Biological markers for early detection and pharmacological treatment of Alzheimer's disease

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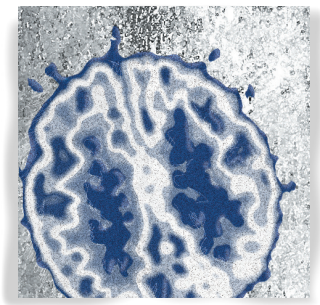

The introduction of biological markers in the clinical management of Alzheimer's disease (AD) will not only improve diagnosis relating to early detection of neuropathology with underlying molecular mechanisms, but also provides tools for the assessment of objective treatment benefits. In this review, we identify a number of in vivo neurochemistry and neuroimaging techniques, which can reliably assess aspects of physiology, pathology, chemistry, and neuroanatomy of AD, and hold promise as meaningful biomarkers in the early diagnostic process, as well as for the tracking of disease-modifying pharmacological effects. These neurobiological measures appear to relate closely to pathophysiological, neuropathological, and clinical data, such as hyperphosphorylation of tau, abeta metabolism, lipid peroxidation, pattern and rate of atrophy, loss of neuronal integrity, and functional and cognitive decline, as well as risk of future decline. As a perspective, the important role of biomarkers in the development of innovative drug treatments for AD and the related regulatory process is discussed.

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A large and growing number of new therapeutic compounds aiming at "disease modification" in Alzheimer's disease (AD) are currently under clinical investigation *(Table I)*. However, these innovative therapeutic approaches require a variety of novel biomarkers with differentiated roles and functions to ensure objectivity and efficiency of drug development, as well as the initiation and monitoring of drug treatment in patients. Accordingly, new guideline documents from regulatory authorities, such as the FDA and EMEA, will most likely strongly recommend thorough validation of biological, as well as imaging, candidate markers as primary end points in upcoming phase II and III treatment trials of compounds claiming disease-modifying properties. In this context, the ideal biomarker would serve at least two purposes.

- First, it would enable early diagnosis, which also relates to early detection of pathophysiology. This is particularly important for "disease modification" and early intervention in a condition that progresses for 5 to 8 years prior to awareness of cognitive loss.

Keywords: *Alzheimer's disease; Alzheimer's Disease Neuroimaging Initiative; biomarker; drug development; disease modification; diagnosis*

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Selected abbreviations and acronyms

AD	*Alzheimer's disease*
MCI	*mild cognitive impairment*
MRI	*magnetic resonance imaging*
MRS	*magnetic resonance spectroscopy*
PET	*positron emission tomography*

- Secondly, the biomarker would enable assessment of objective treatment benefit so that the therapeutic regimen could be adjusted according to patient response. Those biomarkers could also serve as objective end points in clinical trials assessing the efficacy of new compounds.

Large-scale, controlled, multicenter biomarker trials are currently being conducted in US, Japanese, Australian, and European Alzheimer networks (Alzheimer's Disease Neuroimaging Initiative, ie, US-ADNI and E-ADNI) in an attempt to systematically develop and validate core feasible candidate biomarkers in research areas such as neurochemistry and structural and functional imaging.

To date, a large and increasing number of monocenter studies and an increasing number of more or less controlled multicenter trials have investigated biomarker candidates for AD. Potential diagnostic biomarkers are measured against the criteria established by expert consensus conferences.[1,2] These guidelines specify that a biomarker should reflect a neuropathological characteristic of AD and should be validated in patients with a neuropathological diagnosis. The sensitivity of the "ideal" biomarker to detect AD should be at least 85%. Its specificity to differentiate AD patients from controls of the same age and from patients with other forms of dementia should be at least 75%. In clinically diagnosed populations, a higher level of specificity for biomarkers will not be able to be achieved for methodological reasons, as even the gold standard, the clinical diagnostic criteria, cannot be absolutely specific. The same applies to controls of the same age, as some of them might have undetected incipient preclinical AD.[3] In large groups, this will inevitably affect the specificity of the results of even the best mechanistic biomarker.

In contrast to early detection of pathology, application of biomarkers to map treatment effects is still at an early stage. An overview of the current literature provides an initial indication that treatment effects may indeed be reflected at the biomarker level. However, results are still inconclusive. In several cases, biomarker studies have led to unexpected results that opened up new questions; the answers to these questions will probably enhance our understanding of the pathophysiology of AD in the future. Further studies on core candidate markers will probably show that some presumed pathomechanisms of marker regulation and expression are more differentiated and complex than currently supposed.

This paper will present an overview of the most promising findings relating to biomarkers which can be assessed in vivo. A particular focus will be on biomarkers that have already been evaluated on clinical samples (eg, using structural and functional imaging methods or analysis of cerebrospinal fluid and plasma/serum). At the end of the article, a short discussion on the regulatory and industrial perspective of the topic will also be provided.

Biomarkers derived from neuroimaging

Structural magnetic resonance imaging (morphometry)

Hippocampus volumetry

High-resolution magnetic resonance imaging (MRI) determines structural changes in the brain in vivo. Significant atrophy of the hippocampal formation, entorhinal cortex, and parahippocampal gyrus can be demonstrated by MRI, even in the preclinical stages of AD, and predict later conversion to AD with about 80% accuracy.[4-6] Manual volumetric methods are currently the gold standard to determine the hippocampal volume, but they are time-intensive.[7] Hippocampal volumetry is the best-established structural biomarker for AD, particularly for early diagnosis, and appears to be suitable for risk stratification in mild cognitive impairment (MCI) cohorts in treatment trials. Controlled multicenter diagnostic studies are currently being conducted on manual hippocampal volumetry within the German Dementia Network to establish whether this method would be reliable and accurate for broader clinical application.[8] However, the procedure is still time-consuming and involves a great deal of manual work, and therefore is not set to become a routine diagnostic test in the foreseeable future.

