CSF Biomarkers

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Abstract: With an ever growing population of aged individuals who are at risk of developing Alzheimer's disease (AD), there is an urgent need for a sensitive, specific and preferably non-invasive diagnostic standard of disease progression. Diagnosis of AD is still largely based on exclusion criteria of secondary causes and other forms of dementia with similar clinical pictures, than the diagnostic accuracy of AD is low. Recent research focused the attention to biochemical diagnostic markers (biomarkers) as they are very important indicators of normal and abnormal biological processes. Molecular aberrations in the AD brain are reflected in the cerebrospinal fluid (CSF) where three candidate biomarkers have recently been identified: total tau protein, amyloid β-protein 1-42 and tau protein phosphorylated at AD-specific epitopes. The sensitivity and specificity of these data are able for discrimination of AD patients from controls. Here, we review the recent literature on biochemical biomarkers and discuss their predictive value as indicative for disease vulnerability to detect individuals at risk for AD and to determine the clinical efficacy of novel, disease-modifying strategies. According to the literature analysis reported in the present review, we can conclude that the combination of the CSF biomarkers and their ratios may significantly increase the specificity and the accuracy of AD diagnosis.

Keywords: Alzheimer's disease, cerebrospinal fluid, biomarker, tau protein, amyloid β-protein.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia among elderly people and will become a public health crisis within two to three decades if left untreated. The onset of disease is not manifested clinically and little is known regarding the cause of non-familiar AD. Diagnosing AD and distinguishing it from other dementias depends primarily on clinical evaluation, and, ultimately, on investigator judgment [1]. This procedure is time consuming and costly, requiring neurological examinations, neuropsychological testing, neuroimaging, and blood investigations. There is also no definite clinical method to determine which patients with mild cognitive impairment (MCI) will develop AD [2, 3]. A definitive diagnosis of AD can only be made after death, when autopsy can reveal senile plaques and neurofibrillary tangles in brain tissue. Therefore, there is an urgency to develop a novel promising biomarker for early diagnosis of AD [2].

Indeed, the prevailing diagnostic standards in research for AD, the NINCDS–ADRDA criteria, are now being revised in order to introduce neurobiological measures on to the clinically based criteria [4]. The new criteria are centred on a clinical core of early and significant episodic memory impairment and there must also be at least one or more abnormal biomarkers among structural neuroimaging with MRI, molecular neuroimaging with PET, and cerebrospinal fluid analysis of beta-amyloid (Aβ) or tau proteins [4]. These proposed criteria move away from the traditional approach of first identifying dementia according to degree of functional disability, rather they aim to define the clinical, biochemical, structural and metabolic presence of AD.

The testing and ultimate implementation of emerging therapies will require identification of affected and "at-risk" individuals to target them for clinical trials, and to direct and monitor therapy. Thus, fluid and neuroimaging measures are being explored as possible biomarkers for early-stage and pre-clinical AD diagnosis because it is in these initial stages that disease-modifying therapies are likely to have the greatest chance of preserving normal brain function. Much focus has therefore been directed on patients with MCI, which is a syndrome characterized by cognitive impairment but not severe enough to fulfil the criteria for dementia [5]. Even though around 40-60% of patients with MCI develop AD during the first 5 years, many have a stable form of memory impairment [6, 7]. Moreover, early stages of vascular dementia or dementia with Lewy bodies, for example, can be preceded by MCI [7].

Although there are currently no proven therapies that delay the onset or prevent the progression of AD, several promising candidates are being developed. Biochemical changes in the brain are reflected in the cerebrospinal fluid (CSF), and intense research efforts have been made to develop biomarkers for the central pathogenic processes in AD that can be used as diagnostic tools. Early studies indicated that CSF biomarkers could be useful for defining a subgroup of patients with MCI at especially high risk of developing AD [8-10]. The best studied fluid proteins in AD have been CSF levels of Aβ42, the primary constituent of amyloid plaques, and tau protein, the primary component of neurofibrillary tangles. Levels of CSF Aβ42 are typically reduced in AD [11-13] reflecting its aggregation and deposition as amyloid in the brain [14], whereas levels of CSF tau and phosphorylated tau (p-tau) species are increased

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in AD [15, 16] and are hypothesized to reflect the presence of neurofibrillary tangles, neurodegeneration, or both [17, 18] although not all studies support this conclusion [19]. Indeed, there is no proof that plaque formation is reason for Aβ concentration decrease, only correlation. A tau protein/Aβ42 index, usually increased in AD patients, has high sensitivity, high specificity and also high negative predictive value in AD diagnosis [20].

