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REVIEW

Rethinking on the concept of biomarkers in preclinical Alzheimer's disease

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Abstract The neuropathological processes eventually leading to Alzheimer's disease (AD) are thought to start decades before the appearance of clinical symptoms and the clinical diagnosis of AD dementia. The term "preclinical AD" has been recently introduced to identify this "silent stage" of AD, when the disease is already present, but symptoms are not yet clinically evident. Advances in AD biomarkers have dramatically improved the ability to detect AD pathological processes in vivo in cognitively intact subjects, thus demonstrating the presence of AD pathology in the preclinical phase. This review focuses on the recent advances in the field of neuroimaging and CSF AD biomarkers specifically in the preclinical phase of AD, and aims to discuss the significance that such biomarkers could have in cognitively intact subjects. Even though the use of such biomarkers in AD preclinical phase has contributed to improve our understanding of AD early pathological processes, it raised also a number of new challenges that still remain to be overcome, such as a better definition of the clinical and individual significance of currently known biomarkers in preclinical stages and the development of novel biomarkers of different early AD-related events.

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Introduction

The term "preclinical" Alzheimer's disease (AD) has been proposed to identify the "silent stage of AD"—when the disease is already present, but symptoms are not yet clinically evident. This is the stage in the AD pathophysiological continuum, when disease-modifying treatments are supposed to be more effective [1]. The existence of this stage has been supposed for years [2], but the actual identification of preclinical AD has been possible thanks to recent advances in neuroimaging and cerebrospinal fluid (CSF) assays, which made it possible the demonstration in vivo of AD pathophysiological processes. In particular, the possibility to non-invasively study the presence of AD pathological processes has stimulated the creation of large-scale databases, which included also cognitively intact subjects and thus permitted the demonstration of the continuum of AD pathophysiological process, which begins decades before overt dementia symptoms occur.

The criteria for preclinical AD recently proposed by the US National Institute on Aging-Alzheimer's Association (NIA-AA) workgroups, identified three stages [1]:

- Stage 1: evidence of amyloid-β (Aβ) accumulation on positron emission tomography (PET) Aβ imaging or CSF assays (Fig. 1).
- Stage 2: cerebral amyloidosis plus evidence of neurodegeneration, as elevated CSF tau levels or abnormalities on functional or structural neuroimaging.
- Stage 3: amyloidosis plus neurodegeneration with evidence of very subtle cognitive decline that does

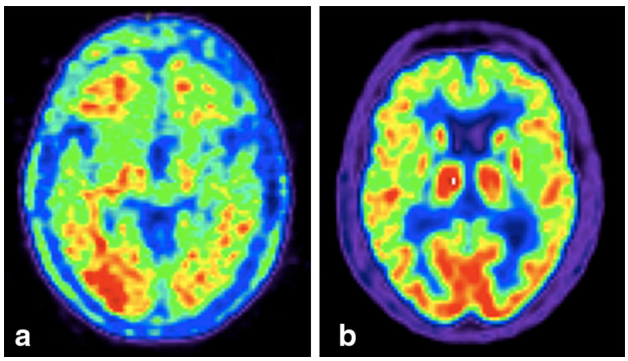

Fig. 1 Example case of cognitively intact subject (74 years old, education: 5 years) with positive amyloid PET (**a**) and negative FDG PET (**b**). **a** amyloid PET shows Aβ accumulation in frontal, temporal and occipital lobes, more pronounced in the right hemisphere. **b** FDG PET scans shows normal brain metabolic activity, particularly in temporo-parietal regions

not yet meet the criteria for mild cognitive impairment (MCI) (Fig. 2).

Two additional categories have further been proposed [3]:

- Stage 0: older individuals with no biomarker evidence of AD pathology (Fig. 3).
- Suspected non-Alzheimer pathology (SNAP): individuals showing biomarkers of neurodegeneration without positive markers of amyloid accumulation (Fig. 4).

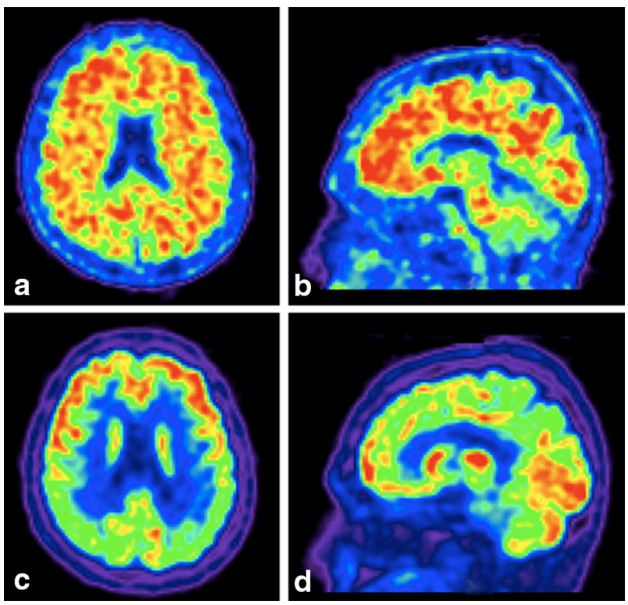

Fig. 2 Example case of a patient (53 years old, education >18 years) with mild cognitive impairment and both amyloid PET (**a**, **b**) and FDG PET (**c**, **d**) positive. **a**, **b** amyloid PET shows widespread cortical Aβ accumulation in both hemispheres. **c**, **d** FDG PET shows reduced metabolic activity in temporo-parietal regions, in precuneus and posterior cingulate cortex

