Biomarkers and preanalytics in Alzheimer's disease

by Dr Jacqueline Gosink

Analysis of biomarkers such as amyloid-beta peptides and tau proteins in cerebrospinal fluid (CSF) is nowadays a core criterion in Alzheimer's disease diagnostics. Test methods such as ELISA and chemiluminescence immunoassay enable robust and reproducible measurement of the analytes in patient CSF samples. Recommendations for preanalytical sample collection and handling have enhanced the standardization of the analyses.

Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of dementia in old age. It is characterized by progressive cognitive impairment caused by degeneration of brain cells. The biological hallmarks of the disease are amyloid-beta (Aβ)-containing plaques and tau-containing neurofibrillary tangles which build-up in major hubs of the brain [1]. Plaques of Aβ1-42 form extracellularly next to the nerve cell ends as a result of disruption in the breakdown of Aβ peptides (Fig. 1). Aggregates of hyperphosphorylated tau protein (P-tau) accumulate inside the cells. It is still unclear if the plaques and tangles cause AD or are a by-product of the disease process.

AD cannot be cured. Traditional therapy focuses on management of symptoms with medication, as well as non-pharmacological interventions such as behavioural therapy. In 2021, the first disease-modifying drug came onto the market. Aducanumab, which is marketed under the name Aduhelm™ and is approved in the USA and United Arab Emirates, reduces Aβ plaques in the brain. A second plaque-reducing drug, lecanemab (Leqembi™), has just received accelerated approval from the U.S. Food and Drug Administration (FDA) and is on the verge of being approved in some other countries. Further promising drugs are in the pipeline.

Disease-modifying drugs aim to delay onset or slow progression of AD, but must be applied in the initial stages of mild cognitive impairment before dementia sets in. Thus, the need for early and accurate AD diagnosis has taken on a new urgency. Timely diagnosis is also important for helping patients and their families plan long-term care and support.

Diagnostics

Diagnosis of AD is based on clinical evaluation, imaging methods such as positron emission tomography (PET) and analysis of biomarkers in cerebrospinal fluid (CSF). Demonstration of Aβ pathology by imaging or CSF tests is essential prior to treatment with plaque-targeting drugs.

Amyloid-PET and amyloid CSF measurements show a good correlation. However, CSF analysis is considered more sensitive than brain imaging methods, detecting pathologic changes years before disease onset. CSF Aβ constitutes the earliest known marker of AD pathology [2]. Moreover, CSF analysis may be the preferred diagnostic method in some situations, for example when other CSF tests are needed at the same time or due to lower costs and fewer side effects. CSF tests are also easier to perform in regions where specialized PET equipment and qualified staff may not be readily available.

CSF samples for analysis are collected by lumbar puncture (spinal tap), which is considered a safe and well-tolerated procedure. Guidelines for the sample collection are available to minimize the risk of complications and discomfort for patients [3,4].

AD biomarkers

On the one hand, AD is associated with a decrease in the isoform Aβ1-42 in CSF, which can occur as many as 5–10 years before the onset of cognitive changes. The concentration of Aβ1-40, on the other hand, remains unchanged and reflects the individual amyloid expression.

The most reliable measurement of Aβ peptides in AD is provided by the ratio of Aβ1-42 to Aβ1-40 rather than the single parameter Aβ1-42. The Aβ1-42/Aβ1-40 ratio is more stable and comparable between patients and is less influenced by external factors. Furthermore, the ratio demonstrates a higher concordance with PET than Aβ1-42 alone. Measurement of the Aβ1-42/Aβ1-40 ratio is now recommended in all notable guidelines. The amyloid ratio is particularly valuable for discriminating AD from other forms of dementia such as vascular dementia.

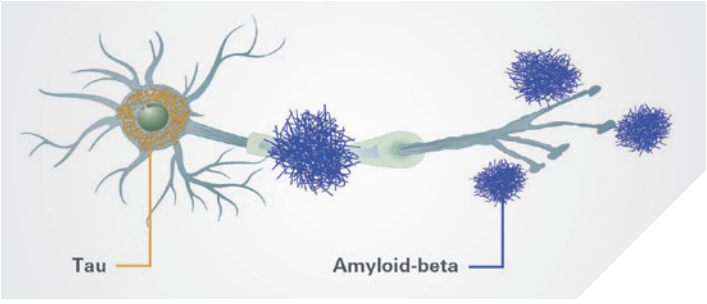

Figure 1. Amyloid-beta and tau deposits in Alzheimer's disease pathology

The concentration of tau proteins in CSF increases as AD progresses. Total tau is a general marker of neuronal injury and is also elevated in other conditions such as traumatic brain injury, stroke and Creutzfeldt-Jakob disease. Phosphorylated tau (P-tau) is a specific indicator of neurofibrillary tangles. Tau phosphorylated at the position threonine 181 is the most studied form and serves as a marker for AD.

Determination of the triad of Aβ1-42/Aβ1-40 ratio, total tau and P-tau in CSF provides vital support in the diagnosis of AD and helps predict progression to dementia in patients with mild cognitive impairment.

Standardization and preanalytics

In recent years, there has been a concerted effort to standardize measurement of Alzheimer's biomarkers in CSF. Aspects such as preanalytical sample handling, detection technology and reference materials can have a significant impact on the analytical results [5]. These issues have been addressed in collaborative work between scientists, clinicians and industry partners such as EUROIMMUN, in particular as part of the Alzheimer's Association Global Biomarker Standardization Consortium (GBSC). The GBSC recently published the first official guideline for preanalytical sample handling, which gives recommendations for collection, transport, handling and storage of CSF samples [4].

