PAVING THE ROAD TO STANDARDIZED ALZHEIMER'S BIOMARKER MEASUREMENT

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Alzheimer's disease (AD) is one of the leading causes of death in many parts of the world. While the cause of the disorder is still unknown, the scientific field has seen several groundbreaking discoveries over the past two decades that tremendously expand our knowledge about risk factors affecting the development and ideal management of the disease. With the first disease-modifying Alzheimer's therapeutic being approved in several countries and further drugs being on their way, a correct differential diagnosis becomes key to a high quality patient care. The road to worldwide implementation of Alzheimer's disease laboratory diagnostics was bumpy since CSF-based biomarker measurement faces several analytical hurdles. Today, through interdisciplinary teamwork, most of these analytical potholes have been filled with knowledge. Here, we discuss the key insights and achievements that today pave the road to standardized Alzheimer's disease biomarker measurement.

The suspicion by an individual or their family members that changes are occurring with their memory, language, or personality must become the trigger for an in-depth search to find the underlying cause of the problem before it irreversibly affects daily living activities.

> *People experiencing cognitive changes now have the opportunity to seek professional medical help to determine if the changes are normal for one's age, reversible or a symptom of Alzheimer's disease or another dementia.*

A COMPLEX AND HETEROGENEOUS PATHOLOGY

Dementia is a syndrome that have distinct pathophysiologies, characterized by a heterogeneous group of clinical features and pathological hallmarks, including but not limited to amyloid plaques and neurofibrillary tangles (Fig. 1). The term neurodegeneration refers to any pathological condition that results in the progressive loss of structure or function of neurons, which may result in dementia in certain diseases. Alzheimer's disease, defined by McKhann et al. (1984), is a neurodegenerative disease and the most common cause of dementia in the elderly (>70%). Due to aging societies, the