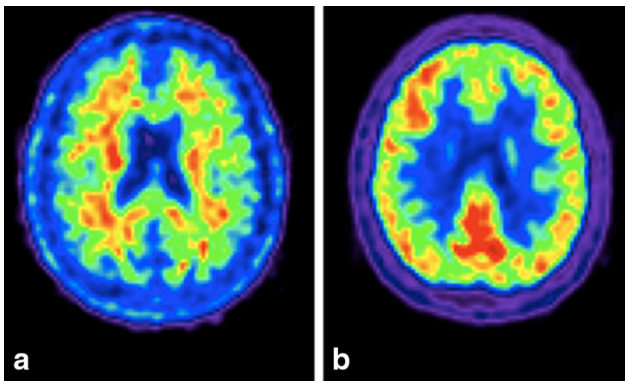

Fig. 3 Example case of cognitively intact subject (58 years old, education: 8 years) with negative amyloid PET (**a**) and negative FDG PET (**b**). **a** amyloid PET shows no tracer uptake in cortical gray matter, but only aspecific uptake in white matter. **b** FDG PET scans shows normal brain metabolic activity, particularly in temporo-parietal regions

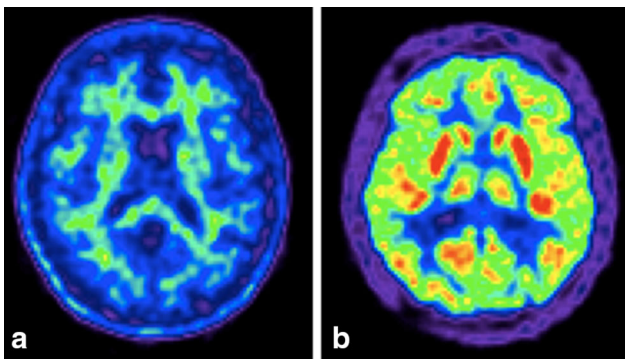

Fig. 4 Example case of cognitively intact subject (55 years old, education: 5 years) with negative amyloid PET (**a**) and positive FDG PET (**b**). **a** amyloid PET shows no tracer uptake in cortical gray matter, but only aspecific uptake in white matter. **b** FDG PET shows hypometabolism in parietal lobes (particularly left parietal lobe), in posterior cingulate and precuneus, and in frontal lobes (particularly on the right side)

The preclinical phase of AD is hence primarily defined by changes in biomarkers, and cognitive and behavioral changes become subtly evident only in its last stage (stage 3). The term "preclinical" is different from and was preferred with respect to "presymptomatic", to underline that symptoms in the form of very subtle cognitive changes could arise several years before the condition of MCI occur [4].

Several studies have shown that the presence of AD biomarkers in clinically normal older individuals is associated with increased risk of cognitive decline. The ability of the recommended NIA-AA criteria for the diagnosis of preclinical AD to predict progression to cognitive impairment [1] has been recently tested in a large cohort of cognitively normal individuals who underwent brain

magnetic resonance imaging (MRI) or [18F] fluorodeoxyglucose (FDG) and [11C] Pittsburgh compound B (PiB) PET. Subjects had global cognitive test scores, and were followed for at least 1 year. Of the 296 initially normal subjects, 31 (10 %) progressed to a diagnosis of mild cognitive impairment (MCI) or dementia (27 amnestic MCI, 2 nonamnestic MCI, and 2 non-AD dementias) within 1 year. The proportion of subjects who progressed to MCI or dementia increased with advancing NIA-AA stage (stage 0, 5 %; stage 1, 11 %; stage 2, 21 %; stage 3, 43 %). Authors hence suggested that despite the short follow-up period, the new preclinical AD recommendations confirmed that advancing preclinical stage led to higher proportions of subjects who progressed to MCI or dementia [5, 6].

The formal definition of the preclinical phase of AD marked a paradigm shift in the way AD is considered: from a debilitating disease affecting more and more people to a disease that is already sub-clinically affecting and will probably affect everyone. Indeed, according to the criteria and the proposed stages of preclinical AD, everyone will enter in the AD trajectory, sooner or later. The question is not whether, it is when. Under the proposed criteria, even cognitively intact subjects that are negative for all known AD biomarkers could not be considered "AD-free", but they could be considered in the stage 0 of preclinical AD. In this scenario, the research in the field of AD biomarkers is crucial, to determine the likelihood and the moment in which a subject could enter in the AD trajectory, or could be considered "AD-free".

The relationship between AD biomarkers and AD preclinical phase is particularly relevant for several reasons; first of all, preclinical AD is by definition characterized by changes in those biomarkers. Moreover, the preclinical phase of AD would hopefully give significant information about disease pathogenesis, mainly on the time sequence of early pathological events. Finally, the preclinical population is the most relevant from a therapeutic point of view, since it may be the only one in which disease-modifying actions could work.

This review focuses on the recent advances in the field of neuroimaging and CSF AD biomarkers specifically in the preclinical phase of AD, and aims to discuss the significance that such biomarkers could have in cognitively intact subjects.

Biomarkers, risk and resilience factors: three different entities around cognitive decline

The definition of "Biological marker" or "Biomarker" provided by the Biomarkers Definitions Working Group is: "…a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [7]. Biomarkers contribute to monitor health status and disease detection, and can provide a basis for the selection of candidates for clinical trials, and for characterization of the subtypes of disease for which a therapeutic intervention is most appropriate [8].

In AD clinical trials, biomarkers can be employed: (1) to improve the selection accuracy of trial participants; (2) for stratification of AD patients; (3) to monitor safety; (4) to identify and monitor the biochemical effects of drugs [9]. Biomarkers can further provide the possibility to characterize and validate drug mechanisms of action, to monitor AD course and progression, and to evaluate therapeutic response/outcome [10]. The development of imaging tools for diagnosis of AD and monitoring disease progression is a clinical research effort of the Biomarker Consortium which was convened by the Foundation for the National Institute of Health under FDA's Critical Path initiative [11].