Several studies have focussed on the temporal rate of change of hippocampal atrophy in AD patients. Atrophy

	Study	Follow-up	Primary outcomes	Assessment	Other outcomes
γ-secretase inhibitor/ modulator	LY-450139 (Eli Lilly, Phase III)[a,c,e]	21 months	Safety and tolerability; rate of cognitive and functional decline in AD over time	Brain-scanning techniques (FDG-PET, vMRI, AV-45-PET); biochemical measures; ADAS-Cog, CDR, MMSE; NPI; Qol-AD; RUD-Lite; EQ-5D Proxy	Determine levels of peptides in blood and spinal fluid that might relate to Alzheimer's disease; evaluate changes in thinking and memory; evaluate changes in daily living activities; determine levels of study drug in blood and CSF
	NIC5-15 (Humanetics, Phase II)[a]	7 weeks	Pharmacokinetics; safety	Pharmacokinetic analysis; safety assessments including vital signs; physical exam; symptom checklist; complete blood count; serum chemistries; urinalysis; electrocardiogram; ADAS-Cog	Clinician's Global Impression of Change (ADCS-CGIC); Mini-Mental Status Exam (MMSE); Activities of daily living (ADCS-ADL); neuropsychiatric inventory; insulin sensitivity and secretion; biomarkers; ApoE genotyping
	GSI-953 (begacestat) (Wyeth, Phase I)[a]	10 months	Safety	Biomarkers amyloid beta 40 and 42 in CSF	Pharmacodynamics and pharmacokinetics
	GSI-136 (Wyeth, Phase I)[a]	6 months	Safety and tolerability		Pharmacokinetics as evaluated from the blood and urine concentrations of GSI-136; pharmacodynamics as evaluated from the levels of select biomarkers in the blood and the administration of a visual analog scale to measure sedation effects
Immuno-therapy (Active)	ACC-001 (Élan/Wyeth, Phase III)[a,e]	24 months	Safety and tolerability	Cognitive and functional measures	Immunogenicity of each dose level of ACC-001 with or without QS-21 in subjects with mild to moderate AD
	CAD-106 (Novartis/Cytos, Phase II)[a]	52 weeks	Tolerability and safety assessments; antibody titers	Physical/neurological examination; ECG; vital signs; standard and special immunological laboratory evaluations; MRI; EEG; AE/SAE monitoring; IgM and IgM titers against amyloid and carrier protein	Immune response; cognitive and functional assessments
	V950 (Merck, Phase I)[a]	4 years	General safety and tolerability		Immunogenicity
	Affitope AD01 (Affiris, Phase I)[a,g]	12 months	Tolerability		Immunological and clinical efficacy (evaluated in an explorative manner)
	Affitope AD02 (Affiris, Phase I)[a,g]	12 months	Tolerability		Immunological and clinical efficacy (evaluated in an explorative manner)

Table I. Potential disease-modifying and amyloid-targeting agents in development. Sources: a, www.clinicaltrials.gov; b, www.neurochem.com; c, www.lilly.com; d, www.cornell.edu; e, www.phrma.org; f, www.regentherapeutics.com; g, www.affiris.com

	Study	Follow-up	Primary outcomes	Assessment	Other outcomes
	PF-04494700 (Pfizer, Phase I)[a]	18 months	Efficacy; safety and tolerability	Adverse events; vital signs; physical exam; neuro exam; 12-lead ECG; lab tests (hematology, blood chemistry, urinalysis); MRI	Effects of PF 04494700 on potential biomarkers of RAGE inhibition and amyloid imaging (AV-45, F18 PET); potential dose response of PF 04494700; Pharmacokinetics and pharmacodynamics
Immuno-therapy (Passive)	NIC5-15 AAB-001 (bapineuzumab) (Élan/Wyeth, Phase II)[a]	24 months	Safety assessments	Vital signs; Weight; Clinical laboratory tests; electrocardiograms [ECGs]; brain magnetic resonance imaging [MRIs]; physical and neurological examinations; infusion site assessments	Blood levels of administered study drug; cognitive and functional assessments
	Gammagard (IvIG) (Baxter/Cornell, Phase III)[a,d]	18 months	Cognition and global function	ADAS-Cog; ADCS-CGIC; 3MS; ADCS-ADL; NPI; GDS; QOL; ADCS; pharmacoeconomic assessment; plasma and CSF anti-amyloid antibody titers; plasma and CSF beta amyloid levels; FDG cerebral glucose utilization; PIB cerebral amyloid distribution (PET); PK11195 microglial activation (PET); adverse event frequency and severity	Activities of daily living, behavior, and quality of life
	LY-2062430 (solanezumab) (Eli Lilly, Phase II)[a]	6 months	Adverse events		To determine the plasma pharmacokinetics of LY2062430; to evaluate the pharmacokinetic/ pharmacodynamic relationships between LY2062430 concentrations and plasma peptide amyloid beta concentrations; to evaluate the changes in thinking and memory
	GSK933776A (GSK, Phase I)[a]	52 weeks	Safety; tolerability; immunogenicity	Physical and neurological examination; brain MRI; cognitive status; laboratory parameters; ECG; vital signs	Pharmacokinetic parameters; pharmacodynamic effects; effect on plasma and CSF biomarkers; titre and neutralizing activity of antibodies
	R1450 (Roche, Phase I)[a]	24 months	Safety; tolerability; pharmacokinetics in plasma; pharmacodynamics	AEs; Laboratory parameters; vital signs	CSF biomarkers; clinical efficacy parameters

Table I. Continued

	Study	Follow-up	Primary outcomes	Assessment	Other outcomes
Aggregation Inhibitor	Tramiprosate (3APS) (Neurochem, Phase III)[a,b] (Pfizer, Phase I)[a]	18 months	Safety, efficacy and disease-modifying potential	ADAS-Cog.; CDR, MRI	Brain volume change from baseline as measured by MRI
	AZD103 (Élan/Transition, Phase II)[a,e]	18 months	Safety and tolerability; cognitive and functional measures		
	ColostrininTM (O-CLN) (ReGen Therapeutics, Phase II)[f]	30 weeks	Efficacy; tolerability	ADAS-Cog; CGIC; IADL; MMSE; Global Deterioration Scale; Geriatric Depression Scale; ADAS-Non Cog; gradation of overall patient response	Cognition (memory, language, reasoning); function (activities of daily living)

Table I. Continued

rates of 3% to 7% per annum were demonstrated,[9-11] while healthy controls show a maximum atrophy rate of 0.9% in old age.[12] Hippocampal volume is thus a core candidate structural progression marker of AD. The hippocampus volumetry method is already being used as a secondary end point in several pharmacological trials. There are indications that volumetric markers might be approved as surrogate end points and primary outcome variables in trials on drugs claiming disease modification by regulatory authorities such as the FDA and EMEA in the future.

