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Review

Perspectives for Multimodal Neurochemical and Imaging Biomarkers in Alzheimer's Disease

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Abstract. The diagnosis of Alzheimer's disease (AD) is presently going through a paradigm shift from disease categories to dimensions and toward the implementation of biomarkers to support identification of predementia and even preclinical asymptomatic stages of the disease. We outline the methodological basis of presently available biomarkers and technological methodologies in AD, including exploratory and hypothesis-based plasma and blood candidates, cerebrospinal fluid markers of amyloid load and axonal destruction, and imaging markers of amyloid deposition, synaptic dysfunction, cortical functional and structural disconnection, and regional atrophy. We integrate biomarker findings into a comprehensive model of AD pathogenesis from healthy aging to cognitive decline, the resilience to cerebral amyloid load (RECAL) matrix. The RECAL framework

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integrates factors of risk and resilience to cerebral amyloid load for individual risk prediction. We show the clinical consequences when the RECAL matrix is operationalized into a diagnostic algorithm both for individual counseling of subjects and for the identification of at risk samples for primary and secondary preventive trials. We discuss the implication of biomarkers for the identification of prodromal AD for the primary care system that seems presently not even prepared to cope with the increasing number of subjects afflicted with late stage AD dementia, let alone future cohorts of subjects searching counseling or treatment of predementia and asymptomatic stages of AD. The paradigm shift in AD diagnosis and its operationalization into a diagnostic framework will have major implications for our understanding of disease pathogenesis. Now, for the first time, we have access to *in vivo* markers of key events in AD pathogenesis integrated into a heuristic framework that makes strong predictions on pattern of multimodal biomarkers in different stages of AD. Critical testing of these predictions will help us to modify or even falsify the currently hold assumptions on the pathogenesis of AD based on *in vivo* evidence in humans.

Keywords: Alzheimer's disease, amyloid, atrophy, biomarker, blood, cerebrospinal fluid, diagnosis, diffusion tensor imaging, hippocampus, mild cognitive impairment, neurodegeneration, neuroimaging, pathophysiology, positron emission tomography, prognosis, resting state functional magnetic resonance imaging, tau, therapy

INTRODUCTION

The recent consensus process toward the definition of new criteria for the diagnosis of Alzheimer's, disease (AD) [1] catalyzes a development that had already started in the 1980 s of the last century. With the availability of novel imaging technologies, including structural magnetic resonance imaging (MRI) and positron emission tomography (PET), research groups worldwide had initiated the search for positive markers of AD [2, 3]. A few years later, imaging markers were complemented by the detection of amyloid-β $(A\beta)_{42}$ and tau protein in the cerebrospinal fluid (CSF) [4, 5]. In the last decade, CSF levels of abnormally phosphorylated tau protein [6, 7] and MRI based measures of functional and structural connectivity using functional MRI (fMRI) and diffusion tensor imaging (DTI) [8–13] have become biomarker candidates for early diagnosis. Finally, since 2004 [14] the detection of amyloid load *in vivo* using Pittsburgh compound B ($[^{11}C]$PIB) allows the identification of early molecular changes of AD. New ^{18}F-labeled compounds will make amyloid PET widely available within the next few years [15].

The wide range of biomarkers of AD, reflecting potential surrogate markers of different stages of disease pathogenesis, has fuelled the definition of new diagnostic categories, including the concept of predementia and preclinical AD [16]. These categories are based on a stage model of disease, where primary amyloid-related molecular pathology over the course of several years leads to destruction of synaptic function and axonal integrity followed by neuronal loss and atrophy and finally clinically detectable cognitive decline [17]. This stage model serves both to guide the development of diagnostic algorithms implementing different levels of biomarkers and as a heuristic paradigm to test hypotheses on the molecular pathogenesis of AD *in vivo*. Without doubt, future studies will modify this stage model of AD. But at least now, for the first time, we have a broadly consented heuristic model which can be tested *in vivo*.

A model of AD spanning preclinical, predementia, and dementia stages puts several demands on clinical research:

1) We will have to integrate multimodal biomarkers using adequate multivariate analysis approaches.
2) The stage model needs to be operationalized into a diagnostic algorithm. This implies that it needs to be implemented in multicenter studies, adding multicenter acquisition as an additional source of variability.
3) A stage model needs to consider modulating factors of state transition. Thus, beside the presence of molecular or environmental risk factors for the transition from healthy aging to cognitive impairment, factors of cerebral resilience have to be integrated that can be quantified using biomarkers.

Figure 1 illustrates a comprehensive stage model of AD operationalized into a diagnostic algorithm and implementing both molecular and environmental risk and resilience factors. This model makes specific predictions which can be tested in future studies. Thus, the model predicts that one will not find a significant number of subjects with a typical pattern of destruction of functional or structural connectivity in the absence of significant amyloid load as detected by PET or CSF. The model also predicts that clinical signs of cognitive decline should not be found before at least beginning structural and functional cortical disconnection.

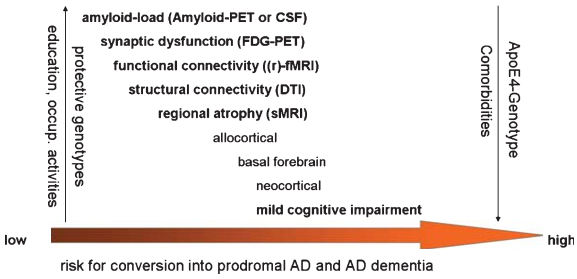

Fig. 1. Stage model for risk stratification. According to the amyloid cascade hypothesis, a molecular event of abnormal amyloid processing and deposition triggers down-stream pathological events from synaptic dysfunction through cortical structural and functional disconnection to neuronal loss and cognitive decline during the development of AD. All these proposed steps of the pathogenesis of AD can now be detected using *in vivo* markers. The stage model makes specific assumptions on the sequence of pathological events where the risk for an individual subject for the clinical manifestation of AD increases with the number of biomarkers showing positive (i.e., AD typical) findings. The individual risk, however, will be modulated by factors of resilience and genetic and environmental risk factors of disease. The stage model makes strong predictions on the temporal sequence of events, where a typical pattern of cortical structural and functional disconnection should not be found in the absence of significant amyloid pathology. These strong predictions render the model a helpful heuristic principle to test the validity of the underlying model of disease pathogenesis.

