In the know

Standardisation of biomarker measurements in Alzheimer's disease diagnostics

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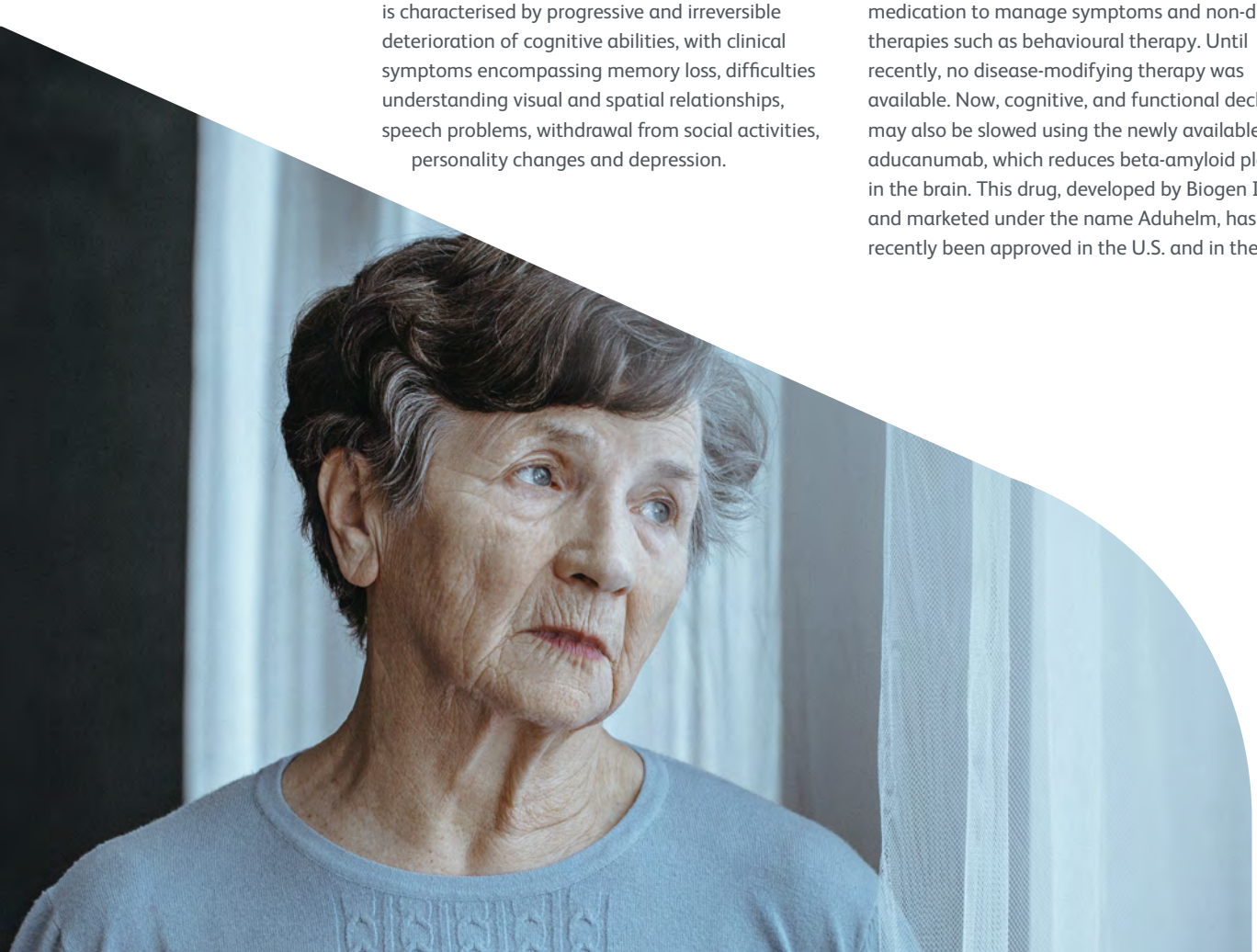
Overview

Analysis of biomarkers such as beta-amyloids and tau proteins in cerebrospinal fluid (CSF) for the diagnosis of Alzheimer's disease has undergone a quantum shift in recent years due to the increased standardisation of tests and procedures. In particular, the ratio Aβ 1-42 / Aβ1-40 is now preferred over the single parameter Aβ1-42 as a more reliable early diagnostic marker. Increased awareness of the impact of preanalytical factors has also led to the introduction of recommendations for sample collection and handling to improve the reliability of results.