The application of hippocampal volumetry might be further improved in the short term by implementing semi-automated and fully automated analysis procedures. Automated methods which have a good correlation with manual measurements and reduce the measurement time from 2 h to 30 min are now becoming available.[13,14] However, the automated protocols of hippocampal volumetry in AD patients still need to be comprehensively validated.

Volumetry of the entorhinal cortex

Another very promising anatomical structure for the early diagnosis of AD is the entorhinal cortex, which lies adjacent to the hippocampus. This area is hypothesized to be affected by the neurodegenerative process at a particularly early stage. Studies have shown that entorhinal cortex volumetry is unlikely to provide any additional benefit in patients with manifest AD[15-18]; however, at the MCI stage, it may gradually improve prognostic efficiency by a few percent compared with hippocampal volumetry.[16,19] However, it should be reflected that entorhinal cortex volumetry is even considerably more laborious than hippocampal volumetry, and that no automated procedures are available for this structure yet. Sufficient data have not yet been obtained to assess whether entorhinal cortex volume does indeed offer an additional benefit over hippocampal volume as a surrogate end point to evaluate the efficiency of a particular treatment.

Automated data-driven neuroimaging methods

Due to the laborious nature of initial manual volumetric methods, various automated methods have been developed over the past years to demonstrate change in brain structure and morphology in AD patients more efficiently, and in some cases using hypothesis- and rater-independent approaches. One of the best-established methods is the automated measurement of the whole brain volume over time, which is already being used as a secondary end point in clinical treatment trials. This method demonstrated an atrophy rate of approximately 2.5% whole brain volume reduction in AD patients over the course of 1 year, compared with only 0.4% to 0.9% in healthy controls. However, the heuristic value of this method is limited, as only global effects can be recorded without providing information about regionally differentiated effects.

Voxel-based volumetry

The most commonly investigated method to date is voxel-based volumetry (VBM),[20] which consistently shows a reduction in the cortical gray matter in the

region of the mediotemporal lobes and lateral temporal and parietal association areas in AD patients.[21,22] In MCI subjects, involvement of the mediotemporal lobe and lateral association areas of the temporal and parietal lobes was demonstrated using VBM.[23,24] Interestingly, significant atrophy of mediotemporal, laterotemporal, and parietal association areas was observed in a genetic risk model, even years before clinical symptoms were manifested, indicating preclinical neurodegeneration in the neocortical association areas.[25,26] This adds to the commonly used neuropathological staging model, which hypothesizes primarily early preclinical mediotemporal changes. One study demonstrated a considerably different pattern of cortical atrophy between patients with MCI who went on to develop AD in the subsequent clinical course and those whose cognitive performance remained stable.[27] The patients who converted to AD showed a pattern of atrophy that was largely consistent with that of early AD.[28] However, VBM offers no direct way of making an individual diagnosis as it is always based on group statistics.

Deformation-based morphometry

While VBM transforms brain images into a standard space, thus compensating for global differences in the position of the head and the size of the brain, but preserving local differences in the distribution of the cortical gray matter that can then be used as a basis for detecting group differences, deformation-based morphometry (DBM) transforms the brain volumes at high resolution to a standard template brain, thus completely eliminating the anatomical differences between the brains. The anatomic information then is no longer found in the MRI images themselves, but instead in the deformation fields that are required to transform the patient's brain into a standard brain. These deformation fields offer a multivariate vector field of localization information, from which regional volume effects can be extrapolated.

In a recent study using multivariate principal component analysis, DBM was used to calculate an individual risk for the presence of AD in MCI subjects. This method allowed a group separation of about 80% between AD patients and healthy controls. Interestingly, the accuracy in distinguishing between MCI subjects who developed dementia over a period of 1½ years, and MCI subjects whose cognitive performance remained stable over time was 70% to 80%. This method may thus be used for individual risk prediction.[29] It has yet to be applied more extensively to a larger number of MRI scans.

Analysis of cortical thickness

Another interesting automated method involves determining the cortical thickness of the neocortical association areas and the entorhinal cortex.[30] Group separation showed an accuracy of more than 90% in distinguishing between AD patients and healthy controls.[31] However, this method has yet to be evaluated in an independent group, and the accuracy of this method in predicting conversion to AD in MCI subjects has not yet been studied.

Imaging the cholinergic nuclei in the basal forebrain

The imaging of structural changes in the region of the cholinergic nuclei of the basal forebrain was recently established using a combination of automated methods with regional information. The cholinergic projections from the basal forebrain to the cortex are affected early on in AD. An MRI-based method showed a signal reduction in the region of the lateral and medial nuclei of the basal nucleus of Meynert for the first time in vivo.[32-34]

Functional magnetic resonance imaging (fMRI)

The utilization of functional magnetic resonance imaging (fMRI) allows for the measurement of brain activation during cognitive tasks at a high level of resolution without any radiation exposure to the patient. There have been many studies that have examined brain activation changes in MCI subjects compared with AD, for the development of a marker of early AD.[35-37] One new approach has been to investigate changes in the functional connectivity between regions of an activated network.[38] Functional connectivity gives a measure of the linear association between two regions and is a function of the phase relationship between the regions' signals.[39] An investigation of functional connectivity in MCI subjects have shown that there are widespread changes in functional connectivity of the fusiform gyrus to other visual processing areas, and areas within the ventral and dorsal visual pathways.[38] The changes in functional connectivity preceded differences in brain activation

between the MCI and healthy control group. Given that cognitive function requires a high level of integration across the network subserving cognitive function, it suggests that the first factor that may be altered in the brain by the putative AD neuropathology is the integration across a neural network. In addition, it has been found that the activation level within the fusiform gyrus was more strongly correlated to the gray matter density in the ventral and dorsal visual pathways compared with the healthy controls, further suggesting that changes in the entire network affect activation within a network region.[40] A study of working memory in AD patients[41] provides further evidence that cognitive decline in AD is due to a breakdown in the integrated activity of a network. When subjects performed a working memory task, the functional connectivity between the frontal lobe and the hippocampus was disrupted in the AD patients and they recruited a different network that included the amygdala, prefrontal regions, and anterior and posterior cingulated gyrus to perform the task. The activation in the frontal lobes of the healthy controls showed strong correlation with posterior cortical areas, while in the AD patients the frontal lobe activity was primarily correlated with other frontal regions. In a follow-up study with semantic and episodic memory task, it was shown that the different network in the AD patients represents a compensatory mechanism as the activity in the network was correlated with memory performance.[42]