The assessment of these markers can also contribute to the differential diagnosis of vascular dementia, but also other forms of primary degenerative dementia (e.g., a frontotemporal dementia). Therefore, the availability of biochemical markers that, at least in part, replace the clinical procedures, is highly desirable.

AMYLOID-β

The discovery that Aβ peptide forms the main component of AD plaques primarily with a length of 42 amino acids [21] and that it is secreted by cells [22] led to investigations of Aβ42 in the CSF for AD. A large number of studies have been conducted on patients and controls, showing a reduction of Aβ42 by about 50% in AD patients compared with non-demented controls of the same age; the diagnostic sensitivity and specificity levels ranged between 80% and 90% [8]. An autopsy study demonstrated an inverse correlation between Aβ42 levels in the CSF and the number of plaques [23]. Reduction in CSF Aβ42, likely reflecting Aβ aggregation in the brain, is associated with brain atrophy in the pre-clinical phase of AD [24]. This suggests that there is toxicity associated with Aβ aggregation before the onset of clinically detectable disease. Thus, CSF Aβ42 may be considered a useful biomarker for the presence of amyloid plaques regardless of clinical status. However, since some neurodegenerative diseases present with low CSF Aβ42 levels in the absence of plaques, other reasons for the reduced CSF Aβ42 levels in AD cannot be ruled out. Recently, it was found that multiple sclerosis goes with reduced CSF Aβ42, which is probably caused by upstream downregulation of β-secretase (BACE1) and amyloid precursor protein (APP) expression secondary to neuroinflammation [25]. Furthermore, reductions in CSF Aβ42 values reflect the presence of amyloid plaques but perhaps also diffuse nonfibrillar plaques and/or concomitant Aβ oligomer formation, both of which could contribute to neurotoxicity [26]. Promising studies using unconventional methods suggest that Aβ oligomers can be measured in CSF in the femtomolar range [27, 28].

Since CSF levels of the shorter Aβ40 isoform are unchanged or increased in AD, it has been also proposed that measurement of the Aβ42/Aβ40 ratio might be more accurate to Aβ42 alone [12, 29-32], even in early stage disease [33]. Moreover, the discovery of shorter Aβ isoforms in brain and CSF has made it clear that Aβ constitutes a large family of peptides with considerable length variations [34]. The shorter peptides likely result from the ability of BACE1 and γ-secretase to execute catalytic cleavages at different positions in APP, as well as the probable involvement of other APP- and Aβ-degrading proteases. More studies are needed to determine the clinical value of CSF measurements of detailed Aβ peptide patterns, as compared to CSF Aβ42 alone.

Recently, a method based on stable isotope labeling and mass spectrometric analysis of immunocaptured Aβ was presented [35]. This technique allows for determination of production and clearance rates of total Aβ in CSF and plasma and may help addressing the fundamental question if patients who develop AD have increased production or decreased clearance of the peptide.

TAU PROTEIN

The main component relating to intraneuronal changes in AD patients is the microtubule-associated tau protein. In AD patients, tau protein is present in a pathologic, hyperphosphorylated form. Tau pathology is also characteristic of other neurodegenerative diseases, but it differs from tau pathology in AD patients at the molecular level [36]. Initially all forms of tau, regardless of their phosphorylation status, were quantified in the CSF involving that they are released extracellularly as a result of the neurodegenerative process. Numerous studies have demonstrated an increase in the concentration of total tau (t-tau) in AD patients by approximately 300% compared with non-demented elderly subjects, and a systematic increase in the concentration with age was observed in the control groups [37, 38].

Using monoclonal antibodies specific for different phosphorylated epitopes of tau, enzyme-linked immunosorbent assays have been developed that sensitively measure concentrations of phosphorylated tau protein (p-tau) in CSF. Indeed, most of the studies to date have investigated tau protein hyperphosphorylated at threonine 231 (p-tau 231P) and at threonine 181 (p-tau 181P), and a few results have been obtained for serine 199 (p-tau 199P). Differences have certainly been observed between the individual p-tau subtypes: p-tau 231P and p-tau 181P show better results than p-tau 199P in distinguishing AD from control groups [39]. Furthermore, p-tau 231P significantly improved differential diagnosis between AD and other non-AD groups, particularly frontotemporal dementia [40], and p-tau 181P has been proposed as a potential marker for discriminating patients with AD from those with dementia with Lewy bodies [39].

