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UPDATE ARTICLE

Mild cognitive impairment (part 2): biological markers for diagnosis and prediction of dementia in Alzheimer's disease

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Objective: To present a critical review of publications reporting on the rationale and clinical implications of the use of biomarkers for the early diagnosis of Alzheimer's disease (AD).
Methods: We conducted a systematic search of the PubMed and Web of Science electronic databases, limited to articles published in English between 1999 and 2012, and based on the following terms: mild cognitive impairment, Alzheimer's disease OR dementia, biomarkers. We retrieved 1,130 articles, of which 175 were reviews. Overall, 955 original articles were eligible.
Results: The following points were considered relevant for the present review: a) rationale for biomarkers research in AD and mild cognitive impairment (MCI); b) usefulness of distinct biomarkers for the diagnosis and prediction of AD; c) the role of multimodality biomarkers for the diagnosis and prediction of AD; d) the role of biomarkers in clinical trials of patients with AD and MCI; and e) current limitations to the widespread use of biomarkers in research and clinical settings.
Conclusion: Different biomarkers are useful for the early diagnosis and prediction of AD in at-risk subjects. Nonetheless, important methodological limitations need to be overcome for widespread use of biomarkers in research and clinical settings.

Keywords: Alzheimer's disease; mild cognitive impairment; biomarkers; neuroimaging; amyloid-β protein; Tau protein

Introduction

In view of growing life expectancy worldwide, Alzheimer's disease (AD), the most common dementing disorder in the elderly, will reach epidemic status within the next decades.[1] This is a natural consequence not only of the age-dependent increase in the number of incident cases of AD, but also of the development of new diagnostic tools that will allow its early identification in elders with very mild symptoms of cognitive dysfunction, or even in asymptomatic individuals. In the current literature, the early diagnosis of AD subsumes the diagnosis of AD at pre-dementia stages. The identification of individuals at risk of dementia with no detectable clinical manifestations of this syndrome parallels the attempts to determine the risk of the future occurrence of major disease events in the light of measurable underlying susceptibility and/or pathological markers at early stages of the clinical trajectory of the disease. This has been the standpoint of good clinical practice in the management of several medical specialty, such as the assessment and modification of the risk of myocardial infarction in patients with dyslipidemia, diabetes mellitus and systemic hypertension in cardiology; the diagnosis of carcinoma in situ in oncology; or the prevention of fractures given the early recognition of osteopenia and osteoporosis in orthopedic surgery.

With respect to AD, there is little doubt that substantial progress has been accomplished in the pursuit of this target in the past decade. From a clinical perspective, it is still a difficult task to clinically differentiate incipient AD from normal cognitive aging and from subtle cognitive changes that arise in other forms of dementia at prodromal phases.[2] In the early stages of AD, patients may present with mild but persistent (and often progressive) cognitive deficits, albeit not severe enough to warrant the diagnosis of dementia (e.g., patients with amnestic mild cognitive impairment [MCI]).[3,4] In this scenario, the assessment of biomarkers (cerebrospinal fluid [CSF] and peripheral biochemical markers, structural and functional neuroimaging and brain amyloid imaging) can help researchers and clinicians increase the sensitivity and specificity of clinical diagnosis. Despite being

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controversial and a matter of much debate in the literature, the systematic assessment of these biomarkers has been incorporated into recent revisions of AD and MCI diagnosis.[5-7] In this review article, we address recent research developments in biomarkers for the early diagnosis of AD and the current limitations to their widespread clinical use. Finally, we discuss challenges and perspectives for the development of pharmaceutical compounds with disease-modifying properties in AD.

Methods

We carried out a systematic search of the PubMed and Web of Science electronic databases using the following broad MeSH terms: mild cognitive impairment, Alzheimer's disease OR dementia, biomarkers. We did not set any time or language limit to this search. We retrieved 1,130 articles, of which 175 were reviews. We reviewed the remaining 955 articles and included and critically summarized the findings of the most relevant articles in the following sections.