The five most well-established biomarkers of AD have been divided into two major categories: measures of brain Aβ deposition and measures of neurodegeneration—defined as a progressive loss of neurons or their processes (axons and dendrites) with a corresponding progressive impairment in neuronal function [12]. Brain Aβ deposition can be assessed by measuring Aβ levels in CSF [13] and by PET amyloid imaging [14]. Measures of neurodegeneration are increased concentrations of CSF total tau (t-tau) and phosphorylated tau (p-tau) [13], hypometabolism at FDG PET [15] and atrophy at structural MRI [16].

Biomarkers should not be confused with risk factors; whilst the formers are indicators of a pathological process, the latters are characteristics of an individual associated to an increased risk of developing a disease. The greatest risk factor for late-onset "sporadic" AD and other dementias is age [17]. In the development of preclinical biomarkers, age should hence be taken into account in determining the threshold for biomarker positivity.

The apolipoprotein E (APOE) ε4 allele is a major susceptibility gene and genetic risk factor for AD [18]. It has been suggested to play an unfavorable role in Aβ deposition, neuronal maintenances, neuronal signaling pathways, and cytoskeletal structure and function [19]. APOE genotype has been associated with an earlier age at onset of AD, with dosage effect according to the number of ε4 alleles [20].

Family history is another major risk factor for developing AD [21]. Moreover, epidemiological studies demonstrated a parent of origin effect, showing that maternal family history may have a greater impact than paternal transmission [22]. The genetic and pathological basis of such increased susceptibility to AD conferred by

maternal family history is not completely known. It has been suggested a role of epigenetic imprinting and mitochondrial DNA transmission [21].

Particular variants of AD are the rare early-onset dominantly inherited forms of AD. Three major genetic loci for familial AD have been identified: APP (amyloid precursor protein) on chromosome 21q, PSEN1 (presenilin 1) on chromosome 14q, and PSEN2 (presenilin 2) on chromosome 1q. Within these three genes over 200 mutations have been found, capable of causing early-onset AD. Studies of the effects of these genetic mutations demonstrated an abnormally increased Aβ accumulation in the brain, and suggested the central role of Aβ in the pathogenesis of AD [23].

There are several differences between risk factors for AD versus biomarkers of AD. Genetic status remains constant throughout life, whereas biomarkers are dynamic as they reflect the current state of pathophysiology [24]. A biomarker-negative individual could therefore become biomarker-positive later in life course, thus entering the pathological trajectory. In AD, risk factors could define the likelihood of entering the AD pathological trajectory at some point and the slope of such trajectory, while biomarkers could suggest the point of the AD trajectory where an individual currently lies (Fig. 5).

Moreover, the interaction between AD biomarkers and risk factors and the point in the AD pathological trajectory when clinical symptoms become manifest, are influenced by resilience factors such as cognitive reserve (CR). CR refers to "differences in cognitive processes as a function of lifetime intellectual activities and other environmental factors that explain differential susceptibility to functional impairment in the presence of pathology or other neurological insult" [25]. CR has been proposed to account for the frequent discrepancy between a person's underlying level of brain pathology (or age-related changes) and the observed functional and/or cognitive deficits that are expected to result from that pathology [25]. The effect of CR on brain metabolism in subjects with preclinical AD was recently explored, showing that higher education was associated with lower brain metabolic activity at FDG PET in amyloid-positive individuals [26]. These results suggest that CR had a compensatory function to sustain cognitive ability in presence of early AD pathology [26].

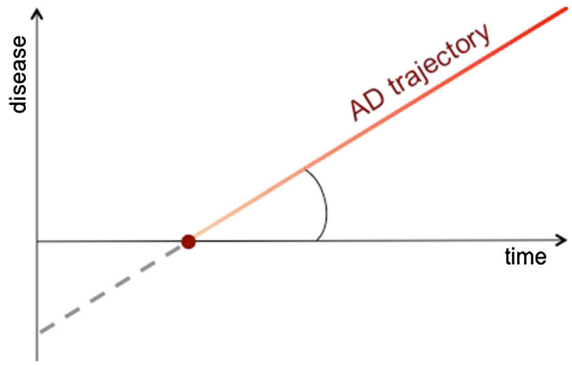

Fig. 5 The hypothesized AD pathological trajectory. Risk and resilience factors could determine both the slope of the AD trajectory and its intercept with the X axis (i.e., the moment of entering the trajectory). Biomarkers may determine at which point of the trajectory an individual lies

These three entities have been firstly studied and largely analyzed in AD patients as separate factors [9, 14, 15], extensively in MCI subjects [13], but only at a limited extent in preclinical stages, obviously because of the difficulty to study these factors in such a large-scale population. However, the study of cognitively intact individuals not only added important information about the "AD trajectory" on the preclinical phase, but such information would also be more crucial in this phase, since the cognitively intact population may be the only one in which disease-modifying actions could work. Lot of work still remains to be done to determine an "individual AD trajectory", based on individual risk and resilience factors and biomarkers levels.

Biomarkers of preclinical AD

Amyloid PET imaging

The use of amyloid PET imaging in AD found its basis on the amyloid cascade hypothesis, which, although controversial, it is the dominant AD etiologic theory [27]. According to this hypothesis, Aβ deposition is the first and initiating event in the pathological process, eventually leading to alterations in neural function and cognitive decline.

The advent of PET Aβ imaging has provided a novel in vivo non-invasive way to visualize some of the brain pathological changes that were previously restricted to invasive or post-mortem studies. The non-invasive nature of such imaging technique has also stimulated its use in large-scale databases including a number of cognitively intact subjects, thus permitting to demonstrate that Aβ deposition is actually a very early event in AD pathological process [28].

PET radiotracers are derivatives of histopathologic stains, and bind specifically to fibrillar forms of Aβ, in particular to sites of the β-sheet structure [29]. Among the many Aβ radiotracers that have been synthesized, only a few of them proved effective for imaging in vivo Aβ [30]. Historically, 18F-FDDNP was the first reported PET tracer to image AD pathology, both Aβ and tau protein depositions [31]. 11C-PiB is currently the most widely studied

tracer for Aβ PET imaging [14]. More recently, several 18F-labeled radiotracers were synthesized, such as 18F-florbetapir, 18F-florbetaben and 18F-flutemetamol [32–35], and are now available for clinical use.

