Diagnosis of neurodegenerative dementia: where do we stand, now?

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Abstract: After many years of large efforts made for understanding the pathogenesis of dementias, the early diagnosis of these degenerative diseases remains an open challenge. Alzheimer's disease (AD) represents the most common form of dementia, followed by Lewy body disease and frontotemporal degeneration. Actually, different pathological processes can determine similar and overlapping clinical syndrome. To detect in vivo the pathological process underlying progressive cognitive and behavior impairment, the Internationals guidelines recommend the use of biological and topographical markers, which can reflect neuropathological modifications in brain. In cerebrospinal fluid (CSF), decrease of amyloid beta 1-42 (Aβ42) and a low ratio of Aβ42 with amyloid beta 1-40 (Aβ42/Aβ40), together with the increase of both total tau protein (t-tau) and phosphorylated tau (p-tau), contribute to define the “Alzheimer's signature”. This review points out on the evolution of the concept for early diagnosis of AD, and on the current use of CSF proteins for research purposes and in clinical setting. Then, we discuss the limitations and drawbacks in wide application of CSF biomarkers for diagnosing degenerative dementias, and on the role of laboratory medicine to convey these biomarkers from “research” toward “clinical practice”.

Keywords: Alzheimer’s disease (AD); diagnosis; dementia; β amyloid; cerebrospinal fluid

Introduction

Dementia is an “umbrella term”, including disorders characterized by loss of cognitive functioning, such as thinking, remembering, and reasoning, and by impairment of behavioral abilities under particular conditions to such an extent that person’s daily life activities are compromised. Alzheimer’s disease (AD) is the first cause of dementia, followed by Lewy body and frontotemporal dementia. The prevalence of dementia is dramatically increasing in late life, and there are no effective interventions that can modify the disease course after clinical onset to date. In this context, early diagnosis, patient’s stratifications and identification of presymptomatic individuals at higher risk of developing a type of dementia, represent the main goals, also in order to “prevent the preventable”, intervening early on the known modifiable risk factors.

The modern concept for diagnosing dementia integrates clinical and neuropsychological examinations, together with evidences from genetics, biochemical and imaging tools, which can provide evidence indicative of the neuropathological process of the disease. Notwithstanding, both clinical and laboratory issues point out a few critical flaws about the integration and the use of cerebrospinal fluid (CSF) biomarkers, especially amongst those physicians that are skeptical on the value of biomarkers for investigating...
the neurodegenerative processes.

This review focuses on the evolution of the concept for diagnosing AD, and the advantage in integrating CSF biomarkers in clinical practice. Then, we will discuss the limitations and drawbacks of wide application of CSF biomarkers, and the role of the laboratory medicine to convey these biomarkers from “research” toward “clinical practice”.

The evolution of the diagnosis of Alzheimer’s dementia

AD is a complex multi-factorial disease and represents one of the greatest epidemic and health challenges among neurodegenerative disorders in the elderly. The most common cases are sporadic, characterized by late onset (LOAD) beyond the age of 65, while up to 5% are rare familiar cases (familial AD, FAD) with onset before 65 years.

For decades, AD was considered as a clinical and pathological entity (1). In 1984, the clinical criteria for the diagnosis of AD were centered around the observation of typical amnestic multidomain symptoms, which were assumed to be related to AD neuropathological changes at autopsy post-mortem, characterized by amyloid plaques and neurofibrillary tangles, the histological hallmarks of the disease. However, when symptoms were not manifest, preclinical neuropathological changes of AD could not be explorable. Following this paradigm, the clinical symptoms may define the presence of probable AD in patients who were alive, while the concept of clinical manifestation and disease were interrelated. These criteria do not include laboratory tests, except eventual instrumental examinations for the exclusion of secondary type of dementia (1).

The diagnostic approach was completely changed after discovering the radiotracer Pittsburgh compound-B (PiB), which can bind to aggregates of the Aβ peptide with high affinity in vitro. Thereafter a positron emission tomography (PET) method was developed for in vivo evaluation of Aβ plaque burden in vivo (2).

In 2006, the observation that plaque amyloid deposition in brain tissue in vivo results in low Aβ42 levels in CSF has revolutionized the idea of biomarkers for AD, thus providing the evidence of a fluid measurable marker of AD correlated with neuropathological process during the course of disease. Moreover, an unimaginable diagnostic opportunity emerged from the observations of amyloid plaque deposition detected by PET imaging associated with low CSF Aβ42 in apparently cognitive normal individuals, thus leading to the hypothesis of potential use of Aβ42 as biomarker of (preclinical) AD antecedent cognitive decline (3). Aside CSF low Aβ42, the high values of both t-tau and p-tau in CSF represent measurable markers of neurodegeneration and are pivotal for the in vivo diagnosis of AD (4).