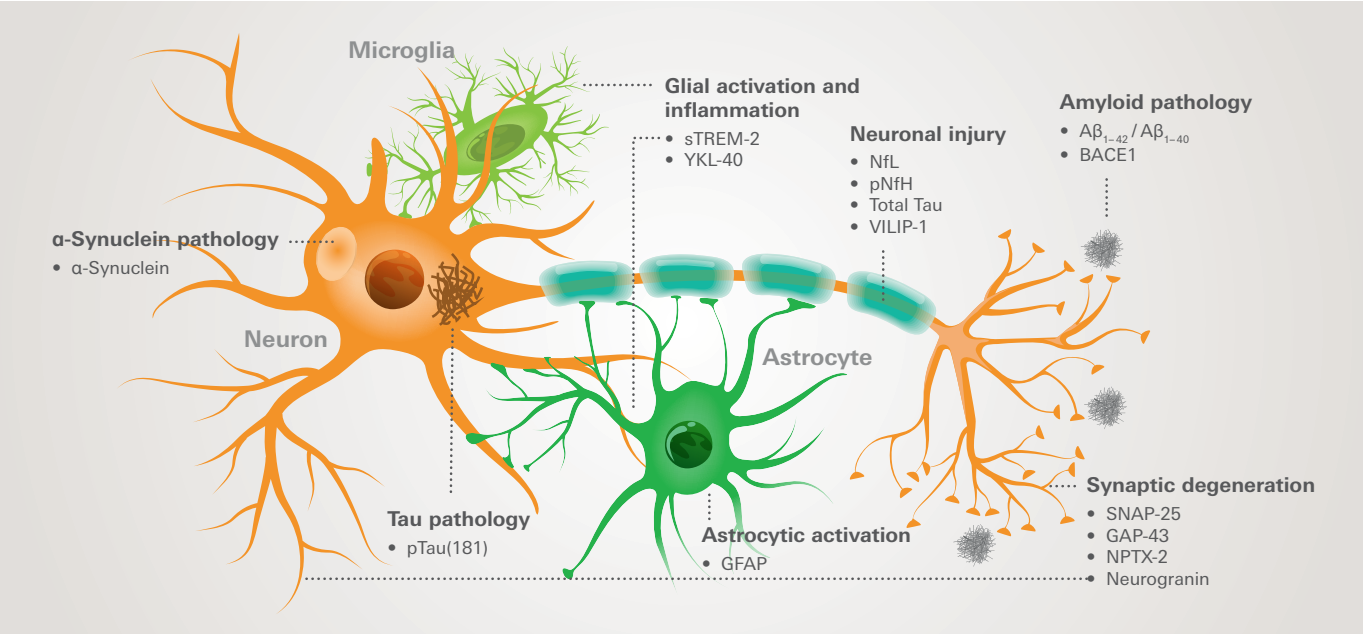

Fig. 1. Biomarkers for neurodegenerative diseases.

worldwide number and proportion of people with AD or other dementia types will grow considerably over the next decades (Alzheimer's Association 2021). Life expectancy after onset of symptoms is 7–10 years in patients aged 60–70 years (Zanetti et al. 2009).

AD is a progressive disease with a long preclinical phase. It is thought to begin more than 20 years before symptoms arise, followed by periods of subjective cognitive decline (SCD), mild cognitive impairment (MCI), and eventually mild, moderate, and severe AD. 15% of MCI patients develop dementia after two years; 32% within five years. Some MCI will revert to normal cognition or will not progress further, especially when the cognitive changes are inadvertently caused by a medication or when MCI is misdiagnosed. Inter-individual differences in the progression rate of the disease or the rate of cognitive decline are modulated in part by age, gender, genetic profile, ethnicity, environmental factors, and comorbid brain pathologies (Dubois et al. 2016). Treatable 'dementia-like' symptoms (e.g., depression, untreated sleep apnea, delirium, side effects of medications, Lyme disease, thyroid problems, vitamin deficiencies, alcohol consumption) are frequently encountered in clinical routine and must be excluded from real dementias in an early diagnostic phase.

> *The substantial clinical heterogeneity of Alzheimer's/dementia reinforces the public health requirement for an accurate disease diagnosis, establishment of appropriate inclusion and exclusion criteria for clinical trials, and the use of pharmacodynamic measures of treatment effects. Biomarker panels will likely be useful to stratify patients, for example, in subgroups with different clinical progression profiles or in subgroups with an enhanced likelihood to benefit from treatment directed to specific protein targets.*

At a certain time and to a certain degree, many people at risk for dementia will obtain a change in the brain associated with more than one pathology. Compared to regular AD, these mixed dementias can differ in the rate of cognitive decline (Fig. 2), the outcome of clinical trials, and optimal treatment (Boyle et al. 2018; Young et al. 2018).

DEMENTIA DIAGNOSIS – THE PAST AND THE FUTURE

In the past, dementia diagnosis was made by way of exclusion, although, in most cases, in a late stage of the disease process when therapeutic interventions are less beneficial for the patient. During the patient's life, the clinician was able to evaluate the mental status and physical health of a person and combine it with neuropsychological testing or physical and neurological examinations. However, cognitive decline is associated with normal aging and cognitive tests are often inconclusive. A definite diagnosis of AD could only be made post mortem via microscopic confirmation of the presence of plaques and tangles in specimens of the brains from affected patients by a pathologist (Scheltens and Rockwood 2011).

The diagnostic field for AD has changed considerably over the last decade due to new scientific discoveries, better design of clinical studies, broader worldwide access to scientific tools and new technologies, as well as by commitments and investments by pharmaceutical and diagnostic companies, including EUROIMMUN. In addition, the recent approval of new therapeutic interventions for AD will have an enormous impact on the diagnostic field.

> *Earlier and more accurate diagnosis is required.*

The willingness of all stakeholders to work together has resulted in a significantly improved understanding of the brain pathology, biological processes, and hallmarks for therapeutic interventions. Examples for these ventures are larger consortia (e.g., Alzheimer's Association, Critical Path Institute, Michael J Fox Foundation), working groups (e.g., Global Biomarker Standardization Group, International Federation of Clinical Chemistry), European projects or funding by philanthropic organizations (e.g., Alzheimer's Drug Discovery Foundation, Weston Brain Institute, Bright Focus). All of these have the ultimate goal of speeding up the availability of treatment possibilities for affected subjects.