Recent work also suggests that cognitive performance is not only a function of a single network, but that the interaction between networks plays a role in cognition.[43] In an associative memory task performed by mild AD patients, MCI subjects, and healthy controls, it was shown that activation of the hippocampus and deactivation of medial and lateral parietal regions was reciprocal.[35] The hippocampus was part of a network that included regions in the occipital-temporal lobes and frontal lobes while the deactivation in the parietal regions was part of the default network[44] that includes the posterior cingulate and medial frontal lobe regions. The activation in the memory network and the deactivation in the default network were linearly correlated, providing evidence that the activation dynamics in the two networks are directly connected. The level of deactivation of the default network during a cognitive task differed among healthy controls, MCI patients, and AD patients.[45]

Investigation of the default network measured during fixation (no task) has shown altered functional connectivity between the left and right hippocampus to the rest of the brain in AD patients compared with healthy controls.[46] This raises the possibility of utilizing the default network to quantify the functional impairment in the brain without using a cognitive task. In particular, Wang and colleagues found that the functional connectivity between the hippocampus and visual cortices was impaired, further supporting the results of impaired functional connectivity found during a visual matching task in MCI patients. In addition, the functional connectivity between the hippocampus and posterior cingulate are strongly disrupted in AD patients.[36]

The network connectivity also can be investigated using diffusion tensor imaging (DTI), which provides a measure of the structural integrity of the white matter tracts connecting regions of the brain.[47] Recent application of DTI with AD patients has found decreases in the structural integrity of the white matter tracts in the corpus callosum, cingulum, and fornix, and frontal, temporal, and occipital lobe white matter areas.[40,48-50] The integration of fMRI with DTI to investigate changes across a neural network has the potential to be a very powerful tool to aid in the development of a marker for AD. However, previous studies assessing the potential of fMRI changes to serve as a marker for early pathology and for potential treatment effects in AD are still in a pilot stage including only small samples. Results need to be replicated in larger samples using prospective and longitudinal study designs.

Magnetic resonance spectroscopy

One common finding reported in the magnetic resonance spectroscopy (MRS) literature as associated with AD is a decrease in N-acetyl-aspartate concentration (NAA) and its ratio to creatine (Cr).[51-54] A positive correlation between NAA, and NAA/Cr, and Mini Mental State Examination (MMSE) scores in neurodegenerative disorders has also been reported.[55] NAA is a free amino acid, present in the brain at relatively high concentrations (8 to 12 mM/kg wet weight). Its function is poorly understood, but it is believed to act as an osmolite, a storage form of aspartate, and a precursor of N-acetyl-aspartate-glutamate. Given that NAA is predominantly intraneuronal, it has been widely used as a marker of neuronal density.[56] Observations suggesting that disruption of mitochondrial energy metabolism leads to a reversible drop in NAA,[57] however, lead to the

conclusion that NAA levels may more accurately reflect neuronal dysfunction rather than neuronal loss.

A second finding reported in the literature as associated with AD is an increase in the myo-Inositol (mI) concentration, as well as its ratio to creatine.[54,58,59] mI is a cyclic sugar alcohol, whose role in the brain is not well understood. It is generally believed that mI is an essential requirement for cell growth, an osmolite, and a storage form for glucose.[60] It has also been proposed as a glial cell marker. Normal concentrations of mI range from 4 to 8 mmol/kg wet weight.

Given the importance of developing surrogate markers for AD diagnosis, ways to improve the performance of MRS-based methods have been proposed. The use of metabolite tailored pulse sequences[61,62] has been proposed for AD diagnosis and treatment. Such pulse sequences are optimized for measurement of some metabolites (eg, NAA and mI) while degrading performance for acquisition of data from others. Although improvements in data acquisition and quantification protocols are bound to significantly reduce measurement variability for MRS data, it is unlikely that such methods will ever acquire the sensitivity and specificity needed to diagnose or monitor treatment in AD on an individual patient basis. The limited chemical shift range for proton MRS (~5 ppm) leads to the existence of a very narrow range for chemical signatures of hundreds of aminoacids and chemical compounds found in human brains. In practice, only a limited number of them (of the order of 10), characterized by large concentrations (>1 mM) and favorable spectral signatures, can be accurately measured in vivo in a clinical setting. This fact can lead to reduced specificity, as many neurological diseases or disease stages can be characterized by similar changes in the concentrations of the metabolites that can be measured accurately. Secondly, MRS measurements performed in vivo can never become more repeatable than measurements performed in phantoms; for most metabolites, measurement repeatability in vitro is limited to 2% to 3%. Consequently, assuming that this limit is reached in vivo, changes on the order of ~5% in metabolite concentrations will be needed on an individual patient basis, in order for this change to be attributed to changes due to disease or treatment. Unfortunately, natural variability of baseline states of different persons is within this range, preventing diagnosis of the disease using this approach. Moreover, such small changes from the baseline state of one person might require more than a few weeks of drug treatment, if trying to decide whether a treatment works or not. On the upside, however, MRS measurements are short, noninvasive, and can easily yield quantitative results with commercially available data analysis programs.[63] Such MRS-based approaches for monitoring disease response to treatment can prove invaluable for phase II clinical trials, by allowing a significant reduction of the number of enrolees.[51,64]

Positron emission tomography

Positron emission tomography (PET) using ^{18}fluorodeoxyglucose (18FDG) is used to study cortical metabolism. In AD patients, 18FDG-PET shows a typical pattern of reduced cortical uptake in the region of the temporal and parietal association cortex, particularly in the region of the posterior cingulum; in mild-to-moderate stages of AD, prefrontal association areas are affected as well.[65]

MCI subjects already show—to a lesser extent—a similar distribution of metabolic deficits which can predict conversion from MCI to AD with an accuracy of over 80%.[66,67] Many researchers regard 18FDG-PET as the gold standard in the in vivo diagnosis of early stages of AD, although this method is not widely available and is relatively expensive. The benefit of 18FDG-PET for differential diagnosis in AD patients is less well validated. Established automated analysis algorithms are already available for PET investigations, providing clinicians with z-score maps for metabolic deviation (for example see ref 68). PET has not yet been used in multicenter treatment trials; however, several monocenter studies have been conducted with PET demonstrating the effect of cholinergic treatment, in particular, on the metabolic pattern in AD patients. A problematic aspect of the majority of the studies is that the analyses are usually based on unblinded treatment arms and that treated responders (according to clinical criteria) were compared with untreated and treated nonresponders.[69] A double-blind study comparing verum- and placebo-treated patients regardless of the clinical effects showed a significant effect of treatment with a cholinesterase inhibitor on cortical metabolism and on the cortical activation.[70] The extent of these effects, however, was considerably smaller than in the previous studies.