Results

AD biomarker development: from translational research to clinical practice

AD is a chronic neurodegenerative disease with well-defined pathological markers, i.e., neuritic plaques (NPs) and neurofibrillary tangles (NFTs), mostly affecting the medial temporal lobe and associative neocortical structures.[8] The main component of NP is aggregated and insoluble β-amyloid$_{42}$, whereas NFTs are the result of hyperphosphorylation of Tau protein in neurons.[9] Current models for the pathophysiology of AD are based on the β-amyloid hypothesis.[10,11]

Briefly, such models support that the primary pathophysiologic event in AD is the overproduction or defective clearance of β-amyloid peptides in the brain, leading to their aggregation into more toxic species (i.e., amyloid dimers and oligomers) and, finally, to the deposition of NPs. These processes trigger the activation of neurotoxic cascades, a local inflammatory reaction, and cytoskeletal changes due to the hyperphosphorylation of Tau protein in neurons, which eventually cause neuronal dysfunction and death.[12]

These pathological processes develop over a long period of time prior to the clinical manifestations of dementia syndrome[13] and may be tracked by different methods, such as visualization of brain amyloid deposition by molecular neuroimaging,[14] biochemical abnormalities in the CSF[15] and serum, and structural and functional brain changes.[16] This framework serves as the basis for the search and ordering of biomarkers that can aid in the diagnosis of AD, the staging of progression of pathological processes, and, most importantly, the identification of patients at the earliest stages of the disease process, who would benefit most from therapies aimed at reducing the risk of progression to the clinical dementia stages of the disease.[17]

The search and development of biological markers of AD pathogenesis

Translational research has been critical for the development of biomarker research in AD. Cumulative knowledge on the neurobiology and pathophysiology of the disease has yielded insights for the discovery of candidate markers of AD pathogenesis. Therefore, markers of intrinsic pathological processes that illustrate distinct phases of AD can be depicted in vivo and be used to support clinical diagnosis, especially at early stages, as well as to ascertain the biological effects of therapeutic approaches (disease modification).[18] To have clinical utility, a good biomarker should reflect core pathological changes that pertain to the disease process, be validated by postmortem studies, and be sensitive to early changes in the disease process. If possible, the determination of such biomarkers should rely on non-invasive, simple to perform, and reliable methods, rendering them adequate for large-scale screenings.[19] So far, no biomarkers tested for the diagnosis and prognosis of AD have achieved universal acceptance, nor fully met the proposed criteria for an ideal biomarker.

AD-related biomarkers can be summarized into three major categories, namely: a) molecular biomarkers that reflect core neuropathological changes in AD, e.g., evidence of significant accumulation of pathological amyloid-β species in the central nervous system (CNS), as shown by CSF analysis, or by in vivo molecular imaging with positron-emission tomography (PET); b) markers of downstream pathological changes triggered by core molecular changes, such as: evidence of neuronal injury, synaptic loss, and cytoskeletal damage (as shown by increased concentrations of total and phosphorylated Tau in the CSF); regional metabolism improved by effective connectivity, perfusional or structural changes affecting brain structures and detectable by neuroimaging methods such as structural magnetic resonance imaging (MRI) (e.g., evidence of medial temporal atrophy; decreased hippocampal or entorhinal cortex volume; rate of change in total brain or hippocampal volume; decreased regional gray- or white-matter volume in voxel-based morphometry [VBM] maps; abnormal pattern in diffusion tensor imaging [DTI]); or abnormal neurochemistry as shown by proton spectroscopy ($^{+}$H-MRS); c) associated homeostatic changes detectable in peripheral fluids, such as markers of inflammation (interleukins, cytokines) and oxidative stress (isoprostanes), plasma Aβ40/Aβ42 ratio, platelet: amyloid precursor protein (APP) ratio, GSK3β activity, and other markers of synaptic damage or neurodegeneration.

Cerebrospinal fluid biomarkers

Given its intimate contact with the CNS, the CSF is a natural source of molecules related to intracerebral pathogenic processes, and has been extensively studied in AD.[20] Several potential diagnostic biomarkers were studied in AD, but the most consistent findings have been obtained with the measurement of CSF concentrations of

β-amyloid$_{42}$ (Aβ_{42}), total Tau (T-Tau) and phosphorylated Tau (P-Tau).[15] These biomarkers reflect core neuropathological (and presumably pathophysiological) features of AD,[21] and have been validated in postmortem studies.[22-24]

The typical pattern for these CSF biomarkers in AD patients is commonly referred to as the AD signature in the CSF, i.e., low concentrations of Aβ_{42} and high concentrations of T-Tau and P-Tau. These findings reflect, respectively, the sinking and deposition of Aβ_{42} in plaques (in detriment of its clearance through the CSF) and axonal dysfunction leading to neuronal degeneration. The latter marker is perhaps specifically associated with AD, given the central role of P-Tau in the formation of paired helical filaments (PHFs) and NFTs.[25] A substantial body of evidence supports that the identification of the AD signature in the CSF has good diagnostic accuracy to differentiate cases of incipient AD from controls, and to predict conversion to dementia in samples of patients diagnosed clinically with MCI.[26] However, the sensitivity/specificity profile for distinguishing AD from other dementia syndromes is significantly lower,[27-29] which calls into question the advantage of routine assessment of these biomarkers in the diagnostic workup of established dementia syndromes.

