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Cerebrospinal fluid biomarkers for cognitive disorders. An introductory overview

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Abstract

The core (established) cerebrospinal fluid biomarkers of Alzheimer's disease (AD), namely amyloid-beta peptide, total tau protein and phospho-tau protein, have become a part of the diagnostic workup of patients with cognitive disorders in many specialized centers, especially for ambiguous cases. Combined, these biomarkers can identify the presence or absence of an AD biochemical process with sensitivities and specificities approaching or exceeding 90% in both dementia and pre-dementia stages of AD. Thus, they have been incorporated in various sets of research or clinical diagnostic criteria and recommendations. Results that are atypical, incompatible with AD, or inconclusive may occur, necessitating the use of other cerebrospinal fluid or imaging biomarkers.

Keywords: Cerebrospinal fluid, tau, phospho-tau, amyloid-beta, Alzheimer's disease, alpha-synuclein, TDP-43, neurofilament light protein

INTRODUCTION

Almost 25 years after their first introduction, cerebrospinal fluid (CSF) biomarkers have become a part of the diagnostic workup of patients with cognitive disorders in many specialized centers. Furthermore, they provide neurochemical information about the disorder underlying each individual patient's clinical presentation, which currently should be viewed as a biological process, sometimes starting many years prior to symptom onset and gradually evolving into a typical or atypical clinical phenotype. This paper provides an introductory, concise review, regarding the current status and future perspectives of CSF biomarker use.

WHY DO WE NEED BIOMARKERS?

Alzheimer's disease (AD) is the most common type of dementia[1], followed by vascular cognitive impairment (VCI)[2], dementia with Lewy bodies (DLB)[3], frontotemporal dementia (FTD)[4] and others. Until relatively recently, diagnosis of AD was made according to clinically based criteria[5]. These criteria may show high diagnostic accuracy, especially when typical cases are examined in specialized centers[6]. However, it is long known that in the community, in early, presenile or atypical cases and in the presence of comorbidities, diagnostic accuracy may drop substantially, with clinicopathological concordance rates sometimes as low as 62.5%[7-9]. Furthermore, it is now recognized that AD, typically presenting as an amnestic dementia syndrome, may rarely have frontal (sometimes frontotemporal-like)[10,11], posterior[10,11], language[10-12] and even corticobasal-like presentations[13,14]. Thus, the same disease may present with different phenotypes, and one phenotype may be caused by different diseases/pathologies. Mixed pathologies are not infrequent in senile cases[15], especially AD mixed with various types of vascular lesions[16], or DLB with concomitant AD pathology[17]. Such mixed pathologies may modify the clinical presentation[18,19] and the rate of disease progression[20]. In addition, some patients present very early, in a symptomatic but pre-dementia stage [mild cognitive impairment (MCI) and MCI due to AD][21]. On the other hand, when the clinical impression is against AD, there is still a 39% chance for pathological verification of AD (co)existence[22].

The above are not uncommon causes of diagnostic confusion in everyday practice. Of course, the gold standard for diagnostic verification is *post mortem* pathological examination. However, correct *ante mortem* diagnosis is necessary since it may help in predicting prognosis and it is likely to affect therapeutic decisions[23]. Thus, biomarkers are needed to serve as objective diagnostic tools during life. In the last 3 decades, various biomarkers have been developed (some being incorporated in various sets of diagnostic criteria), including structural neuroimaging (pattern of atrophy as a marker of neuronal injury), functional neuroimaging with positron emission tomography (PET), either as FDG-PET (hypometabolism as a marker of neuronal injury) or PET at least for amyloid-beta (Aβ), and CSF biomarkers[10,11]. The last have probably received the most attention.

ESTABLISHED (CORE) CSF BIOMARKERS OF AD

In an oversimplified scheme, there are two biochemical processes and pathological hallmarks of AD: (1) misfolding, oligomerization and finally polymerization and extracellular aggregation of Aβ, in the form of amyloid plaques, and (2) intracellular hyperphosphorylation and polymerization of the microtubule-associated protein tau, forming paired helical filaments which in turn aggregate in the form of neurofibrillary tangles[24,25]. The former process mobilizes various mechanisms that are toxic to neurons[26], and the second results in destabilization of microtubules and dysfunction of the cytoskeleton and of axonal transport[27]. Both processes, acting synergistically, lead to neuritic, synaptic and neuronal loss, through a vicious circle of interconnecting final pathways of oxidative stress, excitotoxicity, mitochondrial dysfunction, apoptosis and Ca^{2+}-mediated cell death[28-30], while prion-like spread[31] and neuroinflammation[32-34] are increasingly recognized as important early mechanisms.