Since the earliest amyloid PET studies [14], elevated amyloid tracers uptake has been reported in a significant proportion of cognitively intact subjects, with percentages varying from 21 to 33 %, especially in older individuals. These data are comparable to the age-related frequency of neuropathological AD alterations on post-mortem examination of cognitively intact adults [36, 37].

The spatial distribution of Aβ PET tracers in amyloid-positive cognitively intact individuals is similar to AD patients, demonstrating high cortical binding in the precuneus/posterior cingulate cortex, gyrus rectus, frontal cortex, in lateral temporal and parietal cortices (although to a lesser extent), with relative spare of occipital and sensorimotor regions and minimal binding in the cerebellum [14, 36–38]. Interestingly, it has been noted that this pattern of amyloid deposition overlaps the regions denoted as "multimodal cortex", a network of cortical hubs and brain areas involved in complex cognitive processes [39].

The issue of amyloid positivity of normal individuals has challenged amyloid PET imaging scientific world for years, to demonstrate if it meant the crossing of a threshold in the AD trajectory or not. Some studies tested the significance of "amyloid positivity" in cognitively intact individuals, reporting an increased rate of atrophy [40] and greater clinical worsening [41, 42] in amyloid-positive subjects. Moreover, longitudinal amyloid imaging studies demonstrated higher increase in Aβ deposition in amyloid-positive than in negative subjects [28, 43]. The most significant changes were observed in prefrontal, parietal and lateral temporal cortices [43], and this trend seems to reverse in subjects with very high baseline tracer uptake, consistently with the concept that Aβ deposition is a saturable process [43].

In familial dominantly inherited AD patients, in which the preclinical phase has been extensively studied, amyloid positivity at PET imaging has been documented many years before expected symptoms onset [44–46]. Interestingly, in mutation carriers the cerebral amyloid load pattern differs from that observed in sporadic AD, with predominant tracer uptake in the striatum and thalamus [44–46].

The cognitively intact population is the most suitable to test the interactions between AD risk factors and biomarkers, because there is no confounding variables due to disease severity. The two main AD risk factors, i.e., age and ApoE4, have been shown to have a significant impact on Aβ deposition in cognitively intact subjects. In particular, the prevalence of amyloid-positive PET scans consistently increases with age [38] and with APOE ε4 dose [47, 48]. Besides, a familial, especially maternal, history of AD has been associated with increased Aβ deposition [49].

Limitations of amyloid PET imaging

Although amyloid PET imaging represents at present the only way to visualize in vivo the deposition of fibrillar Aβ, and it has been capable to demonstrate the presence of such pathologic alteration in brains of cognitively intact individuals, putting them in the hypothesized condition of preclinical AD, it is important to acknowledge some limitations of this approach.

First of all, these PET Aβ biomarkers are not sensible markers of soluble oligomeric forms of Aβ, which are thought to be the most toxic species to synaptic function, but visualize only fibrillar forms, which represent the final result of amyloid aggregation [50]. This limitation could be of unique importance in the preclinical phase of AD, since it is a very initial phase when Aβ accumulation is ongoing and oligomeric forms could be more present and convey their most toxic potential.

Besides, the AD pathophysiological model and the amyloid cascade hypothesis, even if based on scientific strong theories, may be incomplete: the exact etiology of AD remains uncertain and mithocondrial, inflammatory, metabolic, cytoskeletal and other alterations could play a key, maybe more important than Aβ, role in the pathogenesis of the disease [51, 52].

So far the approach to amyloid PET studies has been essentially dichotomous, lacking a quantitative evaluation of the amyloid load, which instead represents a meaningful point in neuropathologic assessment of AD [53]. Accurate quantification of amyloid load could be especially meaningful in preclinical AD, hopefully showing the process of Aβ accumulation before reaching the well-known plateau. Further studies are ongoing to develop better radiotracer uptake quantification methods, which could be particularly relevant in cases with intermediate Aβ accumulation and for establishing common cut-off values [54].

Finally, the results in preclinical AD were obtained from cognitively intact subjects from community-recruited studies and selection biases may be present. This could have influenced both the prevalence of amyloid-positive PET scans and the rate of conversion to clinical AD.

Imaging neurodegeneration: FDG PET imaging

FDG is the most widely used tracer for brain PET imaging and measures cerebral glucose metabolism, which reflects brain synaptic activity. FDG PET imaging has been firstly and extensively performed in cognitively impaired patients (with AD or MCI), and it consistently demonstrated significant reduction of cerebral glucose metabolic activity in patients with AD, even at early stages. The specific pattern associated with AD is characterized by hypometabolism in temporo-parietal regions, posterior cingulate cortex,

precuneus and medial temporal lobes [55]. The temporo-parietal pattern of hypometabolism has been considered a biomarker of AD-related synaptic dysfunction and neurodegeneration.

Some cognitively intact subjects may exhibit significant reduction of cerebral glucose metabolic activity involving temporo-parietal regions, and they may be considered to be in the preclinical phase of AD. Such hypometabolic pattern, similar to that observed in AD, can precede the onset of symptoms by many years [56], and it has been considered to reflect the early (maybe reversible) synaptic dysfunction of preclinical AD, and not the massive neurodegeneration (not reversible) typical of advanced AD. Although brain hypometabolism could be observed in absence of clinical symptoms (maybe due to compensatory cerebral circuits), it is not the other way around: AD clinical symptoms rarely, if ever, occur without the presence of brain hypometabolism. The severity, extent and topography of metabolic impairment usually correlate to the severity of cognitive symptoms, after their appearance.

The significance of brain hypometabolism in cognitively intact subjects has been studied in a few longitudinal studies, showing that it represents a "moment" above the hypothesized threshold in the AD trajectory. These FDG PET studies in cognitively intact subjects demonstrated that cerebral metabolic reductions preceded cognitive decline from normal cognition to mild cognitive impairment to AD, with over 80 % accuracy [57].