Alzheimer's disease

Alzheimer's disease is the most common cause of dementia in old age, accounting for 60 to 70 per cent of cases, and represents a substantial burden to patients, carers, and health systems. The disease is characterised by progressive and irreversible deterioration of cognitive abilities, with clinical symptoms encompassing memory loss, difficulties understanding visual and spatial relationships, speech problems, withdrawal from social activities, personality changes and depression.



Disease pathology

In patients with Alzheimer's disease, protein deposits form within and outside the nerve cells of the brain, cause destruction of these cells (Figure 1). Disruption in the breakdown of beta amyloid peptides leads to plaques of Aβ1-42 forming extracellularly next to the nerve cell ends. Aggregates of erroneously phosphorylated tau protein accumulate as neurofibrillary tangles inside the nerve cells, hindering axonal transport. The neuronal damage caused by the plaques and tangles results in loss of synaptic integrity or degeneration of the synapses and consequently cognitive decline.

Therapy

Alzheimer's disease cannot be cured. Traditional therapy options for Alzheimer's patients include medication to manage symptoms and non-drug therapies such as behavioural therapy. Until recently, no disease-modifying therapy was available. Now, cognitive, and functional decline may also be slowed using the newly available drug aducanumab, which reduces beta-amyloid plaques in the brain. This drug, developed by Biogen Inc. and marketed under the name Aduhelm, has recently been approved in the U.S. and in the UAE.

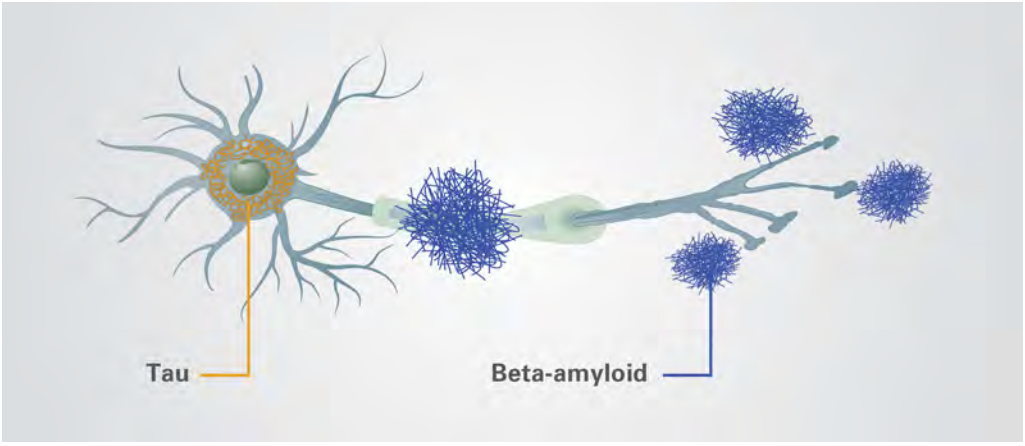


Figure 1. Representation of protein deposits in Alzheimer's disease

Diagnostics

Timely diagnosis of Alzheimer's disease is important for therapeutic decision-making and to enable families to plan appropriate care. In particular, the differentiation of Alzheimer's disease from other forms of dementia is crucial due to differing treatment regimes. It is, however, not possible to diagnose Alzheimer's disease by clinical symptoms alone. In the past, a definite diagnosis was only established post-mortem by autopsy.