A promising approach in PET involves imaging the receptor binding of specific transmitters. By administering positron emitters of labelled receptor agonists or antago-

nists, quantitative measures can be obtained on specific transmitter binding and its kinetics on the basis of biophysical models. Compared with healthy controls, this method can be used to indicate reduced or upregulated receptor expression. In recent years, markers of the muscarinergic system have been developed that demonstrate specific reductions in binding in AD patients, but they have not yet been sufficiently evaluated to allow diagnostic statements to be made.[71] A further interesting marker is the imaging of acetylcholinesterase activity.[72] In one study, a significant effect of treatment with a cholinesterase inhibitor was shown on the expression of acetylcholinesterase in the cortex.[73] Here, sufficient data are not yet available as well to assess the method's potential for diagnostic use or its value as a secondary end point as part of a treatment trial.

Novel markers have recently been developed to image amyloid plaques using PET in AD patients. The most extensively studied radiotracer is Pittsburgh Compound B (PIB), which shows a specifically enhanced uptake in AD patients compared with healthy controls.[74] It is not clear at present, however, whether the diagnostic accuracy of this method may be better than that of the more matured FDG-PET. However, its application in treatment studies to investigate amyloid-modifying strategies as a marker of a biological mechanism would be conceivable.

Biomarkers derived from neurochemical CSF analysis

Amyloid beta peptides

The discovery that amyloid beta peptide forms the main component of AD plaques primarily with a length of 42 amino acids (Aβ42)[75] and that it is secreted by cells[76] led to investigations of Aβ42 in the cerebrospinal fluid (CSF). Around 20 studies have been conducted on some 2000 patients and controls showing a reduction of Aβ42 by about 50% in AD patients compared with nondemented controls of the same age; the diagnostic sensitivity and specificity levels range between 80% and 90%.[77] In healthy subjects, the concentration exceeds 500 pg/mL in all age groups.[78] It is not clear why Aβ42 is reduced in AD patients. Compared with other types of dementia, the specificity level is only approximately 60%.[79] An autopsy study demonstrated an inverse correlation between Aβ42 levels in the CSF and the number of plaques,[80] and it was recently shown that subjects with a positive signal in amyloid positron emission tomography (PET) studies using Pittsburgh Compound B (PIB; see below) had the lowest Aβ42 values in the CSF.[81] Future studies need to take account of the considerable diurnal fluctuations in Aβ levels in the CSF.[82]

Total tau protein (t-tau)

The main component relating to intraneuronal changes in AD patients is the microtubule-associated tau protein. Abnormal aggregates can only be formed if the tau protein is released from its sites of binding.[83] In AD patients, tau protein is present in a pathological, hyperphosphorylated form. Incidentally, tau pathology can also be observed in other neurodegenerative diseases, but differs from tau pathology in AD patients at the molecular level.[84] Tau protein was quantified in the CSF under the hypothesis that it is released extracellularly as a result of the neurodegenerative process. The methods initially available analyzed all forms of tau regardless of their phosphorylation status at specific epitopes, ie, total tau protein (t-tau).

Around 50 studies have been conducted to date with some 5000 patients and controls, and have all demonstrated an increase in the concentration of t-tau in AD patients by approximately 300% compared with nondemented elderly subjects, and a systematic increase in the concentration with age was observed in the control groups.[85,86] The sensitivity and specificity levels were between 80% and 90% for t-tau as well.[77] In subjects younger than 50 years, the concentrations in the CSF are usually lower than 300 pg/mL, in subjects younger than 70 years lower than 450 pg/mL, and in the over 70s lower than 500 pg/mL.[78] Both t-tau and Aβ42 were already significantly altered in subjects with mild cognitive impairment (MCI) who are at increased risk of AD over time.[87] Although the AD group could be differentiated from healthy controls of the same age—with a sensitivity of 85% and a specificity of 86%—using a combination of the two markers, the differential diagnosis (classification) between AD and other primary degenerative dementias was unsatisfactory (sensitivity = 85%, specificity = 58%).[79] Therefore, more specific biomarkers were sought.

Hyperphosphorylated tau protein (p-tau)

Approximately 30 phosphorylation epitopes have been detected in AD. Around 1999, the first methods were published and demonstrated concentrations of hyper-

phosphorylated tau protein in the CSF. Most of these studies to date have investigated tau protein hyperphosphorylated at threonine 231 (p-tau231P) and at threonine 181 (p-tau181P), and a few results have been obtained for serine 199 (p-tau199P). A correlation with neurofibrillary neocortical pathology was demonstrated for p-tau231P in the CSF,[88] but not for p-tau181P.[89] Single studies are available on other epitopes as well.

An increase in p-tau has consistently been found in the CSF of AD patients compared with controls. Around 20 studies have been conducted on some 2000 patients and controls with sensitivity and specificity levels of between 80% and 90%. Differences have certainly been observed between the individual p-tau subtypes in distinguishing between the groups. P-tau231P and p-tau181P show better results than p-tau199P in distinguishing AD from control groups and even from other types of dementia.[90] These and other studies suggest that p-tau is promising in distinguishing AD from frontotemporal dementia (FTD), with sensitivity and specificity rates of 85% to 90%.[90,91] A combination of various p-tau subtypes did not provide improved results in distinguishing between the groups due to ceiling effects. P-tau may also be useful in distinguishing AD from idiopathic normal pressure hydrocephalus (iNPH). A study found similarly altered concentrations of t-tau and Aβ42 in both groups compared with controls, while p-tau181P was considerably higher in the AD group only.[92] The sensitivity and specificity rates were higher than 85%. A systematic review discusses what clinical benefit p-tau might offer. The high negative predictive value of p-tau of approximately 90% appears to be particularly significant. This means that normal values rule out the presence of AD with almost 90% probability.[93]

In MCI subjects, high p-tau231P concentrations correlated with a decline in cognitive performance and conversion to AD.[94] Similar results were established for p-tau181P.[95] The three p-tau subtypes presented above were comparable in this respect.[96] High p-tau231P concentrations at the initial examination also correlated with structural disease progression, measured as the rate of hippocampal atrophy in the course of the disease.[97] A recent European multicenter trial on CSF p-tau231 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. 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.[98] This result is particularly promising regarding clinical use of p-tau by general practitioners or consultants in order to inform patients as early as possible.