In the mid- and late 1990s, several studies attempted to define whether these biomarkers could help in the diagnosis of MCI. Patients with MCI had intermediate CSF biomarker levels as compared to healthy elderly subjects and patients with AD.[30] Large-scale longitudinal studies of MCI cohorts addressing the progression to AD presented consistent findings confirming that, in samples of patients with MCI, the presence of the AD signature in the CSF has good sensitivity ($>$ 80%) to discriminate converters from non-converters and to differentiate such cases from other (non-AD) dementia outcomes.[26,31,32] These data have been extensively replicated in different research settings worldwide,[33-36] and confirmed by meta-analyses.[37,38] A recent study reported that the CSF pathological signature could be identified in subjects with MCI between 5 to 10 years before the clinical diagnosis of dementia.[39]

The aforementioned CSF biomarkers were also investigated in patients with subjective memory complaints (SMC) but no evidence of objective cognitive decline. These studies suggest that the AD signature may help predict the incidence of memory deficits, i.e., amnestic MCI.[40,41] More recently, abnormalities in CSF biomarkers have been correlated with poorer performance in memory and attention tasks[42] and with worse functional performance in the instrumental abilities of daily living,[43] both in cognitively healthy subjects and in those with MCI. In addition, studies of healthy elderly subjects showed that lower CSF Aβ_{42} levels predicted the emergence of SMC up to 3 years of follow-up.[44]

These findings demonstrate that the CSF biomarker pattern observed in AD patients can be identified at early stages of the disease process, starting from prodromal and even pre-symptomatic stages. This pattern distinguishes with good accuracy MCI patients who will progress to dementia from healthy individuals and stable cases of MCI. It may also help to discriminate slow- from rapid-converting cases of MCI, although with lower accuracy,. Therefore, the measurement of AD-related biomarkers in the CSF may be a useful tool to improve the diagnostic accuracy and predictive value of clinical classification of patients according to the definition of MCI.

Notwithstanding, there are still obstacles that need to be overcome to enable reliable use of AD biomarkers in clinical practice. Although the quantitation of CSF concentrations of these biomarkers using enzyme-linked immunoassay (ELISA) or multiplex techniques (e.g., xMAP-Luminex) has low coefficients of intra-laboratory variability (below 10%), high inter-laboratory variation (20 to 30%) is a major obstacle to the comparison of data generated in different settings. Multiple sources of bias include pre-assay (i.e., lumbar puncture protocol, sample handling and aliquot storing prior to experimentation), intra-assay (different methods and protocols for the determination of the actual concentrations of biomarkers), and post-assay variations (e.g., definition of reference ranges for patients and controls to guide the interpretation of results).[45] This situation is a major limitation to the establishment of multicenter cooperation. The establishment of protocols to be shared by all involved laboratories[46] and the recent launch of a multicenter quality control program with over 40 laboratories around the world will hopefully overcome these limitations in the near future.[47]

Peripheral biomarkers

Despite encouraging findings from CSF biomarker research, poor accessibility to samples is a relative disadvantage of this procedure. Although the cumulative experience of expert groups reinforces the large safety window of this procedure, one must acknowledge the fact that lumbar puncture is still an invasive method and requires qualified, trained personnel to perform it. Furthermore, CSF collection may raise ethical concerns and risk-to-benefit issues. Although the information gained from analysis of CSF biomarkers seems to be relevant for diagnostic accuracy, it may be questioned on the basis of the lack of validated therapeutic strategies to prevent AD. Therefore, other sources of AD-related biomarkers need to be defined. Several candidate biomarkers have been studied in plasma and peripheral blood cells, particularly leukocytes and platelets.

Different species of the amyloid-beta peptide may be measured in plasma. In particular, concentrations of Aβ_{40} and Aβ_{42} (in addition to the A$\beta_{42/40}$ ratio) have been extensively analyzed in clinical and epidemiological studies. A significant increase in plasma levels of Aβ_{40}, along with a decrease in Aβ_{42} (and in the A$\beta_{42/40}$ ratio), has been reported to predict cognitive decline in MCI patients and healthy elders.[48,49] Increments in plasma Aβ_{42} have also been reported to predict AD in these subjects.[50,51] Many studies have not found significant differences in the concentrations of these biomarkers in

samples of MCI converters.[52,53] Therefore, the current evidence suggests that measurement of plasma Aβ as a biomarker for AD is still controversial and lacks the consistency observed for CSF biomarkers.