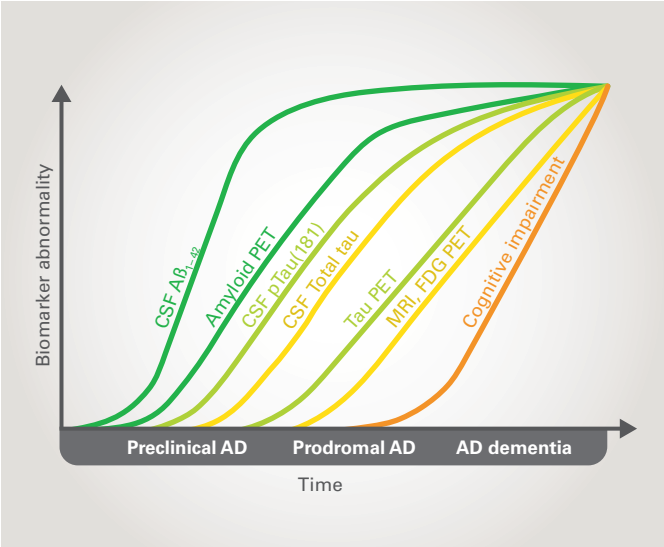

Fig. 2. Biomarker dynamics in Alzheimer´s disease (modified according to Mattsson-Carlgren et al. 2020).

> *The pressure to provide better care and treatment for people with Alzheimer's disease and their families is acute. Affected individuals and their family members must have access to an objective diagnosis about the origin of their memory problems and access to treatment possibilities.*

DIAGNOSIS OF ALZHEIMER'S DISEASE WITH BIOMARKERS IN CSF

Until the early 1980s, AD diagnosis was based solely on clinical evaluations and a wide range of clinical cognitive tests. During the last decade, *in vivo* diagnosis of brain pathology or identification of early signs of the disease process (e.g., amyloid pathology, tauopathy, synucleinopathy, inflammation, oxidative stress) became possible through FDA approval of amyloid-β positron emission tomography (Aβ-PET) imaging and by the worldwide acceptance of methods for accurate quantification of relevant biomarkers in cerebrospinal fluid (CSF). In contrast to PET imaging, analysis of biomarkers in CSF is an excellent diagnostic tool as it readily contains several proteins that directly reflect the key hallmarks of the disease: reduced $A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-40}$ as markers for amyloid plaques; elevated phosphorylated (pathological) tau (pTau) as a measure for tangles and elevated total tau as an indicator for neurodegeneration (Jack et al. 2018). These days, CSF is preferred over Aβ-PET for several reasons. These include but are not limited to the possibility to screen and quantify changes in several key pathologies in parallel (not only amyloid pathology), the lower risks and side effects associated with lumbar puncture, the lower cost for the analysis, less limitations with respect to availability in all regions of the world, and no need for highly specialized instrumentation.

The clinical relevance of using a combination of CSF biomarker proteins in a routine diagnostic environment is unquestionable. At present, they are considered as the gold standard for neurochemical-based diagnosis of AD to identify the presence of amyloid pathology of the brain, to detect early signs of the disease, and for exclusion of other dementia types. As such, measurement of the Aβ and tau proteins are part of the 2011 National Institute on Aging and Alzheimer's Association (NIA-AA) diagnostic guideline (Albert et al. 2011, Jack et al. 2011). In the NIA-AA research framework of 2018, they are grouped according to the amyloid deposition (A), tau aggregation (T) and neurodegeneration (N) classification system (A/T/N), which categorizes the different biomarkers according to the underlying neuropathological hallmark (Jack et al. 2018). A reduction in the ratio of $A\beta_{1-42}/A\beta_{1-40}$ reflects plaque burden in the brain. Increased levels of pTau(181) are correlated with the presence of cortical neurofibrillary. Although higher levels of CSF total tau are not directly correlated with AD, they reflect neuronal injury/degeneration more generally and are, for instance, also increased in traumatic brain injury, stroke, Creutzfeldt-Jakob disease, which all do not show elevated pTau levels.