A Swedish 6-year study investigated the predictive value of the combined t-tau, Aβ42, and p-tau181P (defined as a ratio) for AD in a group of 137 MCI patients.[99] AD was able to be predicted in the MCI subjects with a sensitivity of 95% and a specificity of approximately 85%, both with a combination of t-tau and Aβ42 and with a combination of t-tau and the ratio of Aβ42/p-tau181P.[99] This suggests that a useful combination of markers may optimize prediction in a more heterogenous MCI population over a longer observation period.

The single assay methods have been modified by using the Luminex xMAP® technology (Luminex Corp, Austin, TX) based on flow cytometry, which allows several parameters to be determined at the same time; the three biomarker candidates presented here can thus be measured at once using a relatively small volume of CSF. The first multicenter results are promising.[100] Determination of these parameters is implemented both in the US and the European dementia networks. The first round-robin study is currently being conducted.

Novel and emerging approaches

A particularly promising new approach in the CSF focuses on the detection and quantification of β-secretase (BACE-1), one of the key enzymes responsible for the pathological amyloidogenic cleavage of the amyloid precursor protein (APP). A significant increase was found in BACE-1 concentration and activity in the CSF of MCI subjects compared with healthy controls; subjects with the ApoE ε4 risk allele were found to have the highest concentrations. BACE-1 may have added value in early detection, prediction, and biological activity of AD.[101]

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 levels also increased over time. With regards to their diagnostic precision, the CSF markers isoprostanes and p-tau performed better than memory tests. The isoprostanes even improved the results obtained using hippocampal volumetry to distinguish between the groups.[102] However, due to the very demanding analysis method, isoprostanes should still be regarded as a merely scien-

tific approach. This also holds true for the role of apoptosis, oxidative stress, and mitochondrial dysfunction in lymphocytes as potential biomarkers for Alzheimer's disease which are currently under investigation.[103]

Biomarkers derived from plasma and serum

The efforts to discover and develop diagnostic biomarkers for AD in peripheral blood, plasma, or serum has to date not led to any core feasible candidate markers that are even close to the diagnostic accuracy achieved by CSF biomarkers. The best-studied candidate biomarker in plasma so far is Aβ, but the findings are contradictory. Some groups have reported high concentrations in plasma of either Aβ42 or Aβ40 in AD, although with a broad overlap between patients and controls, whereas most groups find no change.[104] Some studies have also reported high plasma Aβ42 (but not Aβ40) in nondemented elderly people who later developed either progressive cognitive decline or AD.[105,106] Contrary to these data, van Oijen and colleagues recently reported an association between high Aβ40, low Aβ42, and risk of dementia,[107] a result that is in general agreement with the findings of Graff-Radford and colleagues,[108] who observed a weak association between low plasma Aβ42/Aβ40 ratio and risk of future MCI or AD in a healthy, elderly population. Apart from disease-related factors, the opposing results may be due to the fact that Aβ42 is methodologically difficult to measure reliably in plasma. The peptide is very hydrophobic and binds, not only to certain test tube walls, but also to several plasma proteins, including albumin, α2-macroglobulin, lipoproteins, and complement factors.[109] Additionally, it is unclear what effect Aβ oligomerization has on Aβ concentrations in plasma measured by immunochemical assays. Both homo- and heterotypic protein interactions could mask Aβ epitopes, resulting in the measurement of only a fraction of Aβ.[110] This possible confounder might differ between different methods, which could explain some of the contradictory results in the literature. It is still unclear as well whether the disturbed metabolism of Aβ42 in the AD brain is reflected by changes in the levels of A, markers in plasma. In fact, Aβ is produced by many different cells in the body and there seems to be no correlation between the levels of Aβ42 in plasma and CSF.[111,112] Similarly, other investigations have shown that plasma Aβ42 and Aβ40 do not reflect Aβ accumulation in the brains of individuals with AD.[81,113]

Combination of biomarkers

It would seem obvious to combine a specific set of different neurochemical markers or neurochemical markers together with imaging parameters to achieve a more accurate early and differential diagnosis and to compare the validity of the individual methods. In agreement with this view, combined measurements of the CSF t-tau, Aβ42, and p-tau profile, and regional cerebral blood flow[114] or mediotemporal lobe atrophy[115] demonstrate higher predictive power than either diagnostic approach alone in MCI studies.

Particular combinations or ratios of biomarkers may be useful in answering specific questions; in other words, patterns or rates of change at the neurochemical level may ultimately prove to be optimal. Thus, group separation between AD and vascular dementia patients seems promising using the ratio of Aβ42 and p-tau.[116] AD could be distinguished from dementia with Lewy bodies (DLB) using the ratios of Aβ peptides of varying lengths (Aβ42/Aβ38 and Aβ42/Aβ37) and tau protein.[117] There are also indications that the ratios of various Aβ peptides improve the neurochemical profile for potential diagnostic applications.[118,119] A combination of amyloid imaging using PIB-PET and t-tau, Aβ peptides, p-tau and potentially BACE-1 in the CSF has been proposed as a possible way to improve imaging of the underlying neuropathology and to cross-evaluate the neurochemical markers.[120] These approaches are currently being pursued.

The regulatory perspective

The use of biomarkers as end points in earlier stages of drug development is well established for regulators, and there are examples to approve medicinal products on the basis of their effects on validated surrogate markers, eg, antihypertensives, or cholesterol-lowering products.[121] However, these examples have been considered as validated surrogate markers as they allow substitution for a clinically relevant end point. In their validation a link between a treatment-induced change in the biomarker and long-term outcome of the relevant clinical measure was undoubtedly established.