Nowadays, imaging techniques, such as PET, and CSF analysis enable confirmation of diagnosis during the patient's lifetime. CSF biomarkers are now at the core of diagnostic criteria for Alzheimer's disease[1]. Lumbar puncture is considered a standardised and safe procedure for routine diagnostics, and guidelines are available to help minimise the risk of complications[2]. Biomarker determination may be preferred over PET due to the possibility of screening for several pathologies in parallel, lower costs, fewer side effects for patients, and easier establishment in regions of the world where highly specialised instrumentation and trained staff are not readily available.

CSF biomarkers

Beta-amyloids serve as a marker of amyloid burden (amyloid plaques). Establishment of amyloid pathology is important prior to treatment with aducanumab. The CSF of persons who are developing Alzheimer's disease exhibits significantly decreased concentrations of the Aβ1-42 isoform or a decreased ratio of Aβ1-42 to Aβ1-40 already five to 10 years before the onset of cognitive changes. This decrease is detectable even before amyloid-PET becomes conspicuous. Thus, CSF beta-amyloid is the earliest known marker of Alzheimer's pathology.

The concentrations of tau proteins in the CSF increase with progression of Alzheimer's disease. Phosphorylated tau (P-tau) is a specific marker of tauopathy (neurofibrillary tangles). The most thoroughly examined P-tau epitope for Alzheimer's diagnostics is threonine 181 (P-tau (181)). Total tau is a general indicator of neuronal injury and provides a measure of the level of neurodegeneration. It is not, however, specific for Alzheimer's and also rises in other neurological conditions such as traumatic brain injury, stroke and Creutzfeldt-Jakob disease.

Standardisation of biomarker analysis

In the past, the determination of biomarkers in CSF was hampered by factors such as variable preanalytical sample handling, lack of standardisation of detection technologies, and an absence of reference materials. These aspects have been addressed to a great extent by extensive collaborative work between scientists, clinicians, and industry partners such as EUROIMMUN, for example as part of the Alzheimer's Association Global Biomarker Standardization Consortium (GBSC).

Importance of preanalytics

In laboratory practice, the measurement of dementia biomarkers in CSF is affected by different external factors, which have a considerable impact on the measured analyte concentration, especially when they accumulate. These factors encompass different aspects of sample collection, transport, and laboratory processing.

Since beta amyloids bind irreversibly to plastic and glass surfaces, contact of patient samples with consumables such as syringes, tubes and pipette tips must be minimised to limit adsorption of the peptides. It is recommended to collect CSF by

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gravity drip directly into sample tubes with low binding capacity. Sample volumes constituting at least 50 per cent of the tube volume also ensure a lower ratio of contact surface to sample volume, helping to minimise adsorption of the analyte. Unnecessary movement of the liquid in the tubes can be avoided by freezing the samples prior to storage and transport. In the laboratory, transfers of samples into fresh tubes should be minimised to prevent further analyte loss. Repeated freeze-thaw cycles should also be avoided as they can lead to degradation of the analytes.

The GBSC has recently published the first official guidance for the collection and storage of CSF samples[3]. Laboratories are advised to follow these guidelines to achieve the most reliable results.