Total tau protein (τ_T)[35], hyperphosphorylated tau, especially at a threonine residue at position 181 ($\tau_{P\text{-}181}$)[36] and Aβ peptide with 42 amino acids ($A\beta_{42}$)[37] can be quantified in the CSF. In AD, τ_T is increased, and traditionally, this is viewed as a marker of neuronal/axonal injury[38]; $\tau_{P\text{-}181}$ is also increased and this is considered a more specific marker of tangle formation[39]. On the other hand, $A\beta_{42}$ is decreased and this is considered (inversely) a marker of amyloid burden[40]. The above markers are useful in the discrimination of AD from normal aging and other dementias, and even abnormal τ_T alone may show high sensitivity and, in a few diagnostic questions, adequate specificity for the diagnosis of AD[41]. Combinations of the above biomarkers in the form of various formulas (including the Hulstaert formula[42]) or ratios (including $\tau_T/A\beta_{42}$ or $\tau_{P\text{-}181}/A\beta_{42}$[43,44]) further increase their diagnostic value.

HOW EARLY DO THE CLASSICAL BIOMARKERS BECOME ABNORMAL?

Currently, AD is viewed as a pathological or neurobiological entity, characterized by a continuum of 3 stages, starting as a preclinical ("asymptomatic at risk" or "presymptomatic") stage, which later on progresses to a symptomatic but pre-dementia stage (MCI) and finally to the dementia stage[11,45]. It seems that in most cases CSF biomarkers become abnormal during the preclinical stage of AD[45], and on the basis of studies in families with autosomal dominant AD, this may occur even 10-20 years prior to the expected age of symptom onset[46]. In patients with MCI, abnormalities are detected 5-10 years before progression to dementia[47]. Usually, the first abnormality detected is a decrease in $A\beta_{42}$, followed by an increase in $\tau_{P\text{-}181}$ and τ_T, but the reverse order may sometimes be observed[45]. Sometimes, only the $A\beta_{42}$ decrease is seen in the preclinical stage, and the increase in $\tau_{P\text{-}181}$ and τ_T is observed in the pre-dementia (MCI) stage of AD[48]. Thus, in the vast majority of patients, all 3 classical biomarkers are already abnormal when patients enter the dementia stage and in many (if not most), at the beginning of the MCI stage as well. CSF levels may continue to change during disease progression[46,48,49]. Such changes may be important from the neurochemical point of view, and it has been suggested that they may correlate with the stage of the disease[48]. However, from a diagnostic point of view, the changes compared to controls are small, and thus, these biomarkers are considered as state and not stage markers[49].

DEFINITION OF THE ALZHEIMER'S CLASSICAL CSF BIOMARKER PROFILE (SIGNATURE)

In the research diagnostic criteria for AD of the International Working Group (IWG-2), both decreased $A\beta_{42}$ and increased tau protein (either τ_T or $\tau_{P\text{-}181}$) are considered as *in vivo* evidence of AD pathology, with sensitivities and specificities approaching or exceeding 90%[11]. However, more recent recommendations suggest that all 3 biomarkers should be abnormal[50]. Indeed, this may increase specificity, and abnormality of all 3 biomarkers is highly suggestive (and specific) of the presence of AD, while normal values of all 3 biomarkers is highly suggestive of the absence of AD pathology[50]. In patients with MCI, the combination of all 3 markers (τ_T and the $A\beta_{42}/\tau_{P\text{-}181}$ ratio) identified those harboring AD pathology with sensitivities and specificities of 95% and 87%, respectively[51].

In pathologically verified cases, the combination of $A\beta_{42}$ and τ_T identified AD patients, discriminating them from other dementias or controls with sensitivity and specificity of 90% and 89%, respectively[52], while the combination of $A\beta_{42}$ with the more specific $\tau_{P\text{-}181}$ discriminated AD from other dementias with sensitivity and specificity of 80%-88% and 93%-100%, respectively[52,53].