Unfortunately, in AD none of the imaging or neurochemical markers can be considered to be sufficiently validated as a fully developed surrogate end point, thereby making their use as primary outcome measures in pivotal

efficacy trials unlikely at this time. Nevertheless, they are already utilized in ways that allow decision-making on further drug development, they can be used as primary prespecified outcome measures in phase II studies (proof of concept, dose-finding) or to better define patient populations at risk (enriched populations likely to respond to therapy) for efficacy trials. In particular, the better definition of homogeneous patient populations with AD may permit clinical studies to be shorter or smaller in size even in phase II, and ultimately, if any of these biomarkers would be found to be acceptable later on as surrogate end points, definitive efficacy trials may also be considerably shorter and/or smaller than studies using more traditional clinical outcomes.

To validate a biomarker with regard to a possible claim of disease modification of AD, we would look for the link between a treatment-induced change in the neuroimaging or neurochemical biomarker and the desired clinical outcome measure and the link between the treatment-induced change in the neuroimaging or neurochemical biomarker and change of the underlying disease process.[121] Additionally, there should be a high plausibility that based on the assumed mechanism of action of a given medicinal product the disease process will be modified (based on, eg, preclinical models). However, if the biomarkers are not fully validated, it is very unlikely that approval will be granted on such unvalidated surrogates as sole primary outcomes, but benefit-risk assessment will be based on classical clinical outcome trials of reasonable size and duration.

Why are regulators so strict on validation of biomarkers? The reliance on the pharmacological effect on a surrogate that has not been adequately validated (that is, for which there has not been shown a strong correlation between the expected change in the surrogate and a beneficial effect of the drug) is troubled with interpretive uncertainties,[122,123] eg, it is assumed that an efficacious treatment of AD will slow the progression of medial temporal lobe atrophy measured by MRI. However, in the vaccine trial with AN1792 the extent of brain atrophy increased in patients with antibody response and clinical improvement.[124] This outcome was surprising, and provides evidence that the effects of a treatment (even, potentially, a beneficial treatment) on a chosen surrogate marker can be unpredictable. Of more concern, though, are those (potential) cases in which the desired effect on the surrogate is achieved. If the clinical effects are unknown, concluding that the drug has a beneficial effect for the patients would be based on the assumption that the desired effect seen on the surrogate will translate into the desired beneficial clinical effect. This assumption may be quite wrong, resulting in the approval of a treatment that has no beneficial (or even, perhaps, a deleterious) effect on the patient.

Nevertheless, regulatory bodies like the FDA and EMEA have identified development of biomarkers as high priority in general and particularly in dementia.[125,126] Neurochemical and neuroimaging biomarkers are considered useful in refinement of diagnostic criteria of AD—and possibly earlier diagnosis—as well as rendering the natural course of disease. To foster innovation in this field extensive collaboration of the different stakeholders is proactively supported by regulators and hopefully will lead to further improvement in qualification and validation of a least some of the biomarkers towards surrogacy in AD.

Discussion

There are a number of criteria for a biomarker to qualify as a useful tool in the early detection and for the monitoring of treatment effects in AD. It needs to have face validity, ie, measure something known to be involved directly in the pathophysiology. It also needs to be detectable early in the disease process; to be quantifiable by an automated method; and to possess a dynamic range relevant to progression in the "natural course" of disease as well as regression due to therapeutic intervention, with sufficiently low variance to measure changes that are small relative to rates of progression or regression. In clinical trials of drug candidates, such biomarkers would enable enrichment of populations, confirmation of mechanisms of action (MoA), choice of dosing regimen ("dose-ranging"), quantification of treatment benefit, and dose titration to maximize benefit with least risk of adverse events. The enrichment of populations in clinical trials is particularly important due to the long delay in onset of symptoms and the low annual rate of conversion from MCI to mild AD. Because AD progresses slowly and treatment effects may only be manifest as a slowing or halting of progression, precise measurement of small changes is crucial to the design of clinical protocols with reasonable durations of therapy and achievable numbers of patients. Similar factors apply to diagnostic biomarkers being developed for direct patient care. The ability to diagnose AD pathophysiology prior to onset of symptoms will enable ear-

lier intervention, when a patient has the best hope of efficacy and has retained maximum cognitive performance. In addition to their direct clinical benefit for AD patients and caregivers, early-disease biomarkers are also of interest to payers and purchasers of health care. Results of such a diagnostic test can serve as a baseline to quantify treatment benefit by longitudinal comparisons pre- and post-treatment. This will enable individualization of the treatment regimen, leading to optimal outcomes on a per-patient basis. Optimizing outcomes for individuals enables efficient delivery of health care, which in turn frees up resources to broaden access to the latest technology. The projected costs of AD in the US attendant upon aging of the baby boomers are astronomical; it is the development of novel therapeutics and biomarkers, or diagnostics, based on innovative technology, which offers hope to individuals and to society.

In this review, we identified a number of in vivo neurochemistry and neuroimaging techniques, which can reliably assess aspects of physiology, pathology, chemistry, and neuroanatomy of AD and hold promise as meaningful biomarkers in the early diagnostic process as well as for the tracking of disease modifying pharmacological effects. These neurobiological measures appear to relate closely to pathophysiological, neuropathological, and clinical data, such as hyperphosphorylation of tau, abeta metabolism, lipid peroxidation, pattern and rate of atrophy, loss of neuronal integrity, functional and cognitive decline, as well as risk of future decline. On the neurochemical level, CSF concentration of Aβ42, tau, and P-tau can distinguish subjects with MCI who are likely to progress to AD. They also show preclinical alterations that predict later development of early AD symptoms. Studies on plasma Aβ are not entirely consistent, but recent findings suggest that decreased plasma Aβ42 relative to Aβ40 may increase the risk of AD. Increased production of Aβ in aging is suggested by elevation of BACE-1 protein and enzyme activity in the brain and CSF of subjects with MCI. CSF tau and P-tau are increased in MCI as well, and show predictive value. Other biomarkers may indicate components of a cascade initiated by Aβ, such as oxidative stress or inflammation. Other interesting novel marker candidates derived from blood are being currently proposed (phase I). These merit further study in MCI and earlier stages.