Concentrations of all 3 p-tau proteins are equally significantly increased in patients with AD [39-44]. Thus, the high negative predictive value of p-tau of approximately 90% appears to be particularly significant meaning that normal values rule out the presence of AD with almost 90% probability [45].

RATIO Aβ/TAU

The development of refined assays improved the separation of AD from other dementias but did not improve the sensitivity of detecting AD [44, 46]. Thus, it has been investigated the diagnostic usefulness of the CSF ratio of p-tau to Aβ42 instead measuring alone. It is now well established that CSF Aβ42 or CSF Aβ42/Aβ40 in conjunction with t-tau and p-tau, that reflect the axonal degeneration in AD [47], identify and predict AD with sensitivity and specificity of 80-95% [9, 48-51]. Measurement of the CSF ratio of p-tau to Aβ42 provides a biochemical diagnostic aid that may replace some of the current clinical investigational efforts and thereby speed up the diagnostic procedure and reduce its cost. Measurement of

the CSF ratio of p-tau to Aβ42 may also constitute a tool for monitoring disease progression, which has to be investigated within a longitudinal design.

CSF analyses of t-tau, p-tau 181P and Aβ42 are strong and independent risk markers for development of clinical AD in patients with MCI [9, 48, 50, 52, 53]. Namely, CSF high p-tau 231P levels at baseline correlated with the rate of cognitive decline in Mini-Mental State Examination scores in patients with MCI [39]. In agreement with the analysis of rates of cognitive decline, increased levels of p-tau 231P correlated with conversion to AD [39]. A recent European multicenter trial on CSF p-tau 231P in MCI subjects has shown that the results for p-tau in predicting AD in this risk group are indeed stable and consistent throughout multiple centers [54]. In this study p-tau proved to be a powerful candidate predictor of AD in MCI subjects even in a very short mean observation interval of only 1 to 2 years [54].

Besides, it has been demonstrated that the association of all the different combinations of these CSF biomarkers with incipient AD was much stronger than, and independent of, other established risk factors such as age, sex, education, *APOE* genotype, plasma homocysteine, blood pressure, and low performance on brief cognitive tests [48]. All these studies suggest that a useful combination of biomarkers might optimize prediction in a more heterogeneous MCI population during a longer observation period, however, more studies are needed to establish which combination has the best performance.

NOVEL APPROACHES

Several biomarkers for neurodegenerative diseases have been identified in past studies, which include APP, cathepsin B precursor [55] and β–fibrinogen plus Vitamin D-binding protein or ceruloplasmin for AD [56]. Recently, it has also been suggested that signaling proteins in blood plasma can be used to differentiate AD from control subjects [57]. Unfortunately, however, the sensitivity and specificity of any of these identified markers, either alone or in combination, is insufficient to merit routine clinical use [58]. Thus, analysis of proteins in CSF as new clinically useful biomarkers is of great diagnostic importance.

Cystatin C is another marker that has been investigated for the diagnosis of neurodegenerative diseases [59]. This amyloid protein occurs along with Aβ42 in the walls of the arterioles in patients with AD and binds Aβ inhibiting its fibril formation [60]. It seems that very low concentrations of cystatin C, both in the CSF and serum of AD patients, are caused by its accumulation in the reactive astrocytes before amyloid formation [20]. Some authors reported no statistically significant differences between the cystatin C levels in the serum and the CSF in AD and vascular dementia patients when compared to other patients [20, 61]. On the contrary, other studies [62, 63] reported that serum cystatin C levels decrease in AD patients. Lower serum levels of cystatin C has also been associated with higher incidence of AD in elderly men free of dementia at baseline suggesting cystatin C as a marker of future risk of AD [64]. This finding hypothesizes that low serum cystatin C levels precede clinical AD and, possibly, mirror a reduced ability to inhibit neuronal Aβ aggregation [64]. Thus, further studies are needed to investigate this important issue even though, due to these controversial studies, cystatin C probably will not become a new "revolutionary" marker contributing to the differential diagnostics.

Recently, it has been characterised in the CSF and brain of AD the presence of S100A7 [65], a protein previously implicated in inflammatory responses and cell differentiation [66]. S100A7 content is elevated in the CSF of AD dementia cases compared to neurological control cases, and these elevated S100A7 levels in the CSF selectively identify AD clinical severity [65]. It seems that S100A7 attenuate AD amyloid neuropathology through promotion of the "non-amyloidogenic" α-secretase processing of APP. Thus S100A7 could be a biomarker that quickly diagnoses AD before clinical signs have developed, allowing for early symptomatic treatment.