INTEGRATION OF CSF BIOMARKER ASSAYS IN CLINICAL ROUTINE

The acceptance and full integration of the CSF AD biomarker profile analysis into the routine clinical environment has been hampered in the past since the quantification of these biomarkers faces many challenges that are encountered in most established laboratory diagnostic or clinical chemistry settings. Problems arose due to a missing consensus guideline for sample handling such as variabilities in pre-analytical procedures for CSF samples, differences in outcome values depending on the selected technology, and the absence of ref-

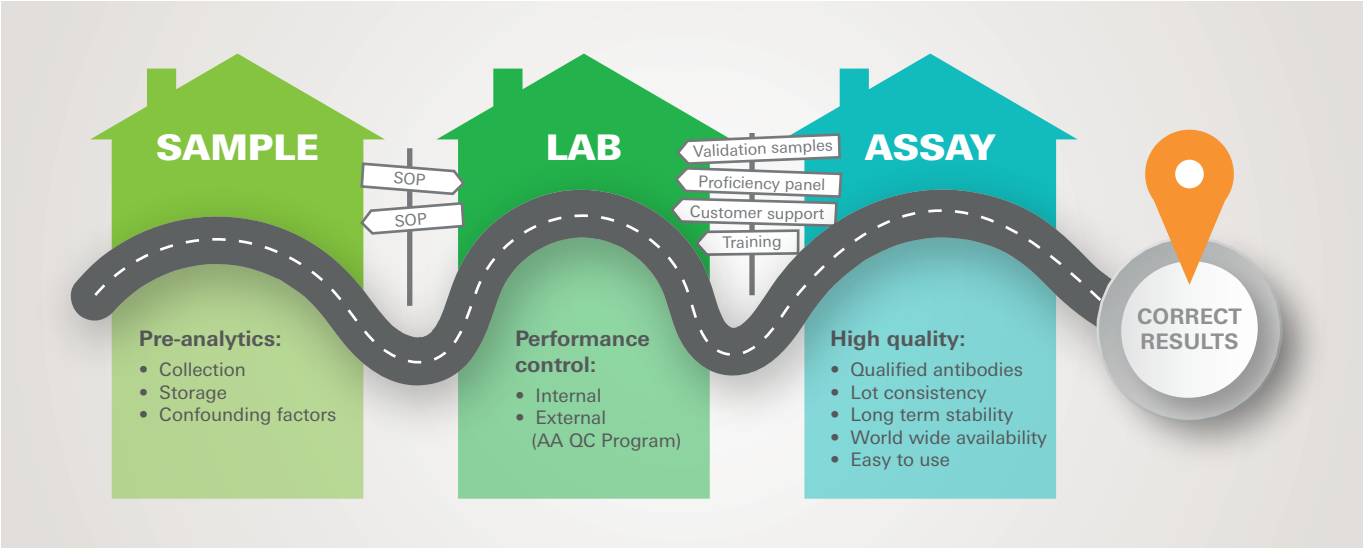

Fig. 3. Roadmap to obtain precision qualified assays. AA: Alzheimer´s Association; QC: Quality control; SOP: Standard operating procedure

erence materials which could be used to harmonize results between different technology platforms. In brief, problems originated from the sample, the assay, and the lab (Fig. 3).

Nowadays, CSF AD biomarker analysis is well established in the clinical routine as most of these pitfalls have been addressed over the past years by dedicated collaboration of IVD manufacturers with KOLs and other stakeholders. EUROIMMUN actively participated in working groups with the Alzheimer's Disease Neuroimaging Initiative (ADNI) and programs from the Alzheimer's Association. Many improvements were integrated in the development of the biomarker assays, taking into account regulatory requirements, the biological relevance of a modification in the production process, and accuracy of the test results. Here, the most important developments are summarized.