Based on this model, we can define a multilevel matrix for the transition from healthy aging to manifest cognitive decline (Fig. 2). Healthy aging is defined as a stage 1 where no pathological mechanisms of AD are present. It is characterized by the absence of molecular changes as detected by amyloid-PET and CSF, intact synaptic function, intact structural and functional connectivity, and normal results in cognitive tests and behavioral function. In the second stage of compensated cerebral aging, we expect dissociation between positive signs of molecular pathology in amyloid-PET or CSF and still intact or only minimally impaired synaptic function and functional and structural connectivity as well as normal cognitive function. This stage represents a key stage for research on mechanisms of cerebral resilience, given the capacity of the brain to withstand progressive molecular lesions based on factors of cerebral reserve. Using a finer resolution, stage 2 of compensated cerebral aging can be split into a stage 2a where synaptic function and cortical functional and structural connectivity is entirely preserved and into a stage 2b where a beginning breakdown of functional and structural connectivity can be detected, however, still in absence of cognitive decline. The third stage, the stage of decompensated aging, is characterized by the convergence of primary molecular changes as detected by amyloid-PET or CSF with impaired structural and functional connectivity as well as beginning or clearly manifest cognitive decline. The classification of an individual subject in this matrix would assist in the clinical counseling of high-risk individuals and the stratification of subjects for clinical trials. Subjects in stage 1 could be counseled to have a low risk for cognitive decline in the following years and they would be the ideal candidates for primary preventive trials. Subjects in stage 2 could be classified as asymptomatic at risk state for AD and advised to participate in secondary preventive trials including both pharmacological as well as non-pharmacological interventions. These subjects have an increased risk to develop AD dementia in the following years depending on their further classification into stage 2a or 2b and on the amount of risk factors and capacity of their cerebral resilience. Subjects in stage 3 are subjects with predementia AD or AD dementia which should be advised to participate in tertiary preventive trials in addition to receiving standard treatment.

With this matrix, we extend the framework of the new diagnostic criteria to integrate risk factors and factors of cerebral resilience and operationalize them for potential clinical application. The clinical validation of such a diagnostic matrix requires the use of multimodal imaging and biomarkers acquired across multiple clinical sites involving the use of multivariate analysis approaches. In the following sections, we will discuss the key modalities of such an approach with specific focus on structural and functional connectivity which is the primary candidate for the stratification of people with molecular pathology into different risk categories and disease stages both for clinical trials as well as clinical counseling. Moreover, structural and functional connectivity is a key index for the integrity of cerebral reserve capacity which modulates the transition from molecular lesions to cognitive decline. Following this, we will describe the implementation of these key markers into multimodal multicenter studies and discuss the application of potential multivariate approaches which will become available for diagnostic algorithms in the near future. Already now, machine learning algorithms and other higher level statistical approaches are being implemented in radiological expert systems which will be part of the next generation MR scanners for the support of the radiological reading of anatomical MRI scans in the elderly. This development will serve as a nucleus for the integration of further biomarker and imaging modalities in future diagnostic expert systems which will first be of interest for the risk stratification and sample selection for clinical trials, but also

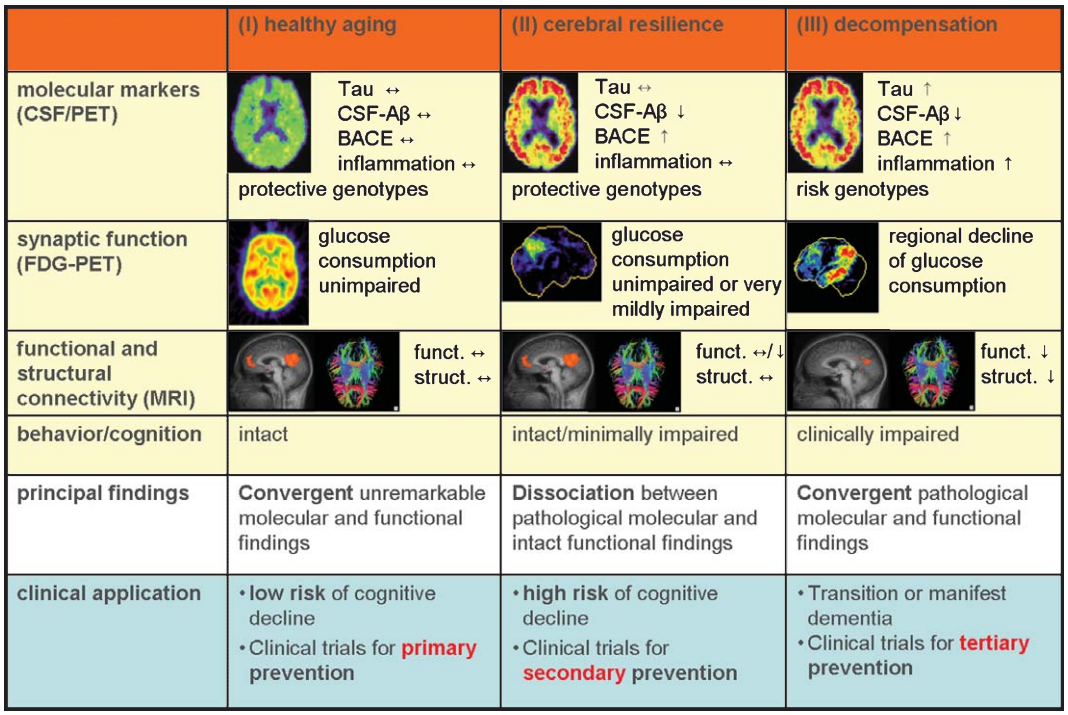

Fig. 2. The RECAL-Matrix: Resilience to Cerebral Amyloid Load. Matrix describing different levels of biomarkers and the expected findings in different stages of AD pathogenesis. In addition, the clinical consequences of the staging of an individual subject in one of the three categories are shortly outlined. This matrix serves as a heuristic model to interpret findings of biomarkers in AD and to help to modify the underlying model of disease pathogenesis.

will become accessible for clinical diagnosis in the mid-term future.