Plasma markers related to inflammatory and neurotrophic cascades have also been addressed in studies with MCI and AD patients. Changes in inflammatory and neurotrophic cascades have been described in the pathogenesis of AD, and can be identified at the prodromal stages of the MCI-dementia continuum.[54,55] Although these homeostatic imbalances may be secondary to core pathological processes, measurement of these biomarkers may still be suitable to investigate risk of AD or to determine cumulative changes that are required for the expression of the dementia phenotype in addition to amyloid and Tau pathology.[56,57] Studies have consistently reported elevated levels of pro-inflammatory cytokines in patients with AD and MCI.[58-60] Longitudinal studies have shown that higher levels of pro-inflammatory cytokines constitute independent and strong predictors of MCI-AD conversion.[61,62] Likewise, lack of neurotrophic support, as indicated by reduced concentrations of brain-derived neurotrophic factor (BDNF), has been demonstrated in patients with AD and MCI, and indicates a higher risk of progression from MCI to AD.[63-65] Nevertheless, these findings may be less specific, since significant changes in these cascades have also been observed in other neuropsychiatric and neurodegenerative conditions, such as major depression, bipolar disorder, schizophrenia, and Parkinson's disease.[66-71]

Blood cells, namely leukocytes and platelets, may also be an important source of AD-related biomarkers, since they can provide insights on the systemic modulation of biological cascades that are supposedly altered in AD. For instance, platelets represent the most important source of circulating APP, and the regulatory loops of peripheral APP metabolism seem to parallel intracerebral homeostasis. The APP ratio is a promising peripheral biomarker that can be readily determined in platelets, addressing the proportion of 130kDA to 110kDa isoforms of the secreted, non-amyloidogenic metabolic product of APP released by secretase cleavage (sAPP). The APP ratio indirectly reflects the equilibrium between amyloidogenic and non-amyloidogenic APP processing. A reduction in APP ratio (indicative of reduced expression of 130kDa fragments, or increased expression of 110kDa sAPP) may reflect the predominant production of shorter (beta-cleaved) secreted products of APP.[72] A reduction in APP ratio has been found in patients with early AD and MCI subjects in cross-sectional analyses[73,74] and predicts the progression from MCI to AD.[75] This finding still requires validation in larger samples and longitudinal studies.

Determination of the enzymatic activity of glycogen synthase kinase (GSK-3β) in distinct biological sources has also been suggested as a promising biomarker for AD. GSK-3β is the main Tau-kinase in neurons, being thus primarily involved in the regulation of the phosphorylation state of Tau. In addition, it has also been shown to participate in the regulation of APP processing.[76] Recent studies have shown a significant increase in GSK-3β activity in the leukocytes and platelets of patients with MCI and AD,[77,78] thus pointing to its potential as a biomarker of early AD. Nonetheless, as is the case for APP ratio, data on GSK-3β activity as a candidate biomarker for AD are limited by the lack of specificity for AD, relative small sample sizes, the lack of longitudinal studies.[79]

Imaging biomarkers

The substantial development of neuroimaging techniques in the last decade has contributed decisively to the search for non-invasive methods to ascertain the pathological changes that evolve in the AD brain. These advances range from new protocols for the analysis of structural MRI (such as volumetric assessments of regions of interest and VBM based on statistical maps) to functional imaging based on PET, addressing metabolic changes and, more recently, in vivo intracerebral imaging of amyloid and Tau.

Structural neuroimaging

The main structural changes observed in AD are global cerebral volume loss, increased ventricular volumes, and more intense regional atrophy in the hippocampal formation and the entorhinal cortex. Topographic gray matter loss correlates with Braak stages and may be already present in patients with very mild AD.[80,81]

Individuals with MCI usually exhibit structural changes that are intermediate to those observed in AD and those seen in healthy controls. Those with MCI usually display mild, but significant, volume loss in specific brain regions, notably the hippocampal and parahippocampal structures, and decreased cortical thickness.[82-84] Longitudinal studies have shown that MCI-converter patients have more intense volume reductions in the hippocampal and parahippocampal structures, and, to a lesser extent, in the posterior cingulate cortex, middle and inferior temporal gyri, fusiform gyrus, posterior cingulate gyrus, precuneus, temporoparietal junction, and frontal cortex.[85-88] Another interesting finding from longitudinal studies is the accelerated gray matter loss in MCI converters as compared to MCI-stable subjects.[89] A recent meta-analytical study showed a consistent finding of decreased left hippocampal volumes in converter vs. stable MCI patients.[90] Altogether, these findings suggest that the characterization of regional volumetric changes in critical brain areas by structural neuroimaging may be a useful biomarker for the identification of subjects at increased risk of developing clinical AD. Of particular interest, the characterization of hippocampal and parahippocampal atrophy as well as acceleration of gray matter loss in longitudinal evaluations are important predictors of dementia outcome at early stages of AD.