Evidence of cerebral glucose metabolic reduction in the preclinical phase has been shown also in presymptomatic individuals carrying autosomal dominant genetic mutations associated with early-onset familial AD [58]. The AD-typical FDG PET abnormalities have been observed several years before the predicted age of onset of cognitive impairment, suggesting that the "threshold crossing" in these particular subjects happens very early in life [59].

Several AD risk factors significantly interact with brain metabolic activity and, as in amyloid imaging, the preclinical phase of the disease is the most suitable one to study such interaction. FDG PET studies demonstrated the AD-typical pattern of cerebral glucose hypometabolism in APOE ε4 allele cognitively intact carriers as compared to non-carriers [47]. Interestingly, such synaptic abnormalities seem to occur independently from the presence of fibrillar cerebral Aβ accumulation as detected by neuroimaging [60]. Moreover, cerebral metabolic deficits in AD-vulnerable regions have been observed in cognitively intact subjects with AD family history, in particular with maternal family history [61].

Limitations of FDG PET imaging

FDG PET imaging is a very well known and studied neuroimaging technique, with a well-established clinical role as a biomarker of several neurodegenerative disorders, both dementias and parkinsonisms.

Although the AD-specific hypometabolic pattern is usually typical of the AD spectrum, from its preclinical phase to the dementia stage, cerebral metabolic reductions cannot distinguish among stages. Besides, there could be heterogeneity in patterns of hypometabolism, which could be due to individual differences (particularly in risk factors and cognitive reserve) or to age-related comorbid conditions (i.e., vascular disease) [62]. Therefore, doubts could remain as to whether cerebral metabolic impairment could be due to AD or to other causes.

Finally, to use FDG PET to document the metabolic decline from preclinical to prodromal AD, and also in the view of using this biomarker to test preventive measures for AD, more accurate measurements of cerebral glucose metabolic activity, such as absolute quantification, may be necessary [63, 64].

Despite these limitations, however, FDG PET still represents an accurate and reliable biomarker of neurodegeneration, even in the preclinical phase of AD, and it remains one of the most widely used neuroimaging techniques useful to follow the disease progression.

CSF biomarkers

CSF biomarkers belong to the category of pathophysiological markers and reflect the two degenerative processes of AD pathology, amyloidosis and tauopathy paths [65]. The typical AD CSF pattern consists of decreased levels of Aβ42 and increased values of *p*-tau and *t*-tau, a pattern commonly referred as the "AD signature" [66]. The AD-signature pattern has been extensively studied and validated in AD patients, but the alterations of such CSF biomarkers can be observed also in cognitively intact subjects, putting them in the condition of preclinical AD. Several studies on familial AD cases confirmed that changes from normal to pathological levels of CSF biomarkers occur 10–15 years before clinical onset of AD [67]. However, the exact time sequence is still under debate. Studies on cognitively intact subjects have tried to provide answers to this question, which is crucial in this preclinical population mainly to establish the best disease-modifying action to start with. Some studies showed decreased CSF Aβ42 levels before the increasing of tau, suggesting that amyloid pathology starts before neuronal dysfunction and neurodegeneration [68]. Other neuropathological studies described intraneuronal tau inclusions (pretangles, neurofibrillary tangles, neuropil threads) years before the deposition of Aβ plaques [69].

CSF Aβ42 levels represent the other biomarker of brain amyloid deposition in alternative to amyloid PET imaging. Several studies reported a general concordance between

low Aβ42 concentration and amyloid PET positivity, suggesting that the decreased CSF concentration of the peptide reflects its deposition in cortical plaques [70]. However, in a small proportion of patient there is discordance between Aβ CSF and amyloid PET results, suggesting that these biomarkers could measure slightly different aspects of AD Aβ pathology [71].

CSF p-tau levels represent another measure that is derived from CSF assays and correlate with the amount of neocortical neurofibrillary tangles pathology and likely reflects the phosphorylation state of tau and the formation of tangles in the brain [72]. CSF t-tau represents the intensity of both acute neuronal damage and chronic neuronal degeneration in the brain. This marker is associated with a faster progression from MCI to AD and to a higher mortality rate in AD patients [73].

Recently, a new CSF marker has been described: the Visinin-like protein 1 (VILIP-1), a neuronal calcium-sensor protein. VILIP-1 concentration predicts rates of whole brain and regional atrophy similarly to t-tau and p-tau and may provide a surrogate for neurodegeneration in early AD and in preclinical phase [74].

Some studies have evaluated whether CSF biomarkers may predict cognitive decline in cognitively intact elderly individuals. A recent meta-analysis shows that the prevalence of amyloid pathology increased from age 50 to 90 from 10 to 44 % in cognitively intact individuals [75]. The presence in normal elderly of CSF AD-signature pattern has been associated with the future development of cognitive decline and dementia, thus suggesting CSF positivity alone could put a subject in the AD trajectory, i.e., in the preclinical phase of AD [76–79].

Limitations of CSF biomarkers

Low Aβ42 levels alone are not specific of AD, since pathological alteration of this biomarker has been found also in non-AD dementias, such as Dementia with Lewy Bodies (DLB) or Vascular Dementia (VD) [80]. The Cochrane Review [76] confirmed that single CSF biomarkers abnormality is not accurate to predict conversion to AD. Among CSF markers, *p*-tau 181 (tau phosphorylated at the threonine 181) is the most specific of AD [81].

Moreover, CSF biomarkers present several biases. CSF values show a high (20–30 %) inter-assay and inter-laboratory variability, and there is not a consensus on the cut-offs [82]. Because of the lack of reproducibility, CSF results from different laboratories cannot be directly compared and longitudinal studies are unfeasible. To define universal cut-off levels, sources of "procedural bias" and "confounding factors" must be considered. For these reasons, inter-laboratory quality control surveys are essential, to standardize measurements [82].

