing the disease. Since clinical diagnosis is difficult, especially in the initial stages, analysis of biomarkers in patients’ cerebrospinal fluid (CSF) is increasingly used as a diagnostic support tool. These neurochemical markers can be measured precisely, reproducibly and independently of matrix effects using a new generation of ELISAs based on well-characterised capture antibodies.

ALZHEIMER’S DISEASE
Alzheimer’s disease was first described in 1906, and is with 60-70% the most common cause of dementia in old age. The risk for developing Alzheimer’s disease doubles for around every five years after age 65, with 30% of persons over 90 suffering from the disease. In contrast to the age-dependent, sporadic form of Alzheimer’s disease, the familial, genetically caused form can occur in young adults from 30 years of age.

The disease course is divided into three consecutive phases; the pre-clinical stage, the mild cognitive impairment (MCI) stage and the dementia stage. Patients usually attend a neurologist initially because of learning difficulties and short-term memory impairment. Cognitive function then declines progressively. In the middle stage, patients struggle to manage everyday life; they become confused and suffer from hallucinations amongst other things. By the final stage, patients are increasingly frail. They need constant support and care and have difficulties in eating and sometimes in swallowing. On average, the life expectancy after the onset of symptoms is seven to ten years.

PATHOLOGICAL CHARACTERISTICS
Alzheimer’s disease is characterised pathologically by the formation of deposits in the neuronal cell body and outside the nerve cell ends (see figure 1). The intracellular deposits (neurofibrillary tangles) consist of hyperphosphorylated tau proteins (P-tau), which form tangled fibre strands. The extracellular deposits (neuritic plaques) contain predominantly the peptides Aβ1-40 and Aβ1-42, which are breakdown products of the membrane-bound amyloid precursor protein. The physiological function of Aβ1-40 and Aβ1-42 peptides is not yet fully understood, but they are presumed to play a major role in signal transmission in nerve cells.

DIAGNOSIS
Definitive diagnosis of Alzheimer’s disease can only be established post mortem. In autopsy the neuropathological changes, namely plaques and neurofibrillary tangles, are visible in the brain of the deceased patient. Tentative in vivo diagnosis of Alzheimer’s disease (‘probable Alzheimer’s disease’) is based primarily on the clinical sign of memory loss and the exclusion of possible reversible causes. Imaging techniques such as MRI, SPECT or PET (amyloid detection) can be used to support early and differential diagnostics, for example exclusion of endocrinopathies and electrolyte disorders. The results from imaging dementia diagnostics are assessed together with other available diagnostic information, including analyses of CSF.

CSF BIOMARKERS
The diagnosis of Alzheimer’s disease in the early and pre-symptomatic stages requires reliable, quantifiable CSF biomarkers, for example soluble Aβ1-42 and tau proteins. The concentrations of these analytes in the CSF reflect the neuropathological changes in the brain. There are currently no blood markers available that show the same clinical value as the CSF markers. Patients with Alzheimer’s disease show a significantly decreased level of Aβ1-42 that is already detectable 5-10 years before the start of cognitive changes. The concentrations of total tau and P-tau, on the other hand, increase when patients show advanced neurodegeneration and cognitive impairment. It is thus possible to discriminate Alzheimer’s patients from healthy persons by means of CSF markers.

In contrast to Aβ1-42, the level of Aβ1-40 remains unchanged in Alzheimer’s patients. Measuring Aβ1-40 together with Aβ1-42 enables calculation of the Aβ1-42 to 1-40 ratio, which helps to increase the efficiency of early diagnostics (see figures 2 and 3). A ratio of under 0.1 indicates amyloid pathology. This ratio might further help to discriminate Alzheimer’s disease from other diseases, such as vascular dementia.
NEW GENERATION ELISAS
The Aβ peptides 1-42 and 1-40 and total tau can be measured in patient CSF samples using standardised ELISAs based on well-characterised antibodies (see figure 4). The ELISAs were developed by EUROMMUN AG in collaboration with ADx NeuroSciences. The Aβ peptides or tau proteins in the patient sample are first bound by a capture antibody and then detected using a labelled secondary antibody. The analyte is literally sandwiched between the two antibodies (see figure 5), hence the term sandwich ELISA. This matrix-independent approach ensures high consistency in results. Lyophilised calibrators and controls provide convenient test performance, high precision and clinical accuracy. Moreover, thanks to interchangeable reagents and identical protocols for the different test parameters, the analyses can be carried out in four hours. The ELISAs are CE labelled and automateable, allowing CSF diagnostics to be easily integrated into the automated routine operations of a diagnostic laboratory.

CLINICAL EVALUATION
Samples from clinically characterised patients with Alzheimer’s disease (85) or vascular dementia (10) and control subjects (18) were analysed using the Aβ 1-42 and 1-40 ELISAs (see figure 3). 82 of the Alzheimer’s patients yielded an Aβ 1-42 value of below 400 pg/ml and an Aβ ratio of below 0.1, in line with the clinical diagnosis. In contrast, patients with vascular dementia exhibited Aβ 1-42 values that were on average twice as high (442 pg/ml) and a ratio of 0.10 to 0.17. The control group showed a mean Aβ 1-42 value of 543 pg/ml and a ratio of between 0.12 and 0.17. These data demonstrate the usefulness of determining Aβ 1-42 and the ratio of the two analytes in the diagnosis and differentiation of Alzheimer’s disease.

PERSPECTIVES
With the current worldwide prevalence of 36 million Alzheimer’s cases expected to double by 2030, the need to reliably identify patients and individuals at risk is critical. The advent of CSF assays for biomarkers, such as beta amyloid peptides and tau proteins, represents a significant step forward in tackling the disease. Molecular genetic risk determination, for example the detection of Alzheimer’s-associatedapolipoprotein E alleles by DNA microarray, may also prove to be helpful in early diagnostics. Neurochemical and genetic indicators are likely to remain at the forefront of Alzheimer’s research as scientists hunt for strategies that allow earlier diagnosis. Predicting Alzheimer’s even before it manifests would offer the best chance of preventing its devastating consequences.