THE SAMPLE

One of the most severe differences between CSF AD biomarker analysis and most established laboratory diagnostics is the immense adverse effect of certain pre-analytical factors. However, problems with CSF samples may arise not only from handling issues, but already during sampling. In many parts of the world, healthcare specialists are not trained to perform lumbar punctures and patients may lack the acceptance to undergo the sampling procedure. These concerns have been addressed by the development and introduction of training videos that help educate both patients and medical professionals (Babapour Mofrad et al. 2017 and 2019).

It is now recommended to collect CSF by means of gravity or aspiration (Doecke et al. 2021) into sample tubes with low protein binding capacity to limit adsorption of the protein to the walls of the tubes. The reason for this procedure is that some proteins and peptides, like Aβ, possess chemical properties that foster interprotein interactions or interactions with plastic surfaces, which can interfere with their accurate quantification. Contact of the sample with plastic surfaces, e.g. during pipetting, when changing tubes, or during the removal of cells in CSF by centrifugation can have a major impact on the clinical interpretation of Aβ levels. Partial normalization can be achieved when using the ratio $Aβ_{1–42}/Aβ_{1–40}$ instead of $Aβ_{1–42}$ alone (Vanderstichele et al. 2017, Vanderstichele et al. 2016). EUROIMMUN was the first vendor to develop an IVD assay for $Aβ_{1–40}$, thus paving the road to a more reliable Aβ measurement.

In addition to the amyloid ratio, heterologous tau-$Aβ_{1–42}$ ratios are in use. However, the ratio $Aβ_{1–42}/Aβ_{1–40}$ is now recommended in all notable guidelines and should be used preferably over heterologous ratios since the pre-analytical impact is higher for Aβ than for the tau proteins. In contrast to $Aβ_{1–42}/Aβ_{1–40}$ as a way of normalizing amyloid levels, these other tau/$Aβ_{1–42}$ ratios can rather be seen as interpretation tools which allow comparison of different biomarker results. $Aβ_{1–42}$ and tau species are of different origin, have different physicochemical properties and reflect distinct pathophysiologies. This results in several issues that are related to pre-analytics and interpretation of the ratios. Tau/$Aβ_{1–42}$ ratios do not normalize adverse pre-analytical effects, thus risking misdiagnosis. The use of tau/$Aβ_{1–42}$ ratios is only feasible in well-defined and centralized, but not in routine settings where samples are shipped from the place of sampling to the analyzing laboratory. It is recommended to aliquot samples for later use and limit the number of freeze-thaw cycles, otherwise cut-offs recommended by the manufacturer of the IVD might not be valid anymore. Hemolytic samples need to be discarded.

Intensive work by the Alzheimer's Association in collaboration with KOLs and diagnostic companies, including EUROIMMUN, has cumulated in the recent publication of the first official guidance for the collection and storage of CSF samples (Hansson et al. 2021).

ABOUT THE AUTHOR

Hugo Marcel Vanderstichele obtained his PhD at the University Hospital in Ghent, Belgium, as an endocrinologist. As a principal investigator, he pioneered industrial AD biomarker research for over 2 decades at INNOGENETICS. In 2011, he founded BIOMARKABLE bv and co-founded ADx NeuroSciences. He is involved in world-wide efforts to qualify and standardize biomarker protein assays (e.g., ADNI, AIBL, CPAD, JPND, MJ Fox Foundation), is a principal investigator in several multi-center projects, and (co-)authored over 150 peer-reviewed papers. He is well known for his valuable work on the pre-analytics of Alzheimer's disease biomarkers.

THE ASSAY

Before successful implementation of AD biomarker analysis in the clinical routine setting, the reliability of measurements suffered greatly from the lack of availability of robust assays. EUROIMMUN closely collaborated with experts from ADx NeuroSciences (Gent, Belgium), pioneers in AD biomarker research and development, for development of their assays. During development of the assay prototypes, special diligence was paid to the robustness of the assays by carefully handpicking highly qualified and extensively characterized monoclonal antibodies. Each of the colorimetric ELISAs quantifies their target analyte with very high selectivity (no interference by other proteins which might be present in CSF), specificity (only one protein isoform is measured), precision (low variability obtained after training of lab staff), and accuracy (harmonized with the certified reference material, as far as available). Prototype immunoassays were transferred to the production environment for development of final IVD assays, including upscaling, establishment of lot-to-lot consistency, optimization for long-term stability, and quality assurance. The subsequent transfer of the assays to automation platforms and the analytical validation thereof ensures the reduction of interlaboratory variation.