In 2011, the recommendation for AD diagnosis have been revised by both the International Working Group (IWG) (4) and the US National Institute on Aging-Alzheimer’s Association (NIA-AA) (5-7). The new criteria abandon the old concept of AD dementia, based on the clinical assessment and autopsy confirmation, introducing a paradigm shift which points to early in vivo diagnosis of AD before development of dementia, thus representing a step forward from clinical-autoptic paradigm, registering the clinical consequences of completed pathological process to a clinical-biological paradigm, which emphasis on measurable in vivo evidences of developing AD pathology (8,9), which can be investigated by both CSF and topographical markers (assessed by PiB-PET and MRI).

The new recommendations created separate sets of diagnostic levels or “clinical” stages of AD, i.e., symptomatic AD, with mild cognitive impairment (MCI) or overt dementia (5,6), and non-symptomatic preclinical AD (7), identifying individuals not yet cognitively impaired but with abnormal AD biomarkers. The criteria for clinical symptomatic phases of AD were conceived to both aid the routine diagnostic procedures and providing a common lexicon to classify the clinical stages of Alzheimer’s pathology (2,3,5).

These last guidelines have been revised in 2018 by a NIA-AA leadership commissioned working group, with the aim to unify and update the 2011 recommendations (10). The absolute revolution of this so called “research framework” is that AD can be now defined in living persons as a biological construct, which can be identified by the assessment of changes of biomarkers that are indicative of disease’s neuropathology, independently by the manifestation of clinical symptoms. Biofluid and imaging biomarkers are grouped into the formula [AT (N)], which included Aβ deposition (A = low CSF Aβ or amyloid PET), pathologic tau (T = increased CSF p-tau and tau-PET) and neurodegeneration (N = increased CSF tau, FDG PET, MRI). Altered Aβ deposition with normal tau biomarkers define “Alzheimer’s pathologic change”, whereas evidence of both Aβ deposition and pathologic tau can identify “Alzheimer’s disease”, thus distinguishing earlier and later
phases of the “Alzheimer’s continuum”. This research framework creates a novel algorithm for identifying and classifying pathological stages across disease’s entire spectrum, to facilitate standardized reporting of research findings across the field. However, it is not intended for general clinical practice, but it constitutes a research framework which have to be tested and modified (if needed) before being introduced into common clinical routine (10).

The CSF biomarker analysis has been currently accepted and adopted in general clinical practice, with some differences and limitations between countries. The Guidelines from the European Federation of Neurological Societies (EFNS) recommend the use of CSF biomarkers for the differential diagnosis in case of both typical and atypical AD (11,12). In Italy, the CSF biomarkers are included in the diagnostic criteria for AD in a limited number of centers, specialized for diagnosing dementia, located in about 65% of the Italian regions (13).

**The use of CSF Biomarkers in clinical setting**

The amyloid β1-42 (Aβ42) and its ratio with amyloid β 1-40 (Aβ40/Aβ42), together with biomarkers of neurodegeneration, total tau protein and phosphorylated tau (t-tau and p-tau), constitute the panel of CSF biomarkers for diagnosing AD (5,10,14). Changes in these core biomarkers allow to identify AD pathology also in the early, prodromal phase of disease (8,15-17). The same biomarkers are used also for the differential diagnosis with other forms of dementia, as exclusion criteria (9,18-21).

Decrease of Aβ42, together with increase of t-tau and p-tau, represent the “AD signature” detectable in the CSF, which is supposed to reflect the main neuropathological hallmarks of this condition, which encompass formation of amyloid plaques and neurofibrillary tangles. Therefore, Aβ is considered a marker of “amyloidosis”, although the concentration observed in the CSF mainly reflect a threshold value, which is indicative of either physiological or pathological status, but it is not associated with clinical phenotype, such as typical or atypical cognitive onset, severity, disease progression or response to treatment (22,23). Recently, the Aβ42/Aβ40 ratio has demonstrated to increase the diagnostic value of Aβ42 (18) by reducing the differences due to inter-individual variability, in particular to those individuals who are over or low producer of total Aβ (24). Moreover, Aβ42/Aβ40 ratio is useful for reducing the bias attributable to preanalytical or analytical factors (25,26). An increased tau value in CSF is considered a marker of neuronal damage, which can be observed in AD as well as in different traumatic, infectious, prion or organic brain disorders. Oppositely, the tau hyperphosphorylation is a specific process characterizing AD pathogenesis, and its increase in CSF is hence pivotal for the diagnosis. Unlike Aβ, enhanced tau value may be related with the degree of cognitive decline and with diffuse neurodegeneration. Indeed, very high levels of both t-tau and p-tau may be associated with a rapid and severe progression (27,28), or to malignant forms of AD with higher risk of mortality (29).