DETECTION OF MOLECULAR AMYLOID PATHOLOGY IN AD

Amyloid PET imaging

Over the last 10 years, PET imaging of cerebral Aβ load has emerged as a powerful biomarker of AD. This reflects the fact that Aβ accumulation represents one of the two histopathological hallmarks of the disease [18]. To date, [^{11}C]PIB [19] is the most widely applied Aβ-targeted PET tracer. Due to the short half-life of the label, however, [^{11}C]PIB does not have the potential to become a tracer for large clinical trials or routine diagnostics. Currently, Aβ PET tracers florbetaben, florbetapir, and flutemetamol, labeled with ^{18}F with a longer half-life, are undergoing late-stage Phase III clinical development [15, 20, 21].

For [^{11}C]PIB, it is known that the tracer accurately discriminates between patients with AD dementia and cognitively healthy controls [19]. It also allows the prediction of the progression from mild cognitive impairment (MCI) to AD dementia [22], rendering Aβ PET a promising tool for the detection of prodromal AD. Cognitively healthy elderly subjects show neocortical [^{11}C]PIB uptake in 10–30% of cases, dependent on the age of the subject [23]. This may indicate that the tracer detects preclinical stages of AD, although the 2-year transition rate in amyloid-positive cognitively healthy subjects to clinical stages of AD is only about 20% [24]. Therefore, for the risk enrichment in clinical trials on disease prevention in cognitively healthy subjects, Aβ PET may need to be complemented by other biomarkers. As compared to late onset sporadic AD cases which demonstrate neocortical tracer uptake mainly in cingulate, frontal, temporal, and parietal cortices [19], early onset autosomal-dominant AD cases exhibit tracer uptake predominantly in striatal areas [25]. In AD dementia, the tracer uptake is a function of ApoE4 state [26]. Of interest, in AD dementia there is no or only limited increase of tracer uptake over time [27]. Both in AD dementia and in cognitively healthy elderly subjects, tracer uptake is not or only weakly correlated with cognition [28, 29]. These results suggest that Aβ PET is not an ideal candidate marker for disease progression. As an alternative, PET

tracers targeting nicotinic acetylcholine receptors might in the future serve this purpose, as the receptor binding is correlated with cognitive function [30, 31]. Despite the fact that [^{11}C]PIB PET has limitations as a marker of disease progression, it was successfully applied to directly monitor effects of Aβ-cleaving therapies on brain Aβ load [32].

Aβ load as measured by [^{11}C]PIB PET has been combined with a range of other biomarkers of AD including: 1) $A\beta_{42}$ levels in CSF; 2) markers of synaptic dysfunction as determined using [^{18}F]FDG PET, 3) markers of neuronal loss as determined using structural MRI, and 4) markers of axonal degeneration using CSF tau.

(i) In AD, CSF Aβ concentration was negatively correlated with brain Aβ load as measured by PET [33], a result which was successfully replicated by other groups. The strength of the correlation between both markers has even led to the proposal to impute [^{11}C]PIB uptake units from CSF Aβ levels [34]. However, in MCI subjects, Aβ PET provided more accurate prediction of progression to AD than CSF Aβ [35].

(ii) Aβ PET has been shown to be more sensitive than [^{18}F]FDG PET to discriminate between AD patients and healthy control subjects [36] and to predict progression to AD in MCI [37]. In general, both biomarkers carry valuable complementary information in MCI and AD dementia subjects [38]. Of interest, the prefrontal cortex of AD patients usually presents with high [^{11}C]PIB uptake, but is relatively spared from glucose consumption deficits [39], a pattern which is so far not fully understood. It suggests dissociation between amyloid deposition and neuronal functional deficits, which may also account for the limited correlation between cognitive decline and amyloid load in cross-sectional studies [28, 29].

(iii) Local brain atrophy in AD dementia as determined by structural MRI was positively correlated with regional and global Aβ brain load as measured by PET [40]. Such a correlation was even found in the inferior temporal cortex of Aβ PET-positive cognitively healthy elderly subjects and was interpreted as a very early sign of asymptomatic preclinical AD [41, 42].

(iv) Conflicting results were reported in the literature on the association between brain Aβ PET and CSF tau and phospho-tau levels in AD dementia [43, 44]. However, in MCI subjects, Aβ PET seems to be more sensitive in predicting the progression to AD dementia as compared to CSF phospho-tau [35].

Compared to other AD biomarkers Aβ PET seems to have certain advantages. Compared to MRI-based markers, Aβ PET directly assesses the supposed primary molecular event in AD pathogenesis; compared to CSF markers, Aβ PET is less invasive and allows determining biomarker expression on a regional level within the brain. Aβ PET plays a key role in the establishing of a multimodal *in vivo* model of AD pathogenesis and will help to determine whether the hypothesis of amyloid processing changes as a primary event in AD pathogenesis can hold or needs to be modified in the future. In addition, Aβ PET has a great potential to serve as a valuable biomarker of AD, especially for early diagnosis and monitoring the effect of Aβ-cleaving therapies. Once the first ^{18}F-labeled Aβ-targeted PET tracers will be approved, they will certainly enrich our clinical routine AD diagnostic toolbox by supplementing cognitive testing towards increased diagnostic confidence.