Functional neuroimaging and in vivo molecular imaging (Aβ and Tau)

The main metabolic changes observed in the AD brain on FDG-PET and SPECT scans are global reductions,

respectively, in cerebral metabolism and perfusion, which occur with greater intensity in the temporoparietal junction, temporal, parietal and frontal lobes, hippocampal formation, and posterior cingulate cortex.[91-93] As is the case for most methods of structural neuroimaging, patients with MCI show an intermediate pattern of changes between healthy elders and patients with AD.[94,95] Likewise, in prospective studies, MCI converters show a pattern of cerebral hypometabolism and/or hypoperfusion that is largely similar to that of patients with mild AD, particularly in the posterior cingulate cortex and the hippocampal regions.[96-98]

The development of new techniques for in vivo visualization and quantitation of Aβ and Tau deposits within the brain is undoubtedly a major achievement in the field of AD biomarker research. The first compound used in humans was the Pittsburgh compound B (PiB),[99] which is a ^{11}C-labeled compound with a high binding affinity for intracerebral Aβ in mature amyloid plaques.[14] Other compounds have subsequently been made available, such as the amyloid-binding compound ^{18}F-BAY94-9172[100] and the dual, amyloid and Tau-binding compound FDDNP, which has the additional property of mapping the occurrence of NFTs and amyloid plaques within the brain.[101]

In patients with AD, there is an increased global cortical retention of PiB (and other compounds), especially in specific brain regions, such as the striatum, cingulate, temporal, and parietal frontal cortices.[102] Studies with amyloid imaging have a very high sensitivity (over 90%) but their specificity is age-dependent, since there is increasing deposition of Aβ in the aging brain.[103] Important studies have shown negative correlations between intracerebral amyloid content (as shown by PiB scans) and CSF concentrations of $A\beta_{42}$ in patients with mild AD (CDR = 0.5 or CDR = 1) as compared with controls.[104] This finding has been recently replicated by the same group of investigators in a larger sample.[105]

As observed in other neuroimaging modalities, PiB retention rates are also increased in patients with amnestic MCI, though at a lower rate than observed in patients with AD. In most studies, PiB retention rates (global and regional) correlate with cognitive performance.[106,107] A positive PiB scan predicts conversion from MCI to AD (55% of PiB-positive patients with MCI progressed to AD in 2 years, vs. 10% of PiB-negative patients).[103] Interestingly, retention of PiB was also observed in elderly subjects without cognitive symptoms, but higher PiB retention at baseline correlated with worse cognitive performance and faster cognitive decline upon follow-up.[108-111] These studies also showed that despite higher PiB retention, some individuals did not have significant brain atrophy.[112] In addition, PiB-positive patients with MCI had a higher rate of conversion to AD than PiB-negative patients with MCI. In MCI converters, the higher the PiB retention rate, the faster the conversion to AD (within 1 year of follow-up).[106,113] Altogether, data from functional imaging also show that changes observed in AD patients can be found not only in patients with MCI, particularly in MCI converters, but also in elderly subjects without cognitive impairment at higher risk of developing dementia.

Temporal dynamics of AD biomarkers

The current most accepted hypothesis of AD pathophysiology posits that increased production or reduced clearance of $A\beta_{42}$ is the initial event that leads to neuronal dysfunction and, ultimately, to the neurodegenerative features of AD.[12] Therefore, it is plausible to hypothesize that changes in biomarkers of cerebral $A\beta_{42}$ would precede changes in other AD-related biomarkers. In fact, low CSF $A\beta_{42}$ ($<$ 500 pg/mL) correlates with reduced hippocampal volumes over 1 year of follow-up[114] and is associated with cognitive decline over time in older adults who were cognitively normal at baseline, predicting cognitive deterioration.[115] In addition, brain deposits of amyloid as demonstrated by PiB scans were shown to be significantly correlated with CSF Tau and Tau/$A\beta_{42}$ ratio, and increased CSF concentrations of Tau have been shown to be associated with reduced brain volumes in mild AD but not in cognitively normal elders.[105] Both increased PiB binding and diminished CSF levels of $A\beta_{42}$ are influenced by increasing age and the presence of the APOE*E4 genotype.[116,117] Decreased $A\beta_{42}$ and increased Tau levels in the CSF are also present in pre-symptomatic familial-AD mutation carriers and in individuals with strong, positive family histories of late-onset AD.[118]