CSF biomarkers' predictive performance could be improved by taking into account confounding factors as age, genotype status and follow-up length. A recent meta-analysis, including such confounding factors, revealed that Aβ42/p-tau ratio can predict with high sensitivity (81 %) and specificity (91 %) the conversion to AD among MCI individuals younger than 70 years, while the predictive power decreases for older MCI subjects [83].

Despite the reported biases, CSF biomarkers are useful in the identification of preclinical AD status. CSF levels of Aβ and tau reflect brain neuropathology and can be useful to monitor the effect of AD therapy in vivo.

Future perspectives

The recent availability of large databases has given the unique opportunity to test AD biomarkers not only in cognitively impaired patients, but also in cognitively intact individuals, thus allowing the recognition and definition of a preclinical phase of AD. Currently available biomarkers proved very effective in the identification of AD pathophysiological processes, however, it is clear that much remains to be discovered.

The challenge with currently available biomarkers (CSF or neuroimaging) is to find a reliable approach to document the transfer from healthy aging to preclinical AD. Biomarker thresholds combined with risk factors could represent the answer; longitudinal large-sample studies are required to test this crucial transition.

The presence of AD biomarkers in cognitively intact individuals confers an increased risk of developing dementia, but an accurate quantification of the likelihood of progression or individual reliable prognosis is still lacking. Such limitation is due to an incomplete knowledge of all AD risk factors, which may accelerate the decline to dementia, and of protective factors, such as cognitive reserve, which could delay the onset of symptoms.

Moreover, research on the exact AD pathological processes is still very active, and the introduction of novel preclinical AD biomarkers is expected. Novel Aβ radiotracers with less not-specific binding and faster wash-out have been synthesized and probably will become available in the next future [84]. Several tau radiotracers are being developed, which will be able to visualize tau pathology not only in AD, but also in other neurodegenerative disorders [85, 86]. The non-invasive monitoring of tau presence in the brain will expand the knowledge of lesion-associated proteins during life span as opposed to post-mortem end-stage pathology.

It is urgent the search of novel biomarkers of AD-related events (e.g., flogosis) that will improve our understanding of the early AD pathological processes, to not only identify

with higher accuracy individuals in the preclinical phase of AD, but also to develop more effective treatments.

Final remarks

The field of AD biomarkers has assisted many advances during the past decade, mainly with the advent of amyloid imaging. Now we know that a significant proportion of cognitively intact individuals (mostly beyond the age of 65) is positive to almost one of the known main AD biomarkers, so we should state that their brain is already showing AD pathology at some degree. What we know up to now is that their prognosis in time is worse than in biomarker-negative individuals, however, we cannot state yet that they will all become ouvert AD before biomarker-negative individuals. Thus, the follow-up of cognitively intact biomarker-positive individuals will continue to give relevant information on the clinical significance of biomarker positivity.

Progressive changes in biomarker levels have been documented during time, ultimately leading to cognitive impairment. Data are still lacking to define the importance of the time profile of these changes in anticipating the advent of clinical symptoms.

Moreover, some points are still obscure: the exact sequence of cause-to-effect relationship between different biomarkers is complex, and not yet fully understood, and their time sequence has been only hypothesized. Researchers are still discussing about the possible role of different pathological processes that could lead to AD [87], and the recent possibility to visualize tau deposits in the brain and to detect the presence of inflammation [88] will probably increase biomarkers impact in the field of AD in general, but mostly in its preclinical phase.

Finally, since the prognosis of each single individual associated with biomarker positivity is still unclear; and ethical issues, such as the idea that we could diagnose AD in normal individuals, have not yet been fully addressed, much work remains to be done: the preclinical AD revolution has just begun.

Compliance with ethical standards

Conflict of interest All Authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Sperling RA, Aisen PS, Beckett LA et al (2011) Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's Dement 7:280–292. doi:10.1016/j.jalz.2011.03.003
2. Braak H, Braak E (1997) Frequency of stages of Alzheimer-related lesions in different age categories. NBA 18:351–357
3. Jack CR Jr, Knopman DS, Weigand SD et al (2012) An operational approach to National Institute on Aging-Alzheimer's Association criteria for preclinical Alzheimer disease. Ann Neurol 71:765–775. doi:10.1002/ana.22628
4. Salmon DP, Ferris SH, Thomas RG et al (2013) Age and apolipoprotein E genotype influence rate of cognitive decline in nondemented elderly. Neuropsychology 27:391–401. doi:10.1037/a0032707
5. Blennow K (2010) Biomarkers in Alzheimer's disease drug development. Nat Med 16:1218–1222. doi:10.1038/nm.2221
6. Knopman DS, Jack CR, Wiste HJ et al (2012) Short-term clinical outcomes for stages of NIA-AA preclinical Alzheimer disease. Neurology 78:1576–1582. doi:10.1212/WNL.0b013e3182563bbe
7. Group BDW (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69:89–95. doi:10.1067/mcp.2001.113989
8. Pupi A, Mosconi L, Nobili FM, Sorbi S (2005) Toward the validation of functional neuroimaging as a potential biomarker for Alzheimer's disease: implications for drug development. Mol Imaging Biol 7:59–68. doi:10.1007/s11307-005-0953-8
9. Hampel H, Lista S (2013) Use of biomarkers and imaging to assess pathophysiology, mechanisms of action and target engagement. J Nutr Health Aging 17:54–63. doi:10.1007/s12603-013-0003-1
10. Vellas B, Carrillo MC, Sampaio C et al (2013) Designing drug trials for Alzheimer's disease: what we have learned from the release of the phase III antibody trials: a report from the EU/US/CTAD Task Force. In: Alzheimers Dement. pp 438–444
11. Parekh A, Buckman-Garner S, McCune S et al (2015) Catalyzing the Critical Path Initiative: fDA's progress in drug development activities. Clin Pharmacol Ther 97:221–233. doi:10.1002/cpt.42
12. Jack CR, Knopman DS, Jagust WJ et al (2013) Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol 12:207–216. doi:10.1016/S1474-4422(12)70291-0
13. Mattsson N, Zetterberg H, Hansson O et al (2009) CSF biomarkers and incipient alzheimer disease in patients with mild cognitive impairment. JAMA 302:385–393. doi:10.1001/jama.2009.1064
14. Klunk WE, Engler H, Nordberg A, Wang Y (2004) Imaging brain amyloid in Alzheimer's disease with Pittsburgh compound-B—Klunk—2004—Annals of Neurology—Wiley Online Library. Annals of …
15. Jagust WJ, Bandy D, Chen K et al (2010) The Alzheimer's disease neuroimaging initiative positron emission tomography core. Alzheimers Dement 6:221–229. doi:10.1016/j.jalz.2010.03.003
16. Dickerson BC, Wolk DA, Alzheimer's Disease Neuroimaging Initiative (2012) MRI cortical thickness biomarker predicts AD-like CSF and cognitive decline in normal adults. Neurology 78:84–90. doi:10.1212/WNL.0b013e31823efc6c
17. Alzheimer's Association (2015) Alzheimer's disease facts and figures. Alzheimers Dement 11:332–384
18. Bertram L, Lill CM, Tanzi RE (2010) The genetics of Alzheimer disease: back to the future. Neuron 68:270–281. doi:10.1016/j.neuron.2010.10.013
19. Gee JR, Keller JN (2005) Astrocytes: regulation of brain homeostasis via apolipoprotein E. Int J Biochem Cell Biol 37:1145–1150. doi:10.1016/j.biocel.2004.10.004
20. Farrer LA, Cupples LA, Haines JL et al (1997) Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and