CSF markers of amyloid load in vivo

In monocentric studies, CSF core feasible AD biomarkers ($A\beta_{42}$, total tau (t-tau) and phosphorylated tau (p-tau)) display predictive and diagnostic sensitivity and specificity in the range of 80–90% [45, 46]. Decreased levels of $A\beta_{42}$ in CSF indicate accumulation of Aβ in the brain and changes of CSF Aβ levels are fully developed as much as 5–10 years before manifestation of dementia syndrome in AD [47]. These findings are in line with theoretical frameworks of biomarker trajectories proposing that changes in amyloid metabolism are among the earliest detectable molecular changes associated with AD pathophysiology [48]. The detection of amyloid pathology via Aβ PET imaging or via changes in CSF Aβ levels is an important defining diagnostic factor for the diagnosis of prodromal [49] and preclinical [50] stages of AD. Moreover, it is accepted as a supportive tool to increase the diagnostic certainty in the determination of AD-related etiology in patients with dementia syndrome [51] and in subjects with MCI [52]. However, CSF based monomeric $A\beta_{42}$ levels (as well as CSF based t-tau and p-tau) have been shown to lack a clear correlation with cognitive and functional state in AD patients [53, 54]. Therefore, recent research has focused on the development of novel approaches in order to assess

those aspects of amyloid-related pathophysiology that may be more closely associated with cognitive and functional impairment, providing the basis for clinically relevant disease-tracking and surrogate marker development. The basis of this research strategy has been the observation that sub-nanomolar concentrations of oligomeric forms of Aβ (Aβ-oligomers) display extreme levels of synaptotoxicity and that their negative effects on synaptic plasticity necessary for learning and memory surpass the effects of monomeric or fibrillar Aβ species [55, 56]. A major problem in the assessment of Aβ-oligomers is their low concentration in CSF but novel methods using ELISAs, nanoparticle based detection methods, or various spectroscopy based methods have recently been developed to successfully assess CSF concentrations of Aβ-oligomers. Flow cytometry based measurement of fluorescence resonance energy transfer (FRET) has proven useful for reliable and valid quantitative measurement of Aβ-oligomers in CSF [57]. So far, several human studies revealed that CSF levels of Aβ-oligomers correlate very strongly with cognitive state in AD patients [58, 59]. Further studies comprising not only demented AD patients but subjects presenting with preclinical and MCI stages of AD are necessary in order to assess the usefulness of CSF Aβ-oligomers to indicate even subtle, subclinical levels of cognitive deficits and to predict cognitive decline in asymptomatic at-risk subjects with confirmed AD pathophysiology.

Another focus of evolving research related to the development of diagnostic and prognostic amyloid based CSF biomarkers is in the assessment of CSF based BACE-1 levels, an enzyme critically involved in the intracerebral generation of Aβ through cleavage of AβPP. Recent human studies suggest that CSF BACE-1 levels are elevated very early during predementia stages of AD [60, 61] and that they are associated with morphological changes of the hippocampus in AD patients [62]. Further research is necessary in order to test whether elevations of CSF BACE-1 may precede the onset of detectable changes in monomeric or oligomeric CSF Aβ species or changed binding to amyloid tracer ligands in PET amyloid imaging. Moreover, the ability to specifically and differentially assess factors related to Aβ generation (as opposed to factors involved in, e.g., Aβ degradation or clearance) is of utmost value for the identification of potential risk states preceding brain amyloid accumulation and of potential endophenotypes of AD.

Despite its somewhat questionable reputation among patients and even among some physicians, lumbar puncture and CSF sampling in a memory clinic setting have been shown to have negligible adverse effect rates and virtually absent neurological or any medical complications [63]. Due to its direct contact with ongoing pathophysiological processes in the CNS, CSF is one of the most promising and worthwhile sources in the quest of diagnostically relevant biomarkers in neurodegenerative diseases. Specific molecular amyloid species beyond monomeric Aβ as well as proteins critically involved in AβPP and amyloid metabolism are important new candidate biomarkers, promising to improve detection and prediction of cognitive decline, and to allow for sensitive tracking of compound and drug effects on aspects of amyloid metabolism relevant to cognitive dysfunction. In line with recent conceptual frameworks of the pathophysiology of AD which propose complex models with heterogeneous upstream molecular pathways ultimately leading to the AD phenotype [64], the identification of potential AD endophenotypes might greatly facilitate the development of disease-modifying compounds specifically designed to therapeutically interfere with specific pathophysiological pathways which are present in some but not all individuals with sporadic AD. CSF candidate biomarkers will likely play a critical role supporting this line of research.

Blood based markers

Lumbar puncture is still regarded as a semi-invasive procedure in many countries worldwide. Therefore, blood-based biological markers would be considered very beneficial for diagnostic purposes due to cost-effectiveness and easy sample collection. Several markers related to AD pathophysiological processes have been studied, such as components of AβPP and Aβ metabolism, cholesterol metabolism, oxidative stress, and inflammation. Particularly $A\beta_{42}$, $A\beta_{40}$, and the $A\beta_{42/40}$ ratio have been investigated in AD patients compared to control subjects. The discriminatory power, however, of all of these markers was insufficient and no single marker or combination of markers has been established so far for diagnostic purposes. Novel proteomic and metabolomic profiling methods seem promising approaches under investigation (for review see [65, 66]) but still have not yielded conclusive results.

Interestingly, blood-based analyses can yield insight in AD-related pathophysiological processes. For example, the conversion rate to dementia of MCI patients treated with antihypertensive drugs was significantly reduced only in patients with elevated

mid-regional pro-atrial natriuretic peptide (MR-proANP) at baseline. This peptide is the propeptide of ANP, which is related to (micro-) circulatory function and hypertension. These data support the notion of a potential impact of microcirculatory function on the development of AD at a prodromal stage. Antihypertensive treatment may reduce conversion rates to AD in MR-proANP stratified subjects with MCI [67]. Another study looked at $A\beta_{42}$ levels in 25 resuscitated patients with severe hypoxia due to cardiac arrest. After a lag period of 10 or more hours, all patients showed significant serum $A\beta_{42}$ elevations. These data indicate that ischemia acutely increases Aβ levels in blood and support the notion that hypoxia may play a role in the amyloidogenic process of AD [68]. These studies support the notion that blood-based markers, if not useful as diagnostic markers, still could contribute within the neurochemical biomarker panel to test hypotheses on the molecular pathogenesis of AD *in vivo*.