There seems to be a specific temporal trend in the evolution of AD-related biomarkers.[119] Amyloid-related biomarkers (CSF $A\beta_{42}$ and in vivo $A\beta_{42}$ imaging) become altered very early in the disease process, as demonstrated by the presence of NP in elderly subjects without cognitive decline (postmortem studies), the evidence that reductions in CSF $A\beta_{42}$ precedes cognitive complaints and decline in healthy elderly subjects, and the evidence of high retention of in vivo $A\beta_{42}$ markers in cognitively unimpaired elderly subjects with no significant brain structural changes. Changes in neurodegenerative-related biomarkers follow the changes observed in amyloid-related biomarkers, with changes in CSF Tau (total and phosphorylated) and in brain metabolism (as measured by FDG-PET) being the first to take place, followed by atrophy in the hippocampus and other brain regions. Hippocampal atrophy, despite occurring later in the temporal dynamics of AD biomarkers, has a closer relationship with the severity of memory deficits and with the progression from MCI to AD. Finally, the subject develops cognitive impairment that is severe enough to significantly impair activities of daily living, warranting the clinical diagnosis of AD.[17]

Nonetheless, given that changes in AD-related biomarkers may begin decades prior to the first dementia symptoms, long-term longitudinal studies are needed to depict the full pathologic cascades of events that lead to dementia.[120] In a recent study, Bateman et al.[121] addressed this issue by estimating the temporal pattern of changes in AD-related biomarkers in carriers of dominantly inherited early-onset AD as a function of

parental age of onset of dementia. They showed that changes in CSF Aβ concentrations appear to decline around 25 years prior to the expected date of dementia onset. This is followed by increased Aβ deposition, as assessed by PiB-PET imaging, increased CSF Tau concentration and brain atrophy 15 years before clinical dementia. Finally, reduced brain metabolism and mild episodic memory impairment take place 10 years before expected symptom onset and global cognitive impairment starts 5 years before expected symptom onset in AD mutation carriers.

It is important to note that the progression of changes in distinct AD biomarkers may be nonlinear and have significant overlap between each other.[17] These aspects render the presentation of biomarker changes in individual patients heterogeneous. In addition, the pattern of progression of these biomarkers (and the emergence of significant clinical symptoms) might be influenced by other factors, such as alterations in neurotrophic, inflammatory, and oxidative stress cascades and genetic and epigenetic alterations, at any stage of disease progression.[122-124] Thus, several factors beyond changes in biomarkers at specific disease stages alone have a significant influence on the relationship between biomarker patterns, disease progression, and clinical phenomena in AD.

Redefining AD

The extent to which available AD biomarkers (and those to be presented in the future) will represent definitive indications of the presence of disease in the absence of clinical symptoms is an important issue to be discussed. The revised NINCDS-ADRDA diagnostic criteria for AD proposed by Dubois et al.[125] anticipate the etiological diagnosis in the presence of evidence of episodic memory impairment plus one or more supportive features provided by validated biomarkers. This is a requirement for the next generation of research into AD, particularly with respect to the selection of patient samples for clinical trials.[126]

A recent working group from the U.S. National Institute on Aging (NIA) and the Alzheimer's Association proposed new diagnostic criteria for AD and its prodromal and preclinical stages. These criteria incorporate the assessment of AD-related biomarkers at all diagnostic levels, either to confirm the diagnosis of dementia in AD or to provide information on the underlying pathophysiologic process and increase the degree of certainty of the diagnosis in the prodromal and preclinical stages of AD.[5-7] Three levels of certainty of the presence of AD pathology have been defined for suspected cases, namely: dementia probably, possibly, or unlikely due to AD. Within these categories, three levels of evidence were considered: a) positive biomarker possibility due to AD etiology; b) identification by PET of amyloid-β deposition in brain tissues, corroborated by $A\beta_{42}$ changes in CSF; and c) evidence of neuronal injury indicated by disturbances of Tau in CSF, as well as by FDG-PET and MRI changes.[5-7]