Beta-amyloid ratio

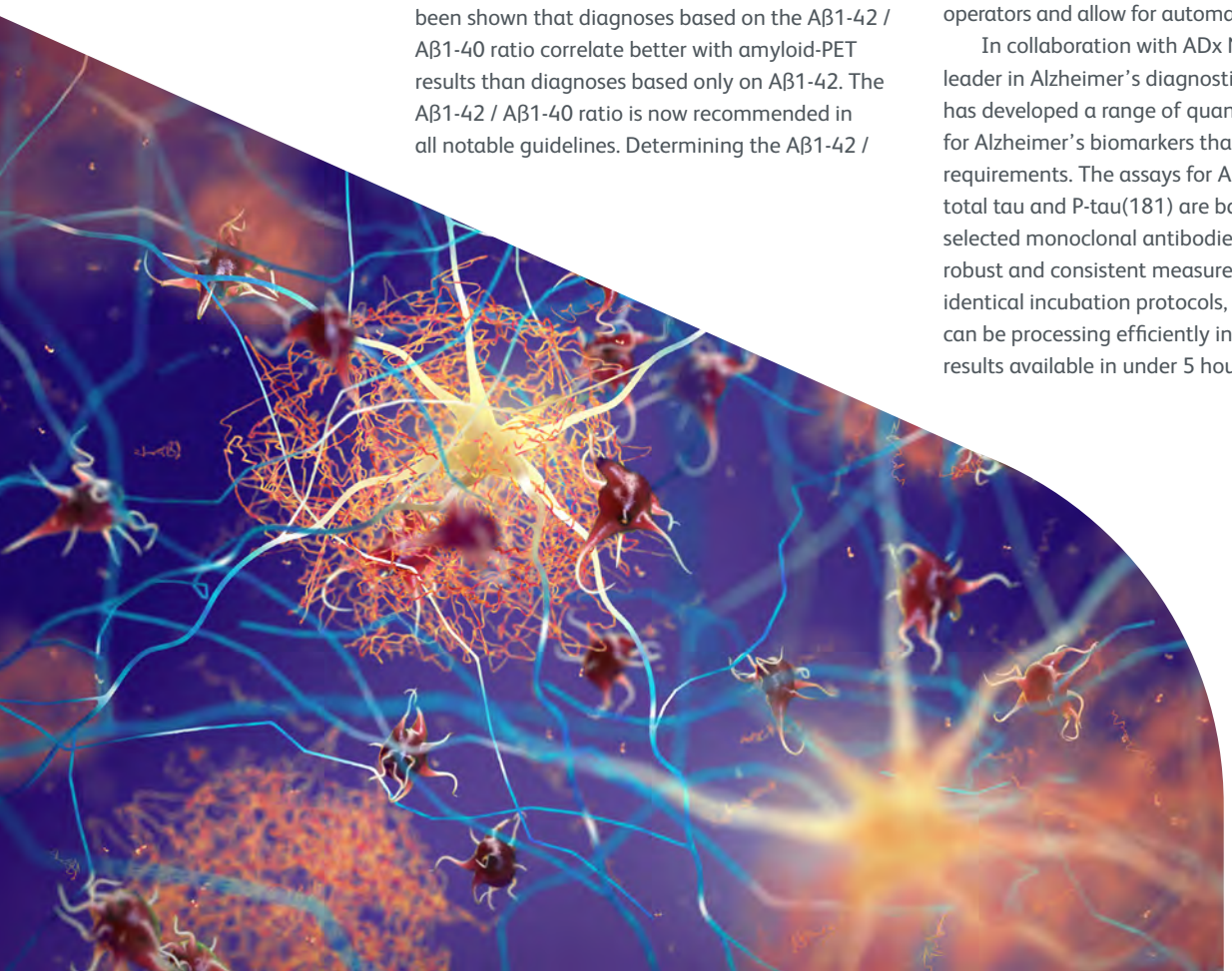
Over recent years the ratio of Aβ1-42 to Aβ1-40 has become established as a more reliable marker for Alzheimer's disease than Aβ1-42 alone. Aβ1-40 is a measure of the individual amyloid expression and remains unchanged by Alzheimer's disease. While the individual beta amyloid level differs from person to person and the measurable concentration of beta amyloid can be greatly affected by external factors, the ratio of the two beta amyloid forms is very stable and also comparable between patients. It has been shown that diagnoses based on the Aβ1-42 / Aβ1-40 ratio correlate better with amyloid-PET results than diagnoses based only on Aβ1-42. The Aβ1-42 / Aβ1-40 ratio is now recommended in all notable guidelines. Determining the Aβ1-42 / Aβ1-40 ratio can improve the efficiency of early diagnosis and is particularly helpful in the clinically difficult differentiation between Alzheimer's disease and vascular dementia. Aβ1-42 / tau ratios should not be used in a routine setting due to the different physiochemical properties and distinct pathophysiologies of these species.

