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Review Articles

Core candidate neurochemical and imaging biomarkers of Alzheimer's disease*

Harald Hampel[a,b,**], Katharina Bürger[b], Stefan J. Teipel[b], Arun L.W. Bokde[a,b], Henrik Zetterberg[c], Kaj Blennow[c]

[a]*Discipline of Psychiatry, School of Medicine and Trinity College Institute of Neuroscience (TCIN), Trinity College Dublin, Trinity Centre for Health Sciences, The Adelaide and Meath Hospital Incorporating The National Children's Hospital (AMiNCH), Dublin, Ireland*

[b]*Department of Psychiatry, Alzheimer Memorial Center, Ludwig-Maximilian University, Munich, Germany*

[c]*Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at Göteborg University, Mölndal, Sweden*

Abstract

Background: In the earliest clinical stages of Alzheimer's disease (AD) when symptoms are mild, clinical diagnosis can be difficult. AD pathology most likely precedes symptoms. Biomarkers can serve as early diagnostic indicators or as markers of preclinical pathologic change. Candidate biomarkers derived from structural and functional neuroimaging and those measured in cerebrospinal fluid (CSF) and plasma show the greatest promise. Unbiased exploratory approaches, eg, proteomics or cortical thickness analysis, could yield novel biomarkers. The objective of this article was to review recent progress in selected imaging and neurochemical biomarkers for early diagnosis, classification, progression, and prediction of AD.

Methods: We performed a survey of recent research, focusing on core biomarker candidates in AD.

Results: A number of in vivo neurochemistry and neuroimaging techniques, which can reliably assess aspects of physiology, pathology, chemistry, and neuroanatomy, hold promise as biomarkers. These neurobiologic measures appear to relate closely to pathophysiologic, neuropathologic, and clinical data, such as hyperphosphorylation of tau, amyloid beta (Aβ) metabolism, lipid peroxidation, pattern and rate of atrophy, loss of neuronal integrity, functional and cognitive decline, as well as risk of future decline. Current advances in the neuroimaging of mediotemporal, neocortical, and subcortical areas of the brain of mild cognitive impairment (MCI) and AD subjects are presented. CSF levels of Aβ42, tau, and hyperphosphorylated tau protein (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 might increase the risk of AD. Increased production of Aβ in aging is suggested by elevation of BACE1 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 might indicate components of a cascade initiated by Aβ, such as oxidative stress or inflammation. These merit further study in MCI and earlier.

Conclusions: A number of neuroimaging candidate markers are promising, such as hippocampus and entorhinal cortex volumes, basal forebrain nuclei, cortical thickness, deformation-based and voxel-based morphometry, structural and effective connectivity by using diffusion tensor imaging, tractography, and functional magnetic resonance imaging. CSF Aβ42, BACE1, total tau, and p-tau are substantially altered in MCI and clinical AD. Other interesting novel marker candidates derived

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**Corresponding author. Tel.: +353-1 896 3706; Fax: +353-1 896 1313.

E-mail address: harald.hampel@tcd.ie; harald.hampel@med.uni-muenchen.de

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from blood are being currently proposed (phase I). Biomarker discovery through proteomic approaches requires further research. Large-scale international controlled multicenter trials (such as the U.S., European, Australian, and Japanese Alzheimer's Disease Neuroimaging Initiative and the German Dementia Network) are engaged in phase III development of the core feasible imaging and CSF biomarker candidates in AD. 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.

Keywords: Alzheimers's disease; AD; ADNI; Aging; MCI; Mild cognitive impairment; Biological markers; Biomarkers; Blood; Plasma; Drug development; CSF; Imaging; MRI; fMRI; DTI; VBM; DBM; Volumetry; PET; diagnosis; Early detection; Progression

1. Introduction

Alzheimer's disease (AD) is one of the most common illnesses of later life. To date, the only treatments available are symptomatic, and they are not administered until advanced stages of the disease process. At present, AD cannot be diagnosed until Alzheimer's dementia has been clinically established and the progressing cognitive deficits affect the patient's ability to cope with functional demands of his or her social and professional life [1]. This clinical "threshold value" varies greatly between individuals; it depends among other things on the patient's premorbid cognitive and intellectual level and is to some extent arbitrary. Hence there is considerable interest in the reliable early detection of AD, possibly in preclinical/predementia stages. Otherwise, preventive and disease-modifying therapies cannot be administered [2,3].