A limitation of AD biomarker measurement is the lack of accessible and binding reference materials for manufacturers. As a result, comparison of biomarker concentrations beyond technology and vendor boundaries is impossible, thus preventing the establishment of universal cut-offs. Over the past years, endeavors have been made to establish certified reference materials (CRMs) for individual biomarkers. EUROIMMUN actively contributed to the international harmonization of test results for CSF $A\beta_{1-42}$ by participating in a working group of the International Federation of Clinical Chemistry (IFCC) and Laboratory Medicine Working Group for CSF proteins (WG-CSF), working closely with the Alzheimer's Association Global Biomarker Standardization Consortium (GBSC) (Kuhlman et al. 2017). The development of CRMs with values standardized by liquid chromatography mass spectrometry (LC-MS) (Leinenbach et al. 2014, Korecka et al. 2014) has now led to the harmonization of $A\beta_{1-42}$ assays. The development of CRM for the remaining AD biomarkers is ongoing.

THE LAB

One of the most critical factors for assuring excellent assay performance is good training of the lab staff. Vice versa, failures in a test run can often be traced back to human errors. Improved assay precision can be obtained by automation of ELISA processing (Gille et al. 2018, Chiasserin et al. 2018) or by the use of fully automated random access systems, thereby reducing the risks for human errors during the analysis.

To further ensure the quality of their analytics, labs need to perform internal and external quality control. Commercially available assays normally come with run validation samples and proficiency panels for internal quality control. In addition, each lab can monitor their test performing capacity by actively participating in external quality assurance schemes (EQAS) such as the Alzheimer's Association Quality Control Program which was already established in 2009 and in which more than 115 laboratories across 26 countries now participate (Mattsson et al. 2013, Lewczuk et al. 2018).

CONCLUSION

Seeing a neurologist early on is key when an individual or their family members notice first signs of memory deficits. A specialist will help identify the cause of the symptoms and can provide an accurate diagnosis. The establishment of a proper diagnosis, which is the very foundation of disease management, is the starting point for better life planning and for tracking the progression of symptoms. It will help determine whether the symptoms experienced are truly due to Alzheimer's disease or some other – perhaps even treatable – condition. Furthermore, a proper diagnosis will enable selection of the ideal medication to slow down the cognitive decline, at least for a few years.

LITERATURE

Alzheimer's Association. **2021 Alzheimer's disease facts and figures.** Alzheimers Dement 17(3):327-406 (2021). https://doi.org/10.1002/alz.12328

Babapour Mofrad R, et al. **Lumbar puncture in patients with neurologic conditions.** Alzheimers Dement (Amst) 8(1):108-110 (2017). https://doi.org/10.1016/j.dadm.2017.04.008

Babapour Mofrad R, et al. **Cerebrospinal fluid collection: An informative animation video for patients and caregivers.** Alzheimers Dement (Amst) 11(1):435-438 (2019). https://doi.org/10.1016/j.dadm.2019.04.005

Chiasserini D, et al. **Performance evaluation of an automated ELISA system for Alzheimer's disease detection in clinical routine.** J Alzheimers Dis 54(1):55-67 (2016). https://doi.org/10.3233/JAD-160298

Doecke JD, et al. **Concordance between cerebrospinal fluid biomarkers with Alzheimer's disease pathology between three independent assay platforms.** J Alzheimers Dis 61(1):169-183 (2018). https://doi.org/10.3233/JAD-170128

Dubois B, et al. **Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria.** Alzheimers Dement 12(3):292-323 (2016). https://doi.org/10.1016/j.jalz.2016.02.002