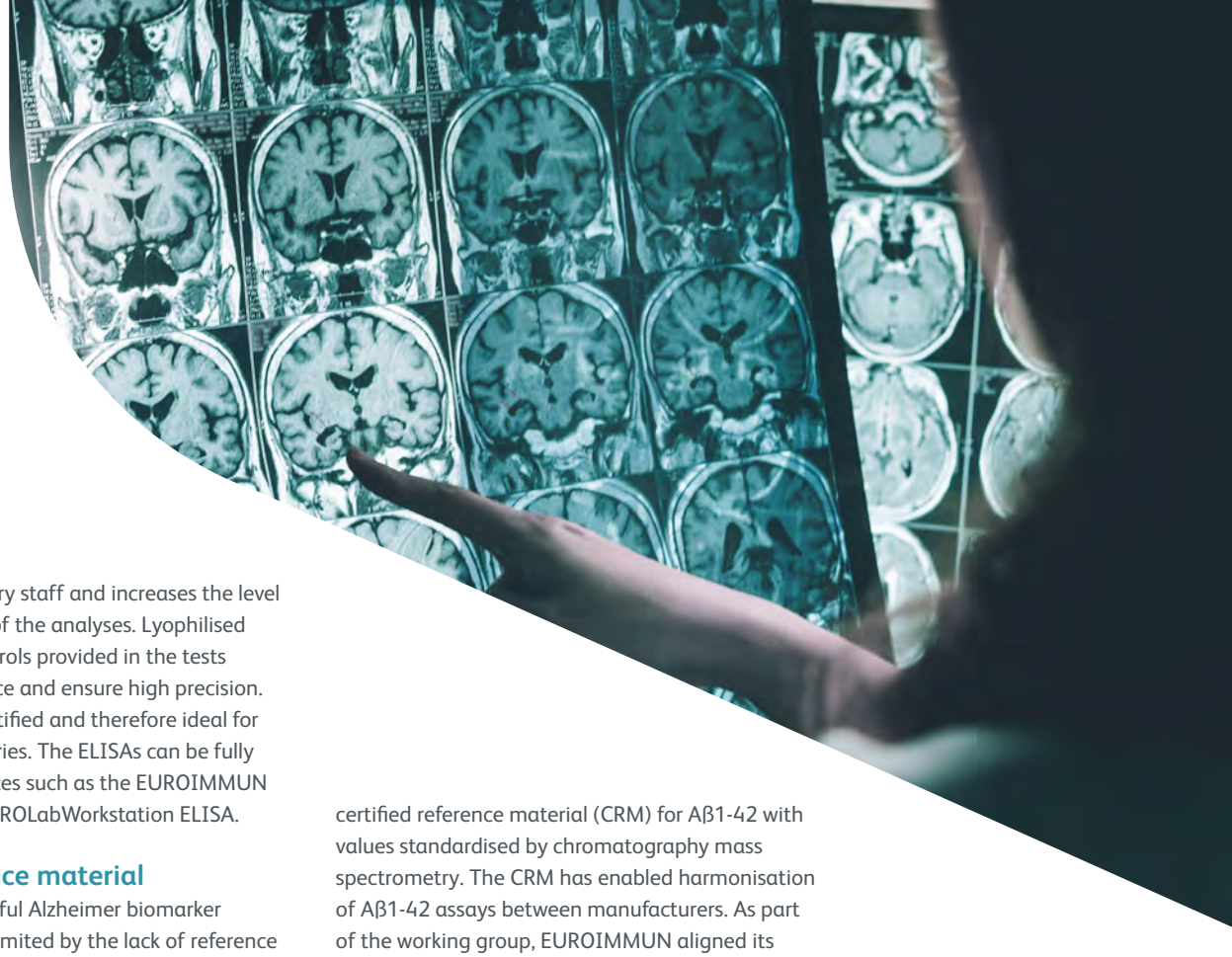
Since the isoforms Aβ1-42 and Aβ1-40 are subject to impact factors to a similar extent, measuring the amyloid ratio helps to normalise pre-analytical effects to a certain extent. The effects of vessel material, sample volume, sample storage and freeze-thaw cycles on the results are demonstrably less severe when determining the amyloid ratio than when measuring Aβ1-42 alone (Figure 2, adapted from ref 4.). For example, the use of polypropylene vessels instead of optimal low-bind vessels resulted in a reduction of 11 per cent in the yield of Aβ1-42, but a reduction of only 4 per cent in the ratio.