CORTICAL FUNCTIONAL AND STRUCTURAL CONNECTIVITY

The concept of cerebral connectivity

The long standing discussions on a modular or network-like organization of human brain function have been integrated into the concept of a complex interplay of distributed neuronal networks subserving higher cognitive functions. The notion of cerebral connectivity has been introduced into the broad discussion since the advent of functional imaging using PET and fMRI. Functional connectivity refers to the covariance of neuronal signals within the time domain [69]. This notion has been critically discussed given its somewhat vague definition. Thus, it can include data from different signal modalities, such as EEG, fMRI, or PET, as well as pooling of data across subjects or across time points within subjects [70]. Still, the concept of functional connectivity can be further qualified by information on modalities and analysis and is very helpful to describe the destruction of neuronal networks which in concert subserve higher cognitive function in AD. A complementary concept is effective connectivity, which combines functional connectivity with a causal model of region A influencing region B [69]. Effective connectivity has become accessible through the use of causal models such as dynamic causal modeling or structural equation modeling of functional imaging data [71]. Closely related to the concept of effective connectivity is the concept of structural connectivity. This concept implies that there exists an anatomical connection between two distant regions of the brain. Indeed, combined DTI and fMRI studies showed that structural connectivity parallels functional connectivity between any of two regions [72]; however, functional connectivity can also be detected between brain regions which have no direct structural connections.

One could assume that functional connectivity would be more sensitive than structural connectivity to neurodegenerative disease progression. This relates to the notion that synaptic dysfunction is among the earliest signs of neuronal dysfunction in prodromal AD where the loss of synaptic function would be detected as a decline of neuronal activity as determined by FDG-PET assessed glucose consumption or the hemodynamic response function of functional MRI. The decline of structural connectivity would imply a more advanced disease stage where axonal structures such as the myelin sheath, axonal membrane, or the intra-axonal cytoskeleton would undergo progressive destruction. This model, however, needs to take into account that compensatory mechanisms of axonal sprouting and neuronal reorganization may lead to the maintenance of functional connectivity (using novel indirect pathways) despite the destruction of direct anatomical pathways and structural connectivity.

The concept of functional and structural connectivity is of high interest in the study of AD progression for several reasons:

1. Since the late 1980 s, a range of post-mortem studies suggested a selective vulnerability of intracortical projecting neurons, particularly layer 3 and 5 large pyramidal neurons, which maintain long-reaching intracortical connections.
2. The concept of cortical connectivity accounts coherently for the development of AD along specific neuronal systems subserving distinct cognitive domains that are early and selectively impaired in the clinical stages of AD.
3. An increasing body of evidence supports the notion that despite progressive neurodegeneration, functional decline can partially be compensated by mechanisms of neuronal restructuring. The underlying bases of these mechanisms are still only partly understood. However, the ability of the brain to maintain functional connectivity despite breakdown of anatomical connectivity is an important feature for the understanding of the natural history of AD.

From a range of studies it is clear that molecular amyloid pathology is an important risk marker for the development of AD in cognitively intact healthy elderly subjects. However, the rate of conversion into cognitive decline and dementia in those subjects with high load of amyloid as detected by PET or CSF amounts only to about 16% over 2 years [24]. This suggests that amyloid load may be a necessary but not a sufficient condition for the development of AD dementia at least over a short time follow-up. Therefore, the study of cortical functional and structural connectivity can significantly increase the accuracy of disease prediction, which is relevant for the selection of at-risk subjects for clinical trials, as well as for the integration of risk and resilience factors into a comprehensive model of AD progression.

Therefore, in the following sections we will outline the current evidence on functional and structural disconnection in prodromal AD and AD dementia based on resting state functional MRI and DTI data. We will focus on these two modalities because they have gained increasing interest over the last years and are the focus of ongoing large scale multicenter studies worldwide.

Functional connectivity in resting state fMRI, a marker of risk and resilience

One approach to the investigation of functional connectivity in the brain is by the measurement of the low frequency coherent networks active while the brain is 'at rest'. Much of the work in this area stems from a pivotal study [73] where intrinsic activity attributed to a baseline state or 'default mode' was first proposed. Deactivations were noted when task conditions were contrasted with a low level, often resting-state, condition which led the investigators to propose that networks of brain activity were present during the baseline state, denoted the default mode network (DMN). Thus, for example, AD patients and amnestic MCI subjects had decreased deactivation compared to healthy subjects during performance of a cognitive task [74–76] and similar alterations between groups were found with functional connectivity [29]. Using resting state fMRI instead of an active cognitive paradigm, significant numbers of baseline networks could be identified, denoted intrinsic connectivity networks (ICNs) [77], yet the earliest and most extensively studied is the DMN. The DMN includes the posterior cingulate cortex/precuneus, dorsal and ventral medial prefrontal, lateral inferior parietal cortices, and medial temporal lobes.

There are several approaches to analyze resting state fMRI data. Among the most widely used methods are functional connectivity analysis, a measure of interregional time-course correlation between regions of interest [9, 78–80] as developed by Biswal and colleagues [81], and independent component analysis (ICA) which automatically isolates networks of synchronized coactivation from the fMRI data in a purely data-driven approach [82–84]. Initial investigations found decreased functional connectivity between the hippocampus and posterior cingulate in the AD group compared to healthy subjects as well as decreased connectivity in amnestic MCI subjects compared to healthy subjects [85, 86]. Alteration of DMN connectivity in AD and MCI is not restricted to the hippocampus and posterior cingulate, but there are significant alterations in connectivity with the other regions of the DMN. One study found decreased functional connectivity between posterior cingulate and medial frontal regions in AD and amnestic MCI subjects [87], while another comprehensive study on functional network alterations in AD patients found decreased intratemporal, temporo-thalamus, temporo-striatal, thalamo-occipital, and thalamo-frontal connectivity, but also increased intrafrontal, frontal-prefrontal, and fronto-striatal connectivity [79]. Similarly, AD patients showed decreased linear correlations between the prefrontal and parietal lobes, but increased positive correlations within the prefrontal lobe, parietal lobe, and occipital lobe [88].

The disruption of the DMN in AD is thought to begin before the appearance of clinical symptoms. Supportive of this idea, healthy subjects with a high amyloid-load deposition in the posterior cingulate had impaired deactivation in the posterior cingulate during performance of a memory task [89]. In addition, another study found that there was increased disruption of functional connectivity in the posterior cingulate/precuneus and temporoparietal cortex across a group of healthy subjects with high amyloid deposition compared to healthy subjects with low amyloid deposition [90].