Large-scale controlled multicenter biomarker trials are currently being conducted in U.S., 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. This article will present the most promising findings relating to such biomarkers developed in the cerebrospinal fluid (CSF) and by using structural and functional imaging methods. Potential diagnostic biomarkers are measured against the criteria established by expert consensus conferences [4,5]. These guidelines specify that a biomarker should reflect a neuropathologic characteristic of AD and should be validated in patients with a neuropathologic 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 methodologic reasons, because even the gold standard, the clinical diagnostic criteria, cannot be absolutely specific. The same applies to controls of the same age, because some of them might have undetected incipient preclinical AD [6]. In large groups this will inevitably affect the specificity of the results of even the best mechanistic biomarker.

Despite the large number of promising results, biologic markers of AD are at various stages of development and clinical evaluation (referred to as development stages I to IV) and have so far not generally been established in clinical routine. Genetic parameters will not be explicitly discussed here; standing alone, they have no diagnostic value in sporadic AD. In this context, the e4 allele of the apolipoprotein E genotype (*APOE* e4) is the best-established risk factor for sporadic AD, but it lacks sufficient validity in individual patients. However, *APOE* e4 is relevant for biomarkers, because it can affect their activity or level of expression and is therefore included as a covariable in almost all biomarker studies.

2. Neurochemical markers

2.1. Amyloid beta peptides

The discovery that amyloid beta (Aβ) peptide forms the main component of AD plaques primarily with a length of 42 amino acids (Aβ42) [7] and that it is secreted by cells [8] led to investigations of Aβ42 in the CSF. Around 20 studies have been conducted on some 2,000 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 ranged between 80% and 90% [3]. In healthy subjects, the concentration exceeded 500 pg/mL in all age groups [9]. It is not clear why Aβ42 is reduced in AD patients. Compared with other types of dementia, the specificity level was only approximately 60% [10]. An autopsy study demonstrated an inverse correlation between Aβ42 levels in the CSF and the number of plaques [11], and it was recently shown that subjects with a positive signal in amyloid positron emission tomography (PET) studies with Pittsburgh Compound B (PIB) had the lowest Aβ42 values in the CSF [12]. Future studies need to take account of the considerable diurnal fluctuations in Aβ levels in the CSF [13].

2.2. Total tau protein

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 [14]. In AD patients, tau protein is present in a pathologic, hyperphosphorylated form. Incidentally, tau pathology can also be observed in other neurodegenerative diseases, but it differs from tau pathology in AD patients at the molecular level [15]. 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 5,000 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 [16,17]. The sensitivity and specificity levels were between 80% and 90% for t-tau as well [3]. In subjects younger than 50 years, the concentrations in the CSF were usually lower than 300 pg/mL, in subjects younger than 70 years they were lower than 450 pg/mL, and in the subjects older than 70 they were lower than 500 pg/mL [9]. 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 [18]. Although the AD group could be differentiated from healthy controls of the same age, with a sensitivity of 85% and a specificity of 86%, by using a combination of the two markers, the differential diagnosis (classification) between AD and other primary degenerative dementias was unsatisfactory (sensitivity, 85%; specificity, 58%) [10]. Therefore, more specific biomarkers were sought.

2.3. Hyperphosphorylated tau protein

Approximately 30 phosphorylation epitopes have been detected in AD. Around 1999, first methods were published and demonstrated concentrations of hyperphosphorylated tau protein (p-tau) in the CSF. Most of these studies to date have investigated tau protein hyperphosphorylated at threonine 231 (p-tau$_{231P}$) and at threonine 181 (p-tau$_{181P}$), and a few results have been obtained for serine 199 (p-tau$_{199P}$). A correlation with neurofibrillary neocortical pathology was demonstrated for p-tau$_{231P}$ in the CSF [19] but not for p-tau$_{181P}$ [20]. 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 2,000 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-tau$_{231P}$ and p-tau$_{181P}$ show better results than p-tau$_{199P}$ in distinguishing AD from control groups and even from other types of dementia [21]. 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% [21,22]. A combination of various p-tau subtypes did not provide improved results in distinguishing between the groups as a result of ceiling effects.

P-tau might also be useful in distinguishing AD from idiopathic normal pressure hydrocephalus. A study found similarly altered concentrations of t-tau and Aβ42 in both groups compared with controls, whereas p-tau$_{181P}$ was considerably higher in the AD group only [23]. 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 [24].

In MCI subjects, high p-tau$_{231P}$ concentrations correlated with a decline in cognitive performance and conversion to AD [25]. The three p-tau subtypes presented above were comparable in this respect [26]. High p-tau$_{231P}$ concentrations at the initial examination also correlated with structural disease progression, measured as the rate of hippocampal atrophy in the course of the disease [27]. A recent European multicenter trial on CSF p-tau$_{231}$ 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 [28]. This result is particularly promising regarding clinical use of p-tau by general practitioners or consultants to inform patients as early as possible.