The above indicates that, ideally, the AD CSF biomarker signature should be defined as abnormal values of all 3 core biomarkers. However, the combination of $A\beta_{42}$ with one of the tau forms (either total or phosphorylated) may be sufficient in everyday practice.

ANSWERED AND UNANSWERED QUESTIONS

Classical AD biomarkers are useful in everyday practice since they can discriminate AD from normal aging[43] and psychiatric conditions[54]. They offer an added diagnostic value in everyday differential diagnosis of dementia patients, since they increase diagnostic confidence[41] and correctly identify the presence or absence of AD in 82% of patients with uncertain clinical diagnosis[55]. They can be useful in the differential diagnosis between AD and FTD[56], and they can identify the additional presence or absence of AD in patients with cerebrovascular disease and dementia[44], including subcortical small vessel disease[57]. These biomarkers may also determine the additional presence of AD in patients with DLB[58] and those with normal pressure hydrocephalus[59]. Additionally, they can identify the presence or absence of AD biochemical process in patients with certain cognitive and/or parkinsonian syndromes such as primary progressive aphasia[12], posterior cortical atrophy[60] and corticobasal syndrome[13].

Of course, CSF AD biomarkers are not standalone tools, and they should be used in conjunction with clinical, imaging, neuropsychological and other biochemical data to reach the correct diagnosis[11]. Keeping that in mind, CSF $A\beta_{42}$, τ_T and $\tau_{P\text{-}181}$ fulfill most of the criteria required for valid biomarkers[61], since they reflect key biochemical mechanisms of AD, and combined, they provide sensitivities and specificities greater than 80%-85%. Sampling needs lumbar puncture, which is less agreeable than urine or blood sampling. However, it is a minimally invasive procedure, usually well-tolerated and with a low incidence of post-lumbar puncture headache (< 4.5%) in dementia patients[43,62]. Thus, the 3 core CSF biomarkers were gradually incorporated in research and/or clinical diagnostic criteria for AD in the dementia (typical or atypical presentations)[10,11,63], MCI[11,64] and preclinical stages[65], and if testing is available, they are currently considered as part of the diagnostic workup of cognitive disorders, especially in ambiguous cases[66-68]. Since new disease-modifying or preventive treatments are currently underway, CSF biomarkers may be used for the selection of patients suitable for clinical trials across all stages of AD (including the preclinical stage) and/or for monitoring treatment effects[69,70].

With time, it has become evident that biomarkers can detect CSF signatures different from the one observed in AD. The term "suspected non-Alzheimer pathophysiology" (SNAP) was introduced for a biomarker profile with normal $A\beta_{42}$ but an abnormal marker of neuronal injury or neurodegeneration, while the term "primary age-related tauopathy" has been used for the tau pathology picture in the medial temporal lobe (hippocampus, entorhinal cortex), with or without minimal Aβ pathology[71]. In patients with normal $A\beta_{42}$, the $A\beta_{42}/A\beta_{40}$ ratio may be used to confirm the absence of amyloid abnormality, since it "corrects" observed $A\beta_{42}$ levels for the total level of $A\beta_{40}$ (the most abundant form of Aβ peptide)[67,72]. When amyloid normality is confirmed, AD becomes unlikely[50] and tauopathies, TDP-43 proteinopathies and other pathologies may be considered to explain SNAP cases[73]. Controversies and questions concerning the "non-AD" biomarker profiles and the underlying pathologies have led to a modification of the 2011 National Institute on Aging and Alzheimer's Association separate recommendations[10,64,65], to a unified biological definition of AD across all stages and incorporating the various possible biomarker profiles and disease categories (AD or non-AD)[74]. This incorporation of "extended" biomarker profiles in diagnostic recommendations, may prove useful in many atypical presentations, including patients resembling or even fulfilling criteria for AD, but without the expected AD CSF biomarker signature, although biomarker levels may remain conflicting in occasional patients.

Another profile which may be observed is characterized by abnormality (reduction) of only $A\beta_{42}$, while τ_T and $\tau_{P\text{-}181}$ being normal. In this case, the $A\beta_{42}/A\beta_{40}$ may be used to confirm or exclude amyloid abnormality. If amyloid reduction is confirmed, AD pathology may be less likely in patients with full-blown dementia, but it is still a possibility, especially in pre-dementia patients[50] and may be compatible with the "AD pathological change"[74]. This profile may also be observed in vascular cognitive decline[57] and in Lewy body synucleinopathies, including PD, PDD and especially DLB[75].