The pattern of increased and decreased connectivity in the DMN among the AD, amnestic MCI, and healthy control groups point to the existence of potential compensatory processes emerging in the early stages of the disease. Cognitive reserve or resilience relates to the capacity of the brain to cope with neuropathology leading to delayed and less severe clinical symptoms of the disease [91]. Bosch and colleagues [92] found that higher cognitive reserve in healthy subjects was found

to be associated with lower deactivations within the DMN and lower task-related activity, which were interpreted to reflect increased neural efficiency. In contrast, the amnestic MCI subjects and AD patients with higher cognitive reserve had greater activity in task-related brain areas and increased deactivations within the posterior cingulate and medial frontal regions compared to those with lower cognitive reserve. Thus, Bosch and colleagues [92] found a greater reallocation of processing resources from the DMN to the neural network engaged in the experimental task, which could reflect increased reliance on compensatory resources to maintain cognitive function.

Several studies have tested and quantified the diagnostic value of the ICNs. One study used the goodness to fit on components of the DMN that were detected by ICA, and found that AD patients and healthy elderly controls could be correctly categorized with a sensitivity of 85% and a specificity of 77% [9]. Koch and colleagues [93] applied both region of interest (ROI)-based interconnectivity analyses and ICA based analyses of the DMN. When used in combination these approaches yielded a diagnostic accuracy of 97% (sensitivity 100%, specificity 95%) in discriminating AD patients from healthy subjects. Fleisher and colleagues [94] directly compared the diagnostic value between altered functional activation during a cognitive task and disrupted DMN connectivity in the resting-state, to separate asymptomatic subjects at high risk for AD (family history and APOE $\varepsilon 4$ allele carriers) from healthy low-risk subjects. A comparison between the two methods revealed that resting state functional connectivity discriminated between the two risk groups with a larger effect size compared with task-related functional activation.

An issue for the use of the DMN as a potential marker for diagnosis of dementia is the stability of the DMN as assessed by ICA within individuals over time. In a study on 18 healthy young subjects, the DMN was reproducible within a single imaging session as well as between imaging sessions twelve hours and one week apart [95]. The reproducibility over time has also been shown for other frequently reported ICNs [96] and using functional connectivity analysis instead of ICA [97]. Finally, ICNs as detected by both functional connectivity analysis and ICA were also shown to be consistent across multicenter data [98]. However, further work needs to be performed in older healthy subjects and longer intervals between scanning sessions to further validate the DMN for clinically oriented uses.

Diffusion tensor imaging as a marker of structural connectivity

DTI entered clinical application around the turn of the century. This technique relies on the diffusion of water molecules where the use of diffusion gradients during the MRI acquisition labels water protons with a spatial coordinate because diffusion will lead to signal attenuation at read-out [99]. Using at least six diffusion gradients, DTI determines the elements of the diffusion tensor which allows the reconstruction of the main directions of diffusion along three orthogonal axes using eigenvectors and the corresponding eigenvalues of the diffusion tensor. Currently, a state of the art DTI sequence for studying patients with dementia will employ at least 30 diffusion gradients and last about 10 minutes. From DTI, we can derive reconstructions of fiber tracts (fiber tracking) as well as scalar indices of anisotropic diffusion, the most widely used being the fractional anisotropy (FA).

Spatial maps of scalar diffusion indices can be analyzed using ROIs in selected brain areas where the decline of diffusion anisotropy and an increase of mean diffusivity indicate microstructural changes in the brain region under study. Using this approach, a range of previous studies has found significant decline of fiber tract integrity in posterior cingulate, corpus callosum, temporal lobe, and parietal lobe white matter [12, 100–109]. Using ROIs in the hippocampus, a study in patients with amnestic MCI showed a more accurate discrimination between MCI and healthy subjects using markers of diffusion anisotropy compared to hippocampus volume [110]. This result agrees with the notion that changes in microstructural integrity of fiber tracts subserving structural connectivity would precede the decline of neuronal density in grey matter areas such as the hippocampus.

More recent studies investigated changes along entire fiber tracts which were reconstructed using selected seed points, for example in the posterior cingulate. Similar to the ROI data, fiber tract integrity was reduced along the cingulate bundle in AD dementia compared to healthy controls [111, 112]. In addition, multivariate approaches which take into consideration the covariance structure of the DTI data showed decline of network connectivity in AD dementia and even prodromal AD [109, 113–115]. Interestingly, while FA is still the most widely used index of microstructural fiber integrity, recent studies suggest that other diffusion indices, including axial, radial, and mean diffusivity may be more sensitive markers of AD-related white matter pathology [116, 117].

DTI also serves to determine mechanisms of cerebral resilience. Already in the 1990 s, epidemiological studies suggested that the incidence of AD is decreased in subjects with higher education [118–120]. The risk reduction in subjects with higher education can be interpreted as the effect of a higher cerebral resilience which helps people to maintain normal cognitive function for a longer time despite progressive cerebral lesions than people with lower education. A potential neurobiological basis for this observation was suggested by studies using FDG-PET [121] and structural MRI [122, 123] who found more severe metabolic dysfunction and atrophy at the same level of cognitive performance in subjects with higher education, suggesting that these subjects could compensate more severe cerebral pathology. Subjects with higher education at the same level of cognitive impairment showed a more severely impaired integrity of structural networks involving far reaching intracortical association areas as detected by DTI [124]. In addition, healthy subjects showed higher anisotropy in intracortical connecting fiber tracts with higher education compared to lower educated cognitively normal subjects. This data suggest that structural connectivity reflects potential mechanism of cerebral resilience, possibly related to the density of intracortical projecting fiber tracts.