Robust biomarker assays

Assays for the determination of Alzheimer's biomarkers in routine diagnostics need to be specific, precise, and accurate. They must detect only the required analyte or specific isoform thereof without interference from other proteins in the CSF, and they should ideally follow a standardised protocol to minimise variability between laboratories and operators and allow for automation.

In collaboration with ADx Neurosciences, a leader in Alzheimer's diagnostics, EUROIMMUN has developed a range of quantitative ELISAs for Alzheimer's biomarkers that meet these high requirements. The assays for Aβ1-42, Aβ1-40, total tau and P-tau(181) are based on carefully selected monoclonal antibodies which provide robust and consistent measurements. Owing to identical incubation protocols, the different ELISAs can be processing efficiently in parallel, with results available in under 5 hours. This yields time

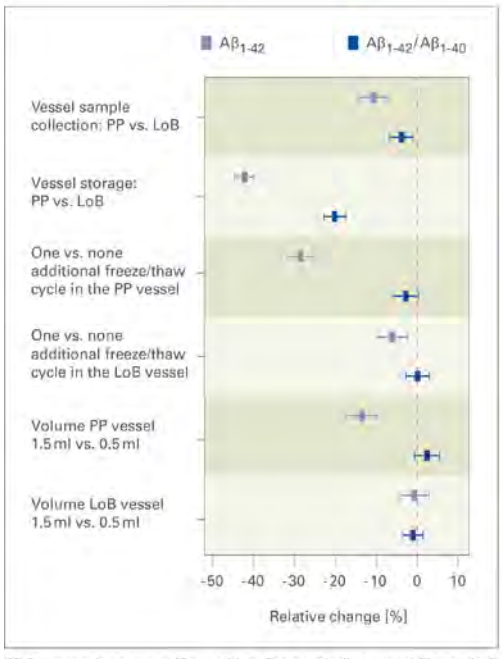




savings for laboratory staff and increases the level of standardisation of the analyses. Lyophilised calibrators and controls provided in the tests enhance convenience and ensure high precision. The tests are CE certified and therefore ideal for accredited laboratories. The ELISAs can be fully automated on devices such as the EUROIMMUN Analyser I or the EUROLabWorkstation ELISA.

Aβ1-42 reference material

Previously, meaningful Alzheimer biomarker measurement was limited by the lack of reference materials to compare measurements from different technologies and platforms. A working group comprising clinical laboratory organisations in collaboration with the GBSC has developed certified reference material (CRM) for Aβ1-42 with values standardised by chromatography mass spectrometry. The CRM has enabled harmonisation of Aβ1-42 assays between manufacturers. As part of the working group, EUROIMMUN aligned its Aβ1-42 ELISA to this international standard.



PP Polypropylene vessel (Sarstedt); LoB: Low-binding vessel (Eppendorf)

Figure 2. Effects of different preanalytical factors on the concentration of Aβ1-42 and the ratio Aβ1-42 / Aβ1-40

Conclusions

With the advent of aducanumab to treat Alzheimer's disease and the expectation of further disease-modifying drugs in the future, it is more important than ever to obtain a reliable diagnosis, especially when patients are in the early stages of the disease. Collaborative work over recent years to standardise biomarker measurement and establish the beta amyloid ratio as a reliable diagnostic tool have contributed significantly to improving diagnostics of Alzheimer's disease. Ongoing research is aimed at further optimising diagnostic tests and procedures to bolster early diagnosis of the disease. Reliable diagnostics also aid pharmaceutical companies in the selection of suitable participants for clinical studies into new drugs.

References

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