Furthermore, there is always the problem of mixed pathologies, especially in the elderly. In a patient with a clinical picture suggestive of DLB, the identification of the typical AD CSF signature, may indicate mixed synucleinopathy with concomitant AD pathology[11,76], but cases of AD with unusual DLB-like presentations have been described[77]. Even in the most common scenario of mixed pathology, the question arises as to whether it represents DLB with some degree of AD pathology, AD with some degree of Lewy-pathology or equally severe pathology of both types[58]. Similarly, in a patient with a FTD-like clinical picture, the identification of the typical AD CSF signature may serve as exclusion criterion for FTD[78], suggesting AD with an atypical clinical presentation (frontal variant)[11], but mixed pathology cannot be excluded, since patients with concomitant FTD and AD do exist[79].

Some patients may show borderline or gray-zone levels in one or more of the classical biomarkers. The $\tau_T/A\beta_{42}$ and $\tau_{P\text{-}181}/A\beta_{42}$ ratios may be of some help in such patients[12], but not always. The "Erlangen Score",

which depends on normal, border-zone or abnormal biomarker levels, has been suggested to determine the level of neurochemical probability for (or against) AD in both dementia[80] and pre-dementia stages[81].

In case of atypical, conflicting or inconclusive CSF biomarker results, other neurochemical and/or imaging biomarkers, and/or later repetition of CSF sampling and analysis may be necessary[50].

VARIABILITY OF BIOMARKER RESULTS

Despite intensive research, there is still a significant inter- and intra-laboratory variability in the results of biomarker level determination, as a result of pre-analytical, analytical, post-analytical and kit-related factors, even between laboratories using the same methods[82-86]. During the last decade, various international initiatives, quality control programs and international workshops have been organized to reduce variability and harmonize the levels of biomarkers[67,82,83], including the "Biomarkers for Alzheimer's disease and Parkinson's disease" project of the Joint Programming Neurodegenerative Disease (JPND-BIOMARKAPD)[87]. As a result, recommendations have been published regarding lumbar puncture, pre-analytical and analytical standardized operating procedures[82,88-90], leading to improvement in diagnostic performance and reduction of measurement errors[91]. Although a measurement error of ± 20% in only one of the three biomarkers may have a minimal effect on overall diagnostic performance in everyday practice (variability ≤ 8%), errors of greater magnitude and/or affecting more than one biomarker, may lead to a significant decrease in diagnostic accuracy[92]. Newer methods for the determination of classical biomarkers may show better repeatability and reproducibility and less inter-laboratory variability[66,93].

NEW AND EMERGING BIOMARKERS FOR AD AND OTHER DISORDERS

Among many molecules studied in AD, neurogranin, neurofilament light (NFL), the ectodomain of triggering receptor expressed on myeloid cells 2 (sTREM2) and visinin-like protein 1 (VILIP-1) may serve as markers of synaptic loss, neuronal/axonal damage, microglial activation and neurodegeneration, respectively[66-68].

Recently, promising results have been published for CSF TDP-43 in patients with FTD and/or amyotrophic lateral sclerosis (ALS)[94-96]. The $\tau_{P\text{-}181}/\tau_{T}$ ratio has been suggested as another marker, which may prove helpful in the identification of FTD pathology[97], but its combination with TDP-43 may increase its diagnostic value even more[95]. NFL may also have some value in patients with FTD and/or ALS[66]. Further studies are needed, and they are in progress, both for validation and standardization of TDP-43 methods and for identifying the optimum combination of TDP-43 with other biomarkers for *in vivo* detection of the FTD subtype.

Alpha-synuclein (α-syn) has been studied as a biomarker of Lewy body synucleinopathies, in the differential diagnosis of cognitive and/or movement disorders[98,99]. Several studies have revealed that in synucleinopathies such as DLB, CSF α-syn levels are reduced, as compared to controls or AD[100,101]. However, increased levels in DLB *vs.* AD[102] or PDD[103] have also been reported, especially of oligomeric α-syn[104], while for PD or PDD, a non-significant reduction was too small to achieve diagnostic significance *vs.* controls and other movement disorders[13] or AD[103]. The above discrepancies indicate that, despite intensive research, there are methodological problems in α-syn quantification. Determination of α-syn needs strict pre-analytical control for confounding factors (especially bloody CSF), while assay parameters such as antibodies used, and forms of α-syn detected, necessitate further studies before one or more robust tests become widely acceptable[99,105].