Alzheimer disease meta analysis Consortium. JAMA 278:1349–1356
21. Mosconi L, Berti V, Swerdlow RH et al (2010) Maternal transmission of Alzheimer's disease: prodromal metabolic phenotype and the search for genes. Hum Genomics 4:170–193
22. Gómez-Tortosa E, Barquero MS, Barón M et al (2007) Variability of age at onset in siblings with familial Alzheimer disease. Arch Neurol 64:1743–1748. doi:10.1001/archneur.64.12.1743
23. Price JL, Morris JC (1999) Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. Ann Neurol 45:358–368
24. Sperling RA, Johnson KA (2013) Biomarkers of Alzheimer disease: current and future applications to diagnostic criteria. Continuum 19:325–338
25. Stern Y (2009) Cognitive reserve. Neuropsychologia 47:2015–2028. doi:10.1016/j.neuropsychologia.2009.03.004
26. Ewers M, Insel PS, Stern Y et al (2013) Cognitive reserve associated with FDG-PET in preclinical Alzheimer disease. Neurology 80:1194–1201. doi:10.1212/WNL.0b013e31828970c2
27. Castellani RJ, Perry G (2014) The complexities of the pathology–pathogenesis relationship in Alzheimer disease. Biochem Pharmacol 88:671–676. doi:10.1016/j.bcp.2014.01.009
28. Vlassenko AG, Mintun MA, Xiong C et al (2011) Amyloid-beta plaque growth in cognitively normal adults: longitudinal [11C] Pittsburgh compound B data. Ann Neurol 70:857–861. doi:10.1002/ana.22608
29. Kundaikar HS, Degani MS (2015) Insights into the interaction mechanism of ligands with Aβ42 based on molecular dynamics simulations and mechanics: implications of role of common binding site in drug design for Alzheimer's disease. Chem Biol Drug Des. doi:10.1111/cbdd.12555
30. Vallabhajosula S (2011) Positron emission tomography radiopharmaceuticals for imaging brain beta-amyloid. Semin Nucl Med 41:283–299. doi:10.1053/j.semnuclmed.2011.02.005
31. Shoghi-Jadid K, Small GW, Agdeppa ED et al (2002) Localization of neurofibrillary tangles and beta-amyloid plaques in the brains of living patients with alzheimer disease. Am J Geriatr Psychiatry 10:24–35. doi:10.1097/00019442-200201000-00004
32. Rowe CC, Ackerman U, Browne W et al (2008) Imaging of amyloid beta in Alzheimer's disease with 18F-BAY94-9172, a novel PET tracer: proof of mechanism. Lancet Neurol 7:129–135. doi:10.1016/S1474-4422(08)70001-2
33. Koole M, Lewis DM, Buckley C et al (2009) Whole-body biodistribution and radiation dosimetry of 18F-GE067: a radioligand for in vivo brain amyloid imaging. J Nucl Med 50:818–822. doi:10.2967/jnumed.108.060756
34. Kung HF, Choi SR, Qu W et al (2010) 18F stilbenes and styrylpyridines for PET imaging of A beta plaques in Alzheimer's disease: a miniperspective. J Med Chem 53:933–941. doi:10.1021/jm901039z
35. Sabri O, Seibyl J, Rowe C, Barthel H (2015) Beta-amyloid imaging with florbetaben. Clin Transl Imaging 3:13–26. doi:10.1007/s40336-015-0102-6
36. Mintun MA, Larossa GN, Sheline YI et al (2006) [11C] PIB in a nondemented population: potential antecedent marker of Alzheimer disease. Neurology 67:446–452. doi:10.1212/01.wnl.0000228230.26044.a4
37. Aizenstein HJ, Nebes RD, Saxton JA et al (2008) Frequent amyloid deposition without significant cognitive impairment among the elderly. Arch Neurol 65:1509–1517. doi:10.1001/archneur.65.11.1509
38. Rowe CC, Ellis KA, Rimajova M et al (2010) Amyloid imaging results from the Australian imaging, biomarkers and lifestyle (AIBL) study of aging. Neurobiol Aging 31:1275–1283. doi:10.1016/j.neurobiolaging.2010.04.007
39. Jagust WJ, Mormino EC (2011) Lifespan brain activity. Trends Cognit Sci 15:520–526. doi:10.1016/j.tics.2011.09.004
40. Schott JM, Bartlett JW, Fox NC et al (2010) Increased brain atrophy rates in cognitively normal older adults with low cerebrospinal fluid Aβ1-42. Ann Neurol 68:825–834. doi:10.1002/ana.22315
41. Resnick SM, Sojkova J, Zhou Y et al (2010) Longitudinal cognitive decline is associated with fibrillar amyloid-beta measured by [11C]PiB. Neurology 74:807–815. doi:10.1212/WNL.0b013e3181d3e3e9
42. Chételat G, Villemagne VL, Pike KE et al (2011) Independent contribution of temporal beta-amyloid deposition to memory decline in the pre-dementia phase of Alzheimer's disease. Brain 134:798–807. doi:10.1093/brain/awq383
43. Villain N, Chételat G, Grassiot B et al (2012) Regional dynamics of amyloid-β deposition in healthy elderly, mild cognitive impairment and Alzheimer's disease: a voxelwise PiB-PET longitudinal study. Brain 135:2126–2139. doi:10.1093/brain/aws125
44. Knight WD, Okello AA, Ryan NS et al (2011) Carbon-11-Pittsburgh compound B positron emission tomography imaging of amyloid deposition in presenilin 1 mutation carriers. Brain 134:293–300. doi:10.1093/brain/awq310
45. Villemagne VL, Ataka S, Mizuno T et al (2009) High striatal amyloid beta-peptide deposition across different autosomal Alzheimer disease mutation types. Arch Neurol 66:1537–1544. doi:10.1001/archneurol.2009.285
46. Berti V, Nacmias B, Bagnoli S, Sorbi S (2011) Alzheimer's disease: genetic basis and amyloid imaging as endophenotype. Q J Nucl Med Mol Imaging 55:225–236
47. Reiman EM, Chen K, Liu X et al (2009) Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. Proc Natl Acad Sci 106:6820–6825. doi:10.1073/pnas.0900345106
48. Fleisher AS, Chen K, Liu X et al (2013) Apolipoprotein E ε4 and age effects on florbetapir positron emission tomography in healthy aging and Alzheimer disease. Neurobiol Aging 34:1–12. doi:10.1016/j.neurobiolaging.2012.04.017
49. Mosconi L, Rinne JO, Tsui WH et al (2010) Increased fibrillar amyloid-{beta} burden in normal individuals with a family history of late-onset Alzheimer's. Proc Natl Acad Sci 107:5949–5954. doi:10.1073/pnas.0914141107
50. Lee H-G, Casadesus G, Zhu X et al (2004) Challenging the amyloid cascade hypothesis: senile plaques and amyloid-beta as protective adaptations to Alzheimer disease. Ann NY Acad Sci 1019:1–4. doi:10.1196/annals.1297.001
51. Pimplikar SW, Nixon RA, Robakis NK et al (2010) Amyloid-independent mechanisms in Alzheimer's disease pathogenesis. J Neurosci 30:14946–14954. doi:10.1523/JNEUROSCI.4305-10.2010
52. Herrup K (2010) Reimagining Alzheimer's disease—an age-based hypothesis. J Neurosci 30:16755–16762. doi:10.1523/JNEUROSCI.4521-10.2010
53. Hyman BT, Phelps CH, Beach TG et al (2012) National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimers Dement 8:1–13. doi:10.1016/j.jalz.2011.10.007
54. Klunk WE, Koeppe RA, Price JC et al (2015) The Centiloid Project: standardizing quantitative amyloid plaque estimation by PET. Alzheimers Dement 11(1–15):e1–e4. doi:10.1016/j.jalz.2014.07.003
55. Mosconi L (2005) Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. FDG-PET studies in MCI and AD. Eur J Nucl Med Mol Imaging 32:486–510. doi:10.1007/s00259-005-1762-7
56. Mosconi L, Mistur R, Switalski R et al (2009) FDG-PET changes in brain glucose metabolism from normal cognition to