Duits FH, et al. **Performance and complications of lumbar puncture in memory clinics: Results of the multicenter lumbar puncture feasibility study.** Alzheimers Dement 12(2):154-163 (2016). https://doi.org/10.1016/j.jalz.2015.08.003

Gille B, et al. **Automation on an open-access platform of Alzheimer's disease biomarker immunoassays.** SLAS Technol 23(2):188-197 (2018). https://doi.org/10.1177/2472630317750378

Hansson O, et al. **The Alzheimer's Association international guidelines for handling of cerebrospinal fluid for routine clinical measurements of amyloid β and tau.** Alzheimers Dement 17(9):1575-1582 (2021). https://doi.org/10.1002/alz.12316

Jack Jr CR, et al. **NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease.** Alzheimers Dement 14(4):535-562 (2018). https://doi.org/10.1016/j.jalz.2018.02.018

Jack Jr CR, et al. **Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.** Alzheimers Dement 7(3):257-262 (2011). https://doi.org/10.1016/j.jalz.2011.03.004

Janelidze S, et al. **CSF Aβ42/Aβ40 and Aβ42/Aβ38 ratios: better diagnostic markers of Alzheimer disease.** Ann Clin Transl Neurol 3(3):154-165 (2016). https://doi.org/10.1002/acn3.274

Korecka M, et al. **Qualification of a surrogate matrix-based absolute quantification method for amyloid-β in human cerebrospinal fluid using 2D UPLC-tandem mass spectrometry.** J Alzheimers Dis 41(2):441-451 (2014). https://doi.org/10.3233/JAD-132489

Kuhlmann J, et al. **CSF Aβ(1-42) – an excellent but complicated Alzheimer's biomarker – a route to standardisation.** Clin Chim Acta 467:27-33 (2017). https://doi.org/10.1016/j.cca.2016.05.014

Leinenbach A, et al. **Mass spectrometry-based candidate reference measurement procedure for quantification of amyloid-β in cerebrospinal fluid.** Clin Chem 60(7):987-994 (2014). https://doi.org/10.1373/clinchem.2013.220392

Lewczuk P, et al. **Cerebrospinal fluid and blood biomarkers for neurodegenerative dementias: An update of the Consensus of the Task Force on Biological Markers in Psychiatry of the World Federation of Societies of Biological Psychiatry.** World J Biol Psychiatry 19(4):244-328 (2018). https://doi.org/10.1080/15622975.2017.1375556

Mattsson N, et al. **CSF biomarker variability in the Alzheimer's Association quality control program.** Alzheimers Dement 9(3):251-261 (2013). https://doi.org/10.1016/j.jalz.2013.01.010

McKhann G, et al. **Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease.** Neurology 34(7):939-944 (1984). https://doi.org/10.1212/wnl.34.7.939

Palmqvist S, et al. **Cerebrospinal fluid and plasma biomarker trajectories with increasing amyloid deposition in Alzheimer's disease.** EMBO Mol Med 11(12):e11170 (2019). https://doi.org/10.15252/emmm.201911170

Scheltens P, et al. **How golden is the gold standard of neuropathology in dementia?** Alzheimers Dement 7(4):486-489 (2011). https://doi.org/10.1016/j.jalz.2011.04.011

Vanderstichele HM, et al. **Recommendations for cerebrospinal fluid collection for the analysis by ELISA of neurogranin trunc P75, α-synuclein, and total tau in combination with Aβ(1–42)/Aβ(1–40).** Alzheimers Res Ther 9(1):40 (2017). https://doi.org/10.1186/s13195-017-0265-7

Vanderstichele HM, et al. **Optimized standard operating procedures for the analysis of cerebrospinal fluid Aβ42 and the ratios of Aβ isoforms using low protein binding tubes.** J Alzheimers Dis 53(3):1121-1132 (2016). https://doi.org/10.3233/JAD-160286

Zanetti O, et al. **Life expectancy in Alzheimer's disease (AD).** Arch Gerontol Geriatr 49(1):237-243 (2009). https://doi.org/10.1016/j.archger.2009.09.035

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