Manual hippocampal volumetry is currently the best-established biomarker for AD in the field of structural imaging, but due to the laborious nature of the procedure it will only be used in clinical studies for risk stratification of study populations and as an end point for treatment effects in the foreseeable future. Automated data-driven and rater-independent methods are currently being investigated to detect regional changes, namely VBM, DBM, and the measurement of cortical thickness. In the medium term, particularly in combination with multivariate statistical analysis methods, analysis algorithms are likely to be identified that are at least as effective as hippocampal volumetry in the early detection of AD in MCI subjects and will therefore be used in pharmacological studies. However, if secondary preventive treatment approaches are approved in the coming years, the use of these kinds of automated methods for the early detection of AD will be of socioeconomic importance in routine diagnostic practice as well. Besides structural neuroimaging, pilot studies using other neuroimaging approaches such as PET (FDG and PIB), DTI and MRS yielded promising results and should be prospectively applied to larger samples.

Apart from hippocampal volumetry, whole-brain volumetry is currently being investigated as a secondary end point in several clinical studies, and other studies are beginning on whole brain volumetry; however, the validity of this marker is limited. PET has been used as an end point in single-center studies.[70] Tau protein has also been used as a secondary end point in clinical studies. In an immunization study, discontinued due to serious side effects, a reduction in t-tau in the CSF was observed in the group of antibody responders (development of a defined high antibody titer after vaccination) compared with the placebo group.[127] Interestingly, MRI showed a decrease in whole brain volume in the responder group in this study.[124] Amyloid reduction with consecutive changes in the CSF space is being discussed as a cause, although this interpretation is controversial. Changes in the concentrations of the Aβ peptides in the CSF and plasma were reported after administration of a γ-secretase inhibitor, a potential drug that may modify amyloid pathology.[2] Furthermore, various combinations of neurochemical and neuroimaging biomarkers are currently used in several ongoing clinical trials on substances with potential disease-modifying properties *(Table I)*.

Conclusions and perspectives

A number of neuroimaging candidate markers are promising, such as hippocampus and entorhinal cortex

volumes, basal forebrain nuclei, cortical thickness, deformation and voxel-based morphometry, structural and effective connectivity using DTI, tractography, and fMRI. CSF Aβ42, BACE-1, total tau, and P-tau are substantially altered in MCI and clinical AD. Biomarker discovery through proteomic approaches requires further research. Despite the large number of promising results, biological markers of AD are at various stages of development and clinical evaluation (referred to as development stage I-IV), and have so far not generally been established in clinical routine. In order to approach this goal, large-scale international controlled multicenter trials (such as the US, European, Australian, and Japanese ADNI, and the German Dementia Network) are engaged in phase III development of the core feasible imaging and CSF biomarker candidates in AD. Also, biomarkers are in the process of implementation as primary outcome variables into regulatory guideline documents regarding study design and approval for compounds claiming disease modification. Specific medium-term tasks in biomarker research include validation of the markers in autopsy-confirmed patient groups, determination of the benefit of biomarkers in the risk stratification of clinical study populations using medico-economic models, and the controlled application of biomarkers in primary care. The aim should be to have early diagnostic markers ready in clinical practice when disease-modifying treatments become available so that those patients who would benefit from these strategies can be identified and treated in time.

To this end, there is a need for thorough and rigorous codevelopment of biological marker candidates with various functions and roles during all stages of drug development. This can only be achieved through planned synergistic collaboration between academic and industrial research partners. Biomarker research in neurodegenerative disorders is a fascinating and fast-developing area; however, much can still be learned by more matured interdisciplinary fields, such as oncology, immunology, and cardiovascular research. ❑

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Marcadores biológicos para la detección y el tratamiento precoz de la Enfermedad de Alzheimer

La introducción de marcadores biológicos en el manejo clínico de la Enfermedad de Alzheimer (EA) no sólo mejorará el diagnóstico relacionado con la detección precoz de la neuropatología y los mecanismos moleculares subyacentes, sino que también proveerá herramientas para la evaluación de los beneficios objetivos del tratamiento. En esta revisión se identifican varias técnicas neuroquímicas y de neuroimágenes in vivo, que pueden evaluar de manera confiable aspectos fisiológicos, patológicos, químicos y neuroanatómicos de la EA y se destacan prometedores biomarcadores significativos en los procesos del diagnóstico precoz, como también para el monitoreo de los efectos farmacológicos que van modificando la enfermedad. Las mediciones neurobiológicas parecen relacionarse estrechamente con datos fisiopatológicos, neuropatológicos y clínicos, tales como la hiperfosforilación de tau, el metabolismo beta-amiloide, la peroxidación de lípidos, los patrones y frecuencias de la atrofia, la pérdida de la integridad neuronal, y la declinación cognitiva y funcional, como también el riesgo de futuros deterioros. Con una visión a futuro se discute el importante papel de los biomarcadores en el desarrollo de innovadoras terapias farmacológicas para la EA y los procesos relacionados con las agencias reguladoras.

Marqueurs biologiques de détection et traitement précoces de la maladie d'Alzheimer

L'introduction des marqueurs biologiques dans la prise en charge clinique de la maladie d'Alzheimer (MA) devrait non seulement améliorer le diagnostic grâce à une détection précoce de la neuropathologie et de ses mécanismes moléculaires sous-jacents, mais également fournir des outils pour l'évaluation des bénéfices objectifs du traitement. Nous individualisons dans cet article un certain nombre de techniques de neurochimie et de neuro-imagerie in vivo, *qui permettent d'évaluer de façon fiable les aspects physiologiques, pathologiques, chimiques et neuroanatomiques de la MA et qui semblent prometteuses comme biomarqueurs du diagnostic précoce, et comme outils de surveillance des effets pharmacologiques modifiant la maladie. Ces mesures neurobiologiques sont étroitement liées aux données cliniques, neuropathologiques et physiopathologiques, telles que l'hyperphosphorylation de tau, le métabolisme de abêta, la peroxydation lipidique, le modèle et le taux d'atrophie, la perte d'intégrité neuronale et d'autonomie et l'altération cognitive ainsi que le risque de déclin futur. Nous nous proposons donc d'analyser le rôle important des biomarqueurs dans le développement de traitements innovants de la MA et les dispositions réglementaires qui s'y rapportent.*

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