A Swedish 6-year study investigated the predictive value of the combined t-tau, Aβ42, and p-tau$_{181P}$ (defined as a ratio) for AD in a group of 137 MCI patients [29]. 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-tau$_{181P}$ [29]. This suggests that a useful combination of markers might optimize prediction in a more heterogenous MCI population during 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 with a relatively small volume of CSF. The first multicenter results are promising [30]. Determination of these parameters is implemented in both the U.S. and the European

dementia networks. The first round robin study is currently being conducted.

2.3. Novel approaches

A particularly promising new approach in the CSF focuses on the detection and quantification of β-secretase (BACE1), one of the key enzymes responsible for the pathologic amyloidogenic cleavage of the amyloid precursor protein (APP). A significant increase was found in BACE1 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. BACE1 might have added value in early detection, prediction, and biologic activity of AD [31].

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 with hippocampal volumetry to distinguish between the groups [32]. However, because of the very demanding analysis method, isoprostanes should still be regarded as a merely scientific approach.

The efforts to discover and develop diagnostic biomarkers for AD in peripheral blood, plasma, or serum have 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 found no change [33]. Some studies have also reported high plasma Aβ42 (but not Aβ40) in nondemented elderly people who later developed either progressive cognitive decline or AD [34,35]. Contrary to these data, van Oijen et al [36] recently reported an association between high Aβ40, low Aβ42, and risk of dementia, a result that is in general agreement with the findings of Graff-Radford et al [37], 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 might 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 [38]. In addition, it is unclear what effect Aβ oligomerization has on Aβ concentrations in plasma measured by immunochemical assays. Both homotypic and heterotypic protein interactions could mask Aβ epitopes, resulting in the measurement of only a fraction of Aβ [39]. 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 Aβ 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 [40,41]. Similarly, other investigations have shown that plasma Aβ42 and Aβ40 do not reflect Aβ accumulation in the brains of individuals with AD [12,42].

3. Neuroimaging

3.1. Magnetic resonance imaging

3.1.1. Hippocampus volumetry

High-resolution magnetic resonance imaging (MRI) determines structural changes in the brain in vivo. Significant atrophy of the hippocampal formation can be demonstrated by MRI even in preclinical stages of AD and predict later conversion to AD with about 80% accuracy [43,44]. Manual volumetric methods are currently the gold standard to determine the hippocampal volume, but they are time-intensive. Hippocampal volumetry is the best established structural biomarker for AD, particularly for early diagnosis, and appears to be suitable for risk stratification in 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 [45]. 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 focused on the temporal rate of change of hippocampal atrophy in AD patients. Atrophy rates of 3% to 7% per annum were demonstrated [46,47], whereas healthy controls showed a maximum atrophy rate of 0.9% in old age [48]. 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 pharmacologic 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 Food and Drug Administration (FDA) and European Agency for the Evaluation of Medicinal Products (EMEA) in the future.

The application of hippocampal volumetry might be further improved in the short-term by implementing semiautomated and fully automated analysis procedures. Automated methods that have a good correlation with manual measurements and reduce the measurement time from 2 hours to ½ hour are now becoming available [49,50]. However, the automated protocols of hippocampal volumetry in AD patients still need to be comprehensively validated.

3.1.2. Volumetry of the entorhinal cortex

Another very promising anatomic 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 [51–54]; however, at the MCI stage, it might gradually improve prognostic efficiency by a few percent compared with hippocampal volumetry [52,55]. 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.

3.2. Automated data-driven neuroimaging methods

Because of the laborious nature of initial manual volumetric methods, various automated methods have been developed during the past years to demonstrate change in brain structure and morphology in AD patients more efficiently and in some cases by 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 during 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, because only global effects can be recorded without providing information about regionally differentiated effects.

3.2.1. Voxel-based volumetry

The most commonly investigated method to date is voxel-based volumetry (VBM) [56], 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 [57,58]. In MCI subjects, involvement of the mediotemporal lobe and lateral association areas of the temporal and parietal lobes was demonstrated by using VBM [59,60]. Interestingly, significant atrophy of mediotemporal, laterotemporal, and parietal association areas was observed in a genetic risk model even years before clinical symptoms were manifest, indicating preclinical neurodegeneration in the neocortical association areas [61,62]. This adds to the commonly used neuropathologic 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 during the subsequent clinical course and those whose cognitive performance remained stable [63]. The patients who converted to AD showed a pattern of atrophy that was largely consistent with that of early AD [64]. However, VBM offers no direct way of making an individual diagnosis because it is always based on group statistics (Figure 1).