CONCLUDING REMARKS

Classical CSF biomarkers of AD are useful tools in the (differential) diagnosis of patients with cognitive decline, especially in early or atypical cases [Table 1]. They are useful in differentiating AD from normal

Table 1. Levels of classical cerebrospinal fluid Alzheimer's disease biomarkers in various cognitive disorders

	$A\beta_{42}$ or $A\beta_{42}/A\beta_{40}$	Total tau (τ_T)	Phospho-tau*
Alzheimer's disease	Decreased	Increased	Increased
Vascular cognitive impairment	May be decreased in some patients	May be increased in some patients	Normal
Frontotemporal dementia	May rarely be decreased	May be increased in some patients	May be increased in some patients
Dementia with Lewy bodies	Frequently decreased	May be increased in some patients	Normal
Creutzfeldt-Jakob disease	May be decreased in some patients	Extremely increased	Normal
Normal aging	Normal	Normal	Normal
Psychiatric disorders	Normal	Normal	Normal

Based on the references cited throughout the text. *Usually for $\tau_{P\text{-}181}$, others have also been suggested

aging, psychiatric disorders such as depression, pure vascular cognitive impairment, pure DLB and FTD, and they can identify atypical and misleading clinical presentations of AD, or the coexistence of AD in other primary (such as VCI or DLB) or secondary cognitive disorders[12,14,44,54,56-59]. However, they should always be used in combination with clinical, neuropsychological and imaging data[15], and due to variability of measurements, each laboratory should establish their own normal or cut-off values[66].

CSF biomarkers detect normal or abnormal biochemistry, offering (during life) an alternative to post-mortem pathology and showing a very good concordance with pathological diagnosis[66]. Thus, many, if not most, of patients can be correctly diagnosed. However, borderline or inconclusive results may occur in some patients, requiring repetition of measurements and/or use of additional biomarkers[50,96,102]. Furthermore, classical CSF biomarkers cannot accurately detect mixed degenerative pathologies, which are not unusual in older patients. For example, the identification of an AD biomarker profile in a patient with dementia, parkinsonism and hallucinations, may indicate an atypical clinical presentation of AD, AD with some additional Lewy bodies, DLB with some additional AD-type pathology, or a severe degree of both pathologies[58]. This further necessitates the use of additional biomarkers (in the above case, α-syn). Unfortunately, methodological issues requiring further investigation prevent some of the newer biomarkers such as α-syn and TDP-43 to be currently considered "established".

The 3 classical AD biomarkers (τ_T, $A\beta_{42}$ and $\tau_{P\text{-}181}$) become 4 by adding $A\beta_{40}$. Adding NFL, α-syn, TDP-43 and others increases the number to at least 7. Adding them to structural and functional neuroimaging and possibly to genetic biomarkers, leads to a tempting increase of available data for patients; unfortunately, there is an even more substantial increase in cost, while the diagnostic accuracy may not be equally increased in some patients. Instead of an "all for all" approach, a personalized, precision medicine approach may be more appropriate[106], while blood biomarkers may be adequate for some patients[107].

The ability to detect the AD CSF biochemical signature in pre-dementia and especially in pre-symptomatic subjects, raises some ethical issues[108]. Communication of a positive result in a non-demented subject may have adverse effects in quality of life and trigger significant emotional reactions[109]. Since the time of appearance of the initial vague symptom(s) is usually unpredictable, many authorities consider it inappropriate to perform such diagnostic tests in the majority of asymptomatic subjects (including families with autosomal dominant AD). However, other subjects prefer disclosure of the results, so that they can adjust their life accordingly (including measures for secondary prevention) or make important decisions before dementia affects their judgment[110]. Such parameters should be taken into consideration before determining CSF biomarkers and/or communicating results, especially in research settings[108].

On the other hand, early detection is important in correct classification of subjects in trials of disease-modifying approaches, which may be effective when given in pre-symptomatic stages of AD. Since such trials are usually multicenter, stability, robustness and harmonization of methods and results, regulatory guidance, operator training, quality control programs, strict adherence to recommendations for

standardized operating procedures and harmonization of diagnostic criteria used, as well as well-organized and secure patient data sharing, are all required and pose challenges that should be faced by specialized centers[111].

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