Future DTI studies will focus on its direct diagnostic application; measures of structural connectivity have entered the state of multicenter trials. Within the framework of the European DTI study in dementia (EDSD), a clinical and physical phantom study suggested an about 50% higher variability of multicenter acquired DTI data compared to classical anatomical MRI scans [125]. In addition, the variability of diffusion indices is higher in fiber tracts with lower anisotropy, suggesting that multicenter studies need to take into account the systematic variation across different types of fiber tracts. In respect to clinical application across 300 subjects, DTI derived measures of structural connectivity using univariate analysis showed relatively lower diagnostic accuracy discriminating AD dementia from healthy subjects compared to anatomical MRI data (Teipel et al. in press). The use of multivariate machine learning algorithms including support vector machine increased diagnostic accuracy by about 9%, but still structural connectivity reached at most identical accuracy compared to anatomical MRI (Dyrba et al. submitted). Based on the model of disease progression in AD (Fig. 1), one would expect a higher accuracy of DTI-based measures of structural integrity compared to measures of atrophy in prodromal stages of AD. This has already been shown in monocenter studies [110], but not yet in multicenter studies.

Multimodal imaging studies

Both the power and diagnostic accuracy of clinical studies, as well as the heuristic value of imaging studies, is increased by the use of multimodal data with multivariate statistical approaches. One of the most interesting approaches is the combination of resting state fMRI with DTI data. In healthy adult subjects, the combination of DTI with resting state fMRI showed that the presence of anatomical connections as suggested by DTI based fiber tract integrity is almost always correlated with functional connectivity in the corresponding brain regions [126]. In contrast, the presence of functional connectivity between distinct brain regions is not necessarily implying the presence of a direct anatomical connection [127]. Within the DMN, it has been found that functional connectivity is predefined by the structural connectivity in fiber tracts connecting the key nodes of this network. This finding has been replicated using both fiber tractography [128, 129] and multivariate analysis of anisotropy index maps [130]. When applied to patients with AD dementia, one can show that the decline of functional connectivity is paralleled by a decline of fiber tract integrity in fiber tracts connecting the key nodes of the DMN (Likitjaroen et al., submitted). These data suggest that the decline of functional connectivity in AD is related to the decline of underlying fiber tract integrity where the combination of both markers can help to discriminate between two types of functional changes, (i) break-down of functional and structural connectivity and (ii) compensatory reallocation of neuronal networks.

In recent years, machine learning algorithms to determine complex patterns of covariance between multidimensional data sets have become available for the analysis of imaging data based on improved hardware and software resources. Among the applied algorithms, support vector machines (SVM) have found increasing interest, because they enable the identification of binary outcomes based on a very efficient algorithm [131, 132]. Thus, recent studies that used combined information from spatial atrophy and DTI based changes in structural connectivity to train a SVM classifier, could show a high accuracy in discriminating between AD patients and control subjects as well as between patients with MCI and healthy elderly controls [115] (Dyrba et al., submitted). Another study used linear discriminant analysis to optimally combine

multimodal MRI measurements (T1- and T2-weighted contrasts and DTI) for the distinction between AD patients and cognitively normal controls. These AD-specific multimodal MRI indices in combination with the discriminant function yielded a highly accurate model for the distinction of patients with MCI that later converted to AD from those that remained stable over follow up [133]. Machine learning algorithms are already now being developed for the software of scanner consoles as radiological expert systems based on anatomical MRI data. They employ SVM algorithms or related techniques, all of which allow the integration of a multitude of image and biomarker modalities.

These approaches will also gain further relevance for the attenuation of effects of multicenter acquisition when imaging is used in the context of clinical trials or routine diagnosis across a range of different scanning platforms.

THE MULTICENTER ASPECT OF MULTIMODAL IMAGING STUDIES

A multicenter study design allows the recruitment of a relatively large number of subjects, such as that required for Phase II and III clinical trials, while keeping the time to completion of the study short. In addition, multicenter studies provide the opportunity to examine the reliability of diagnostic biomarkers such as MRI neuroimaging derived biomarkers for the diagnosis of AD [134]. Calibration studies on multicenter MRI have shown that inter-center variability of scanner performance is an important factor that can influence the assessment of regional grey matter volume measurement in a multicenter study [135, 136]. Whereas the test-retest reliability of repeated MRI measurements on the same scanner—even in face of scanner changes regarding field inhomogeneities, voxel scaling factors, or scanner upgrades—is relatively high, the differences in MRI measurements between scanners are significant [137]. The portion of variability that can be attributed to scanner differences for the measurement of neuroimaging markers such as the hippocampus volume was reported to be as large as 16% when assessed in patients with MCI and AD dementia [138]. Larger MRI multicenter studies such as the Alzheimer's Disease Neuroimaging Initiative (ADNI) which includes 90 MRI scanners at 58 sites to examine neuroimaging and other types of biomarkers of AD (http://www.adni-info.org/), have therefore developed a specific MRI multicenter protocol to minimize multicenter MRI effects. Such a protocol includes, for example, the introduction of a standardized MRI image acquisition protocol, the restriction to a selected number of scanner models, phantom-based scanner calibration, and image correction [139, 140]. Even with such an optimized MRI acquisition protocol, a study showed that for volumetric measures the between-scanner variance was still up to 10 times higher than the intra-scanner variance of repeated measures [136]. Encouraging results come from multicenter studies including studies such as ADNI that show that the effect size and thus the statistical power to detect regional brain atrophy, when using manual volumetry or automated cortical thickness measurements, are not significantly diminished in a multicenter study design compared to single-center assessments [135, 137, 141, 142]. Furthermore, the multi-center variability does not seem to be different in patients with AD when compared to healthy controls, suggesting that a multicenter design does not introduce a disease-specific bias [143, 144]. Estimations of the statistical power to detect brain atrophy in AD have been conducted for multicenter studies. Taking multicenter MRI variability of hippocampus volume measurement into account, Jovicich and colleagues estimated that about 36 subjects per treatment arm are required in order to detect a 50% treatment-induced reduction of the hippocampus atrophy rate in patients with AD, at a statistical power of 90% (alpha = 5%) [137]. The statistical model to control for multicenter effects may also have a significant impact on the power to detect treatment effects [145]. Together, these results suggest that overall a multicenter MRI study design is associated with acceptable MRI measurement precision and demonstrates its utility for clinical trials. However, it should be emphasized that despite relatively good precision of multicenter MRI measurements, the use of different scanners can introduce a systematic bias in measurement [137] so that treatment groups should be distributed equally across different scanners and repeated longitudinal MRI assessments should be done on the same scanner using the same imaging protocol [137]. Furthermore, it should be cautioned that the use of optimized MRI acquisition protocols such as employed in the ADNI may lead to an underestimation of scanner effects if MRI findings are to be generalized toward scanners and acquisition protocols outside such monitored clinical research networks, i.e., within a wider clinical context [144]. Calibration methods based on phantom measurements need to be further investigated for the derivation of scanner specific correction factors to

increase the reproducibility and accuracy in a clinical context.