Interestingly, tau CSF increase is sometimes not associated to low Aβ42 levels, and this finding may represent a specific biochemical pattern of “suspected non-amyloid patient or non-Alzheimer’s disease pathophysiology” (SNAP), which represent a novel concept of distinct Tau pathology separated from AD (30). This is an interesting issue for both clinical classification and patient stratification.

Changes in the concentrations of these biomarkers may be observed in CSF decades before the onset of cognitive symptoms, thus representing useful predictive tools, with decrease of Aβ eventually occurring even twenty years before clinical manifestation, in healthy stages, while tau change later on (6).

The sensitivity reported in different studies is variable, ranging from 76% to 96% for Aβ42 (31), and combined with 65–80% specificity and 40–86% sensitivity for tau (9,32). Thus, combinations of fluid and imaging biomarkers may help improving the diagnostic accuracy and have a better predictive value also for disease severity (33). Different ratios have been proposed, with several and novel candidate markers to increase the diagnostic performance and to follow the variation of each of them throughout the disease course (34). In this view, the collection of data emerged from different omics technologies have changed the approach in our knowledge of AD at multiple levels, thus integrating data from genomic, transcriptomic, epigenomic, proteomic and metabolomic. Then, the Omics era has opened new frontiers toward the development of personalized diagnostic and therapeutic tools (35). Several major genetics or proteomics research programs are nowadays ongoing, then there is a great hope for novel discoveries over the coming years.

Evidences suggest that different genetic factors may play a crucial role in the onset of sporadic cases of AD. The apolipoprotein E (ApoE) gene codes for an apolipoprotein and a transporter of cholesterol that is found in the brain, and represent the main known genetic risk factor for AD. Three different allelic variants, epsilon 2 (ε2), 3 (ε3) and 4 (ε4)
(ε4), have different frequency in the general populations and in AD patients, in particular, carriers of ApoE ε4 are 3 to 4 folds more likely to develop AD respect to non ε4 carriers (36). Actually, several genome wide association studies (GWAS) have identified novel promising risk genes for AD, but they have not yet been included in diagnostic or screening procedures.

The laboratory in the validation of CSF Biomarkers

Integrated clinic-biological diagnosis for AD includes the measurement of the neurodegenerative biomarkers in the CSF (37-40) (Figure 1). Nevertheless, the interpretation of test results needs expertise and caution. Clinical discrepancies of results and analytical issues lowered the accuracy of CSF markers. For example, variability due to the operator or methodological platforms, pre and post analytical procedures, represent the major criticisms for the diffusion of CSF biomarkers as diagnostic tool.

The role of laboratory is crucial for the standardization and harmonization of analytical procedures and criteria for interpretation and use of test results, thus also including cut-off values and decision limits (41).

CSF biomarkers can be measured by using different single or multi analyte test (e.g., enzyme-linked immunosorbent assays (ELISAs) or xMAP technology). Recently, fully automated chemiluminescent methods have been developed, that are expected to reduce the variability compared to manual measurement, and could further facilitate the accreditation of these measurements according to quality regulations (42,43). Several national and international initiative are ongoing, with the common aim to standardize the use and interpretation of AD CSF biomarkers. Since 2009, the Alzheimer’s Association Quality Control (AA QC) program, aim at monitoring analytical variability for CSF biomarkers between laboratories worldwide (44). Moreover, the BIOMARKAPD (biomarkers for AD and Parkinson’s disease) project, that is supported by the European Joint Program Neurodegenerative Disease Research (JPND) consortium, focuses on the definition of certified reference materials for harmonizing assays (45). However, the great effort of all these initiatives is to reach the reproducibility and consistency of measurements, the worldwide comparison of the results, a consensus on the use of CSF biomarkers and finally to reduce debate in clinical value and interpretation for diagnosing AD (46).

Concluding remarks

Early and timely diagnosis of AD is a big challenge. The analysis of biochemical and molecular biomarkers gives a unique opportunity to investigate the underlying pathophysiological mechanisms of neurodegeneration, thus providing an added value in terms of diagnosis, prognostication and therapeutic strategy. Advanced analytical techniques and several international quality control programs may help harmonizing assays procedures and reducing assays variability across centers in a limited timeframe, thus encouraging routine uses of CSF biomarkers for characterizing the biological signature of neurodegenerative disorders in clinical setting.

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Footnote

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