There is a high probability of diagnosis of AD (i.e., probable AD) when the core clinical features of dementia are supported by persistent and progressive decline of episodic memory, with amnestic syndrome being the most common cognitive manifestation. Cognitive decline is accompanied by impairment in activities of daily living. Even in early dementia, neuropsychiatric symptoms can emerge and may be considered. In addition, diagnosis of probable AD is highly likely when pathophysiological biomarkers of AD, such as brain amyloid-β deposition evidenced by PET, reduced concentrations of $A\beta_{42}$ peptide in CSF, occurrence of neuronal injury indicated by increased phosphorylated Tau in CSF, reduction of brain metabolism on FDG-PET, and brain atrophy documented by MRI are present. Diagnosis of possible AD comprises an atypical clinical course of dementia with an etiologically mixed presentation characterized by unavailable, conflicting or indeterminate amyloid-β biomarkers, and markers of neuronal injury. Concomitant cerebral disease or symptoms and signs suggesting other neuropsychiatric disorders, as well as intake of medications with an unfavorable impact on cognition, may create uncertainty as to a more precise and reliable diagnosis. Dementia unlikely due to AD should be considered when the clinical course provides sufficient support for another condition requiring distinct diagnostic approaches, or when additional investigation is negative for biomarkers indicative of brain amyloid-β deposition, neuronal injury, and brain atrophy.

Using the same approach, biomarker information has also been incorporated in the diagnostic workup of MCI due to AD.[5-7] The probability that clinically defined MCI be in fact considered a case of pre-dementia AD will be high in the presence of core AD biomarkers, such as evidence of intracerebral accumulation of amyloid-β (as identified by molecular imaging with PET), reduced CSF concentrations of $A\beta_{42}$, increased phosphorylated Tau in CSF, and brain atrophy as shown by MRI scans. Conversely, the diagnostic certainty of MCI due to AD is lower when investigation of these biomarkers is not available or provides indeterminate, conflicting or negative results. Longitudinal and periodic assessments could improve diagnostic accuracy.

The characterization of preclinical AD[5-7] is hindered by several important challenges, such as the lack of definitive knowledge concerning specific roles of amyloid-β species as etiological agents of sporadic, late-onset disease. Moreover, disturbances of this protein can be associated with other pathophysiological processes. To date, the impact of brain amyloid-β deposition on neurodegenerative mechanisms remains to be elucidated. Ethical and clinical aspects are another important question for discussion. Nevertheless, biomarkers should also be incorporated in preclinical diagnosis of AD because of the prolonged nature of the preclinical phase, which could represent a crucial opportunity to change the disease course. If investigations of biomarkers reveal cerebral amyloidosis, neuronal injury, and subtle cognitive, functional or behavioral decline, the possibility of preclinical AD is high. This possibility is lower when biomarkers are uninformative or conflicting.

There are several challenges to the widespread application of biomarkers for the diagnosis of AD in clinical practice. To date, there is no consensus about the best biomarker combination and when to start the diagnostic assessment. Furthermore, standardized laboratory methods and well-validated, population-based, reference values for discriminating pathological vs. non-pathological biomarker patterns, which are mandatory to their implementation in clinical practice, are still unavailable.[46,127] Finally, there are important clinical and ethical implications of the identification (or misidentification) of cases in the prodromal and preclinical stages of the disease in view of the absence of approved or well-established treatments to prevent or slow the progression of AD to its full-blown dementia stage.

Biomarkers and impact on treatment

The search for treatments with disease-preventing or modifying properties is the Holy Grail of AD research. Despite great efforts toward the achievement of this goal, either with well-established drugs for the treatment of AD (e.g., cholinesterase inhibitors)[128-131] or with newly developed agents (e.g., amyloid-β aggregation inhibitors, gamma-secretase modulators, active and passive immunization),[132] interventions tested thus far have demonstrated little or no clinical benefit in the prevention of progression from MCI to AD.[133-137]

Therapeutic strategies for patients with AD tend to be tailored to the clinical stage of the disease, underlying mechanisms of disease, and putative clinical and biological markers.[133-137] The first phase of the disease process should be considered as the preclinical stage of AD, which probably occurs decades before the onset of symptoms. Although patients have no clinical manifestations at this stage, they exhibit amyloid-β accumulation within the brain, as demonstrated by molecular neuroimaging with PET, and autosomal dominant mutation involving genetic components affecting the APP or presenilin (PS) genes (PS1 and PS2). Potential therapeutic strategies at this stage will comprise a broad intervention spectrum, including the well-established strategy of cognitive reserve improvement by complex intellectual stimulation, appropriate lifestyle modifications such as regular physical exercise, adequate nutrition, and stress reduction, reduction of risk factors for cardiovascular diseases, and management of comorbidities. Despite insufficient evidence, anti-amyloid therapies are forthcoming, based on anti-Aβ immunotherapy, modulation of beta- and gamma-secretase, antifibrillization agents, and chelators. Several drugs have been designed to interrupt potential therapeutic targets along the disease course. These outcomes are expected to be achieved in the prodromal clinical stage as well.