Neuronal pentraxin receptor (NPR) and α-dystroglycan and 120 kDa isoform precursor of neural cell adhesion molecule 1 (NCAM-120) have been also identified as candidate biomarkers for neurodegenerative diseases [67]. More importantly, NPR has been found to be a candidate biomarker specific for AD [67]. However, large-scale validation studies need to be performed to confirm the association between NPR and AD and also to understand how and whether NPR participates in the pathogenesis of AD.

A particularly promising new approach in the CSF focuses on the detection and quantification of BACE1, one of the key enzymes responsible for the pathologic amyloidogenic cleavage of the APP. A significant increase was found in BACE1 concentration and activity in the CSF of MCI subjects compared with healthy controls [68].

Finally, isoprostanes are also being studied as candidate markers of lipid peroxidation. An increase was found in the CSF of MCI subjects compared with controls, and isoprostanes and p-tau performed better than memory tests [24]. The isoprostanes even improved the results obtained with hippocampal volumetry to distinguish between the groups [69]. However, because of the very demanding analysis method, isoprostanes should still be regarded as a merely scientific approach.

In recent years, there has been a growing interest in applying proteomics to research on clinical diagnostics and predictive medicine of neurodegenerative disorders [70]. Proteomics enables the study of many proteins simultaneo-usly and can thereby unravel pattern of changes in neurodegenerative disorders, e.g. patterns that are disease specific. Proteomic profiling of cerebrospinal fluid provided a novel panel of potential biomarkers for prediction of MCI progression to AD and for the differential diagnosis of AD versus normal aging and frontotemporal dementia [71-74].

Many efforts have also focused on serum and CSF levels of antibodies to Aβ that naturally are found in the CSF and plasma of patients with AD as well as healthy control subjects [75]. To date, differences between diseased and control subjects has been highly variable especially because, in biological fluids, antibodies and antigens are in a state of dynamic equilibrium between bound and unbound forms that is concentration dependent. Namely, there is a recent study that demonstrates the relevance of measuring bound and unbound antibody against Aβ in serum of patients affected

by AD [75]. This finding suggests that dissociated Aβ antibody levels are of significant diagnostic value at the onset of the neurodegenerative process and, thereafter, may be a useful biomarker for disease progression.

CONCLUSION

As disease-modifying therapies for AD are being developed, there is great need to identify biomarkers that will serve as surrogates of underlying disease pathology. In the eventual clinical setting, such biomarkers may be used to improve the accuracy of clinical diagnosis and to track disease progression. As an immediate application, biomarkers may be useful in the design and evaluation of clinical trials; for example, to assess the effect of a therapy on its intended target in early phase studies, to optimize patient enrollment in prevention trials and to track disease progression.

Intense research has led to the development of CSF biomarkers reflecting different aspects of AD pathogenesis. Currently, validated and reliable biomarkers exist for amyloid pathology and Alzheimer-type axonal degeneration. Measuring CSF Aβ42, t-tau and p-tau alone or in combination may be especially useful for the selection of pre-symptomatic individuals with known pre-clinical AD pathology for enrollment in prevention trials of disease-modifying therapies. Furthermore, reliable methods to measure Aβ oligomers that may be specifically related to AD onset and progression would be a valuable tool in AD diagnostics. There are also indications that the ratios of various Aβ peptides improve the neurochemical profile for potential diagnostic applications [76, 77].

A potential limitation for the widespread use of CSF biochemical markers in general practice lies in collecting CSF at lumbar puncture. However, the technique of lumbar puncture has considerably improved and, as a consequence, the incidence of headache after lumbar puncture in elderly patients is 2% or less [78]. The evaluation of CSF Aβ42, t-tau, and p-tau in memory clinics will result in some false-positive cases, as well as false-negative cases, and the biomarkers may therefore be useful primarily as screening tools, selecting individuals for a detailed further clinical follow-up. Moreover, they may be useful in enriching study populations for clinical trials of future disease-modifying AD treatments.

It is unrealistic to expect that both sensitivity and specificity could be set at a level higher than 90% by measuring Aβ peptides and tau proteins alone, because post-mortem analyses of brains with AD revealed a variety of additional lesions, such as infarcts, gliosis, argyrophilic grains, and Lewy bodies. In addition, other dementing conditions display at least some neuropathological features that overlap AD, such as tau-positive filamentous lesions. In the future, a biochemical marker pattern reflecting the whole spectrum of abnormal proteins deposited in the brain will most likely provide a more accurate diagnosis of AD, comparable with the current criteria for the neuropatho-logical classification.

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