Multicenter diffusion tensor imaging

Several multicenter studies on DTI have documented the susceptibility of DTI derived measures to multicenter scanner effects [146–150]. The multicenter effects on DTI measures are in general stronger when compared to volumetric MRI measures of regional grey matter volume [150]. Interdependencies between DTI acquisition parameters, scanner hardware, spatial normalization approaches, and fiber tract properties result in a measurement variability that is not well understood [151, 152]. Consequently, no clear recommendations on the optimization on DTI protocols for multicenter acquisition have yet emerged. The multicenter variability of DTI derived FA values is especially high in brain regions that show low FA values, e.g., in small structures where partial volume effects are stronger or structures where crossing fiber are prevalent [152]. White matter regions such as the corpus callosum, which show high FA values and are relatively large, show in general the best reproducibility across centers [150]. Statistical approaches have been proposed to reduce the brain-region dependent influence of multicenter variability. Based on a bootstrapping technique called wild bootstrapping [153, 154] that allows for the assessment of the precision of the tensor estimation, voxel values that show high imprecision of diffusion tensor estimation due to inter-scanner variability can be identified. In proportion to the estimated multicenter variability, such data points of high variability are being weighted less in group comparisons of DTI or when computing average DTI voxel values within a particular white matter ROI. These approaches have been shown to significantly reduce the multicenter variability in ROI values of common DTI parameters such as FA [152], and thus increase the utility of DTI derived measures for clinical application. However, compared to MRI volumetric measures of grey matter, the standardization and establishment of DTI derived measures with a robust performance within a multicenter context is less well developed. Therefore, DTI measures have not yet been recommended as neuroimaging biomarkers for the research diagnosis of AD [51].

CONCLUSIONS

Already now, the discussion on the new diagnostic criteria has pushed the development of novel study designs for preventive trials in at risk and prodromal stages of AD. In the future, the integration of more than one imaging or biomarker modality will increase the accuracy of risk prediction and therefore increase the power of clinical trials to detect disease modifying or preventive treatment effects. At the same time, these markers will serve to define different stages on the transition from healthy aging to AD dementia so that individual counseling becomes possible. Similar to other areas of research, what used to be a sophisticated measure in single expert centers or dedicated international networks now has won increasing interest from industry, including the development of diagnostic expert systems on a new generation of MR scanners and the marketing of PET, CSF, and eventually blood biomarkers of AD [155]. Therefore, the development of multimodal imaging biomarkers has gained a strong momentum and confronts us with several challenges:

1. The broader availability of non-invasive imaging markers and risk markers of neurodegenerative disease has already led and will increasingly lead to the use of these markers outside of clinical care or clinical studies for the counseling of cognitively healthy subjects who want to learn about their risk of dementia at an age of maybe 50 to 60 years. Today, it is not sufficiently discussed how these people should be advised if amyloid accumulation is detected. There is not yet sufficient evidence to suggest if these relatively young subjects indeed have an increased risk for the development of neurodegenerative disease over the following decade and even less is known if any measure could be advised for them to decrease such a potential risk. This lack of established clinical validity renders uncritical application of advanced imaging techniques for "screening" of AD risk an ethical challenge.
2. The availability of non-invasive imaging markers will considerably influence the rate of diagnosis of dementia and prodromal AD in the population because imaging is often easier accessible for primary care physicians and their patients than is complex neuropsychological testing. If this tendency should continue over the next years, one has to discuss the question whether the broad availability of new diagnostic markers will lead to a de-compensation of the present health system which already now has difficulties to cope with an increasing number of subjects who are only clinically—and, hence, at a very late stage

according to the new paradigm—diagnosed. Given that today at most 40 to 50% of the subjects with dementia receive a diagnosis of dementia within the primary care system [156], one wonders what will happen if this proportion will increase significantly. The number of patients will increase even further when predementia stages will be identified, a presumably much larger group in the population. The health care system in its present state will be challenged to adequately support a multiple of the current number of diagnosed subjects and at present there is no evidence to guide the provision of the appropriate, if any, therapy. This renders the development of disease modifying and preventive treatments the key issue in future dementia care. It is here, where the access to accurate instruments to precisely define and select at risk samples for clinical trials becomes imperative. The novel multidimensional paradigm provides a considerable potential to improve future clinical trials, and, thus, the chance to validate effective treatment modalities.

In the future, we might expect to find constellations of imaging and biomarkers in subjects who later develop clinical AD which disagree with the proposed model of a primary molecular lesion and subsequent downstream effects. Thus, one may find subjects which display low levels of amyloid, but exhibit a typical pattern of cortical structural and functional disconnection and later develop clinical signs of AD. It is the great advantage of an *in vivo* model of stage-specific biomarker dynamics to enable us to falsify or modify this model on AD pathogenesis based on clinical and epidemiologic evidence. This will increase the heuristic value of each single multimodal imaging and biomarker study in AD, because all these studies then will contribute a little piece to the same jigsaw-puzzle and will no longer stand alone as an isolated finding.

This integrating function of a multimodal stage model of AD based on biomarkers could become one of the most important advances in clinical research on AD of the last decade. It provides us with a heuristic paradigm to test the validity of biological models on AD pathogenesis. It also allows for a systematic diagnostic algorithm for the risk stratification of samples for clinical trials. Finally, the multimodal stage model laid down provides a potential future instrument to inform and counsel elderly subjects with cognitive impairments.

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