The prodromal stage of AD encompasses features of the disease process that are detectable at its pre-dementia phases. Diagnosis in the prodromal stage should capture the earliest clinical manifestations before the occurrence of functional impairment severe enough to be consistent with the diagnosis of dementia. These clinical markers include episodic memory impairment consistent with amnestic MCI. Specific biomarkers can support diagnosis of prodromal AD, including reduced concentrations of $A\beta_{42}$ in the CSF and molecular imaging with PET showing amyloid-β deposition in brain tissues. The presence of an autosomal dominant mutation involving specific genes such as APP and PS1 or 2 could reinforce the underlying AD pathology. Another way to understand the prodromal stage of AD is via neurodegenerative processes associated with Tau-related pathology, which is characterized by elevated concentrations of phosphorylated Tau in CSF, medial temporal lobe atrophy on MRI, and diminished brain metabolism on FDG-PET. Pharmacological intervention approaches targeting this stage are also forthcoming, and should encompass neuroprotective strategies (antioxidants and anti-inflammatory drugs) and neurorestorative agents (stem cells, BDNF, and nerve growth factor). Tau-related therapies involving pharmacological interventions with GSK inhibitors and lithium[134] could constitute promising strategies. Clinically, patients tend to exhibit multiple-domain amnestic MCI with subtle impairment of episodic memory and other cognitive functions, but no impairment in functional activities. A healthy lifestyle including appropriate nutrition, regular aerobic exercise, psychosocial engagement, and cognitive stimulation remains an important resource for improving brain and cognitive reserve, and contributes to delaying disease progression.

The clinical dementia stage is markedly characterized by the occurrence of NP and NFT in brain tissues. Neuropathology becomes strongly evident with reduced $A\beta_{42}$ and elevated phosphorylated Tau in CSF; significant atrophy, mainly in temporo-parietal regions, on MRI; strong amyloid-β uptake on molecular imaging with PiB-PET; and hypometabolism on FDG-PET. Clinically, patients present significant decline of episodic memory and other cognitive functions, which cause decline of functional activities, as well as behavioral disturbances. Treatment remains with anti-dementia drugs (cholinesterase inhibitors, memantine), cognitive training, functional rehabilitation as far as possible, and psychoeducation for caregivers.

The adequate selection of patients that would benefit most from these interventions is a major challenge in clinical trial design, and poor patient selection is a common explanation for negative results.[138] Most trials selected patients based solely on clinical data or the diagnosis of MCI. As reviewed above, this strategy yields a highly heterogeneous sample that decreases study power to identify the potential benefits of interventions. When included in trial designs, biomarkers are usually evaluated for secondary outcome analysis, as proof of concept of drug mechanism of action, or to stratify the sample in secondary analyses. In opposition to this secondary role, biomarkers could be used as primary inclusion criteria, along with clinical diagnosis, in clinical trials. This strategy would enable several methodological advances, as it allows the selection of more homogeneous study samples, consequently increasing the power to detect clinically significant benefits of interventions with

disease-modifying properties. Nonetheless, to date, no large, published clinical trial that included biomarkers as criteria for patient inclusion.[139,140]

Conclusion

Knowledge about the pre-dementia stages of AD has progressed substantially in the last decade. AD has a long preclinical phase that can last 10 to 15 years, during which time amyloid plaques accumulate within the brain, but symptoms are absent or minimal and indistinguishable from the cognitive changes that occur in normal aging or in the prodromal phases of other neuropsychiatric conditions that are prevalent in this age group. Although patients with MCI are still a heterogeneous group, MCI is not a benign entity, which renders the precise, early diagnosis of dementing diseases essential to guide clinical and therapeutic decisions. Major developments in the understanding of the early pathophysiological features of AD have allowed the development of biomarkers that could identify such changes in different biological substrates. These clinical-biological insights led to identification of the AD signature in the CSF, which is a very consistent finding. Nevertheless, these developments did not translate into immediate improvement of clinical strategies for the early diagnosis of AD. Urgent needs include definition of the clinical validity and reliability of a panel of biomarkers; the use of such an instrument to detect AD at initial, preclinical stages; testing of this model in proof-of-concept trials with disease-modifying compounds; and, finally, design of AD prevention trials. Major challenges to be faced in the coming years are the integration of developments in preclinical (experimental) and clinical research to enable development of widely available tools for the early diagnosis of AD and selection of patients that would benefit most from therapeutic interventions. This, in turn, would allow a more personalized approach to patient care, improving therapeutic effectiveness and quality of life.

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Disclosure

The authors report no conflicts of interest.

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