As Aβ peptides bind irreversibly to plastic and glass surfaces, contact of patient samples with consumables such as syringes, tubes and pipette tips must be minimized throughout the entire analysis. This limits adsorption and thus loss of the peptides and increases result reliability.

The guideline recommends collecting CSF by gravity drip directly into sample tubes with low-binding capacity. The sample should fill at least 50% of the tube volume to ensure a low ratio of contact surface to sample volume. Freezing of samples prior to transport is advisable to avoid unnecessary movement of liquid in the tubes. In the laboratory processing, transfers of samples into fresh tubes should be minimized to prevent further analyte loss. Moreover, repeated freeze-thaw cycles should be avoided as they can result in degradation of the analytes.

Notably, the effects of preanalytical factors are less severe when the recommended Aβ1-42/Aβ1-40 ratio is measured rather than Aβ1-42 alone [5]. Since the isoforms Aβ1-42 and Aβ1-40 are subject to the impact factors to the same extent, measurement of the ratio helps to mitigate the preanalytical effects. This can be observed, for example, for sample vessel, storage, freeze-thaw cycles and sample volume (Fig. 2).

Aβ1-42 reference material

A lack of reference material previously hampered efforts to standardize CSF analyses in Alzheimer's diagnostics. Certified reference material (CRM) for Aβ1-42 has recently been developed to enable more meaningful comparison of measurements between different laboratories and detection platforms. The CRM was developed by a working group comprising clinical laboratories in collaboration with the GBSC. The value was standardized by chromatography mass spectrometry. As a member of the working group, EUROIMMUN has ensured that its Aβ1-42 assays are aligned to the reference material.

Detection technologies

Aβ peptides and tau proteins can be reliably measured in CSF samples using methods such as enzyme-linked immunosorbent assay (ELISA) or chemiluminescence immunoassay (ChLIA). Key requirements for the assays are high specificity and accuracy, as well as standardized protocols for simple processing and automatability.

In collaboration with ADx Neurosciences, a leader in Alzheimer's diagnostics, EUROIMMUN has developed a range of quantitative ELISAs and ChLIAs that provide robust and highly reproducible measurement of Aβ1-42, Aβ1-40, total tau or P-tau. The assays are based on highly specific monoclonal antibodies, which are coated onto microplates (ELISA) or magnetic particles (ChLIA). The incubation procedures for each method are identical, so that the different parameters can easily be processed in parallel. The ELISA procedure takes approximately 5 hours, while the ChLIA analysis takes just 25 minutes until the first result. Lyophilized calibrators and controls supplied in the kits enhance convenience and ensure high precision.

Fully automated processing of the tests increases the efficiency and standardization of the analyses. The EUROIMMUN ELISAs can be processed on devices such as the EUROLabWorkstation ELISA or the EUROIMMUN Analyzer I or I-2P. The CHLIAs can be processed on the benchtop random access instruments IDS-i10 or IDS-iSYS Multi-Discipline Automated System.

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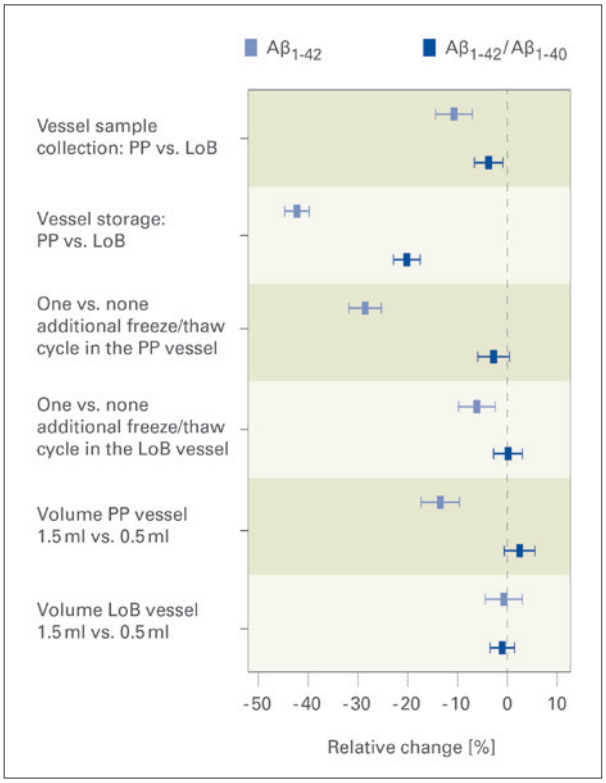

Figure 2. Effects of preanalytical factors on the measurement of amyloid-beta (Aβ)1-42 and the Aβ1-42/Aβ1-40 ratio
PP, polypropylene vessel (Sarstedt); LoB, low-binding vessel (Eppendorf).

Outlook

The disease burden of AD is predicted to increase in the future in all parts of the world due to aging populations. The advent of the first disease-modifying drugs for AD has awakened the possibility of curbing disease progression. These drugs are, however, only effective when applied in the prodromal stage. CSF biomarker measurements together with imaging can help to identify individuals who are not yet substantially impaired but are on the AD pathway and may thus benefit from novel therapies. The tests also aid scientists and pharmaceutical companies in selection of the most suitable participants for clinical trials of new therapeutics.

CSF is currently the only recommended biofluid for AD biomarker determination in diagnostics, with blood measurements limited to research settings. Serum-based detection requires assays with much higher sensitivity due to the very low concentrations of the brain proteins in blood. As detection techniques are honed, biomarker testing using less invasive blood sampling may in the future become feasible for diagnostic purposes.

References

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