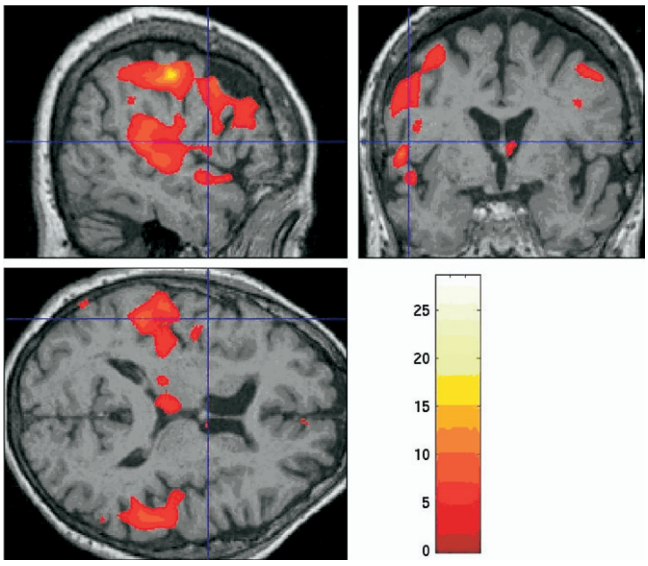

Fig. 1. Multicenter voxel-based assessment of grey matter differences between AD and MCI subjects [45]. Brain areas indicated decreased grey matter volume in AD when compared with MCI ($P < .001$, uncorrected).

3.2.2. Deformation-based morphometry

Whereas 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 anatomic 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 with 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 during a period of 1½ years and MCI subjects whose cognitive performance remained stable over time was 70% to 80%. This method might thus be used for individual risk prediction [65]. It has yet to be applied more extensively to a larger number of MRI scans.

3.2.3. Analysis of cortical thickness

Another interesting automated method involves determining the cortical thickness of the neocortical association areas and the entorhinal cortex [66]. Group separation showed an accuracy of more than 90% in distinguishing between AD patients and healthy controls [67]. 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.

3.2.4. 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 by 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 [68,69].

3.2.5. Summary of structural MRI

In summary, manual hippocampal volumetry is currently the best established biomarker for AD in the field of structural imaging, but because of 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 pharmacologic studies. However, if secondary preventive treatment approaches are approved during 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.

3.3. Functional magnetic resonance imaging

The use 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 [70–72]. One new approach has been to investigate changes in the functional connectivity between regions of an activated network [73]. Functional connectivity gives a measure of the linear association between two regions and is a function of the phase relationship between the regions' signals [74]. An investigation of functional connectivity in MCI subjects has 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 (Figure 2) [73]. 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 might 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 grey 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 [75]. Further evidence for network alterations comes from studies showing white matter fiber tract decreases by using diffusion tensor imaging (DTI) in AD compared with healthy controls [76–78]. The approach of examining changes across the network supporting cognitive function shows promising results for development of a marker for early diagnosis of AD, thus meriting further research in MCI and groups at high risk for AD.

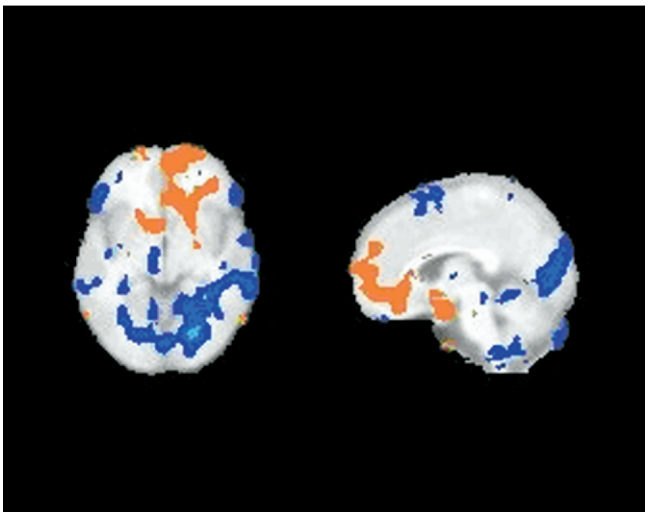

Fig. 2. Differences in functional connectivity between MCI and HC subjects during a face matching task [73]. The images show two areas of the brain with stronger positive connectivity in the HC compared with the MCI and stronger negative functional connectivity in HC compared with MCI.

4. Positron Emission Tomography

Positron emission tomography (PET) with ^{18}fluorodeoxyglucose (^{18}FDG) is used to study cortical metabolism. In AD patients, ^{18}FDG-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 [79].

MCI subjects already show—to a lesser extent—a sim-

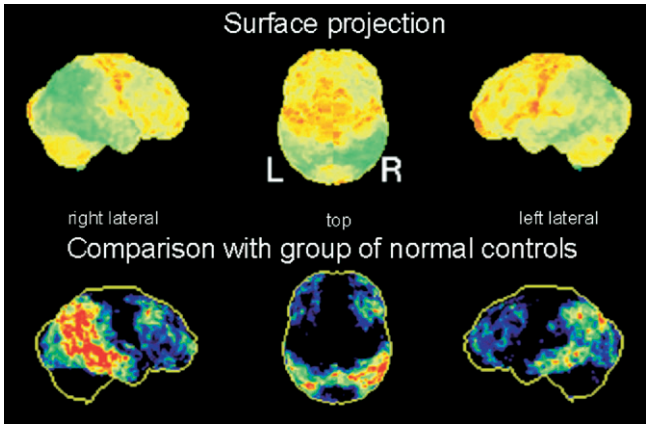

Fig. 3. ^{18}FDG-PET in AD (courtesy of P. Bartenstein and S. Förster, Department of Nuclear Medicine, Ludwig-Maximilian University Munich).

ilar distribution of metabolic deficits that can predict conversion from MCI to AD with an accuracy of greater than 80% [80]. Many researchers regard ^{18}FDG-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 ^{18}FDG-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 [81] (Figure 3). 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 [82]. 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 [83]. 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 labeled receptor agonists or antagonists, 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 up-regulated 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 [84]. A further interesting marker is the imaging of acetylcholinesterase activity [85]. In one study, a significant effect of treatment with a cholinesterase inhibitor was shown on the expression of acetylcholinesterase in the cortex [86]. 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 by using PET in AD patients. The most extensively studied radiotracer is PIB, which shows a specifically enhanced uptake in AD patients compared with healthy controls [87]. It is not clear at present, however, whether the diagnostic accuracy of this method might 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 biologic mechanism would be conceivable.

5. 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 [88] or mediotemporal lobe atrophy [89] demonstrate higher predictive power than either diagnostic approach alone in MCI studies.

Particular combinations or ratios of biomarkers might be useful in answering specific questions; in other words, patterns or rates of change at the neurochemical level might ultimately prove to be optimal. Thus, group separation between AD and vascular dementia patients seems promising by using the ratio of Aβ42 and p-tau [90]. AD could be distinguished from dementia with Lewy bodies (DLB) by using the ratios of Aβ peptides of varying lengths (Aβ42/Aβ38 and Aβ42/Aβ37) and tau protein [91]. There are also indications that the ratios of various Aβ peptides improve the neurochemical profile for potential diagnostic applications [92,93]. A combination of amyloid imaging with PIB-PET and t-tau, Aβ peptides, p-tau, and potentially BACE1 in the CSF has been proposed as a possible way to improve imaging of the underlying neuropathology and to cross-evaluate the neurochemical markers [94]. These approaches are currently being pursued.

6. Discussion

In summary, biologic marker research is most advanced in the area of AD diagnosis. Several neurochemical (t-tau, p-tau, Aβ42) and MRI-based markers (hippocampal and whole brain volumetry) are currently undergoing multicenter evaluation in controlled diagnostic phase IIb studies to determine the sensitivity and specificity of the markers and to make an initial assessment of their positive and

negative predictive values. A number of other markers are undergoing phase I or IIa studies, particularly Aβ peptides, BACE1, isoprostanes, analysis of cortical thickness, DBM, VBM, DTI, markers in MR spectroscopy, and potentially diagnostic paradigms with fMRI.

In comparison, application of biomarkers to map treatment effects is still at an early stage. Thus, 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 [83]. Tau protein has also been used as a secondary end point in clinical studies. In an immunization study discontinued as a result of 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 [95]. Interestingly, MRI showed a decrease in whole brain volume in the responder group in this study [96]. 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 might modify amyloid pathology [5].

Overview of the current literature provides an initial indication that treatment effects might indeed be reflected at the biomarker level. In several cases, biomarker studies 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. An impressive example is the finding of pronounced diurnal fluctuations in Aβ levels in the CSF of nondemented subjects [13].

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 by 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.

New guideline documents of regulatory authorities such as FDA and EMEA will most likely strongly recommend thorough validation of biologic as well as imaging candidate markers as primary end points in upcoming phase II and III treatment trials of compounds claiming disease-modifying properties. To this end, there is a need for thorough and rigorous co-development of biologic 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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