pathologically verified Alzheimer's disease. Eur J Nucl Med Mol Imaging 36:811–822. doi:10.1007/s00259-008-1039-z
57. Mosconi L, De santi S, Li J et al (2008) Hippocampal hypometabolism predicts cognitive decline from normal aging. Neurobiol Aging 29:676–692. doi:10.1016/j.neurobiolaging.2006.12.008
58. Nacmias B, Berti V, Piaceri I, Sorbi S (2013) FDG PET and the genetics of dementia. Clin Transl Imaging 1:235–246. doi:10.1007/s40336-013-0028-9
59. Mosconi L, Sorbi S, De Leon MJ et al (2006) Hypometabolism exceeds atrophy in presymptomatic early-onset familial Alzheimer's disease. J Nucl Med 47:1778–1786
60. Jagust WJ, Landau SM, For the Alzheimer's Disease Neuroimaging Initiative (2012) Apolipoprotein E, not fibrillar—amyloid, reduces cerebral glucose metabolism in normal aging. J Neurosci 32:18227–18233. doi:10.1523/JNEUROSCI.3266-12.2012
61. Mosconi L, Brys M, Switalski R et al (2007) Maternal family history of Alzheimer's disease predisposes to reduced brain glucose metabolism. Proc Natl Acad Sci 104:19067–19072. doi:10.1073/pnas.0705036104
62. Stern Y, Alexander GE, Prohovnik I, Mayeux R (1992) Inverse relationship between education and parietotemporal perfusion deficit in Alzheimer's disease. Ann Neurol 32:371–375. doi:10.1002/ana.410320311
63. Berti V, Vanzi E, Polito C, Pupi A (2013) Back to the future: the absolute quantification of cerebral metabolic rate of glucose. Clin Transl Imaging 1:289–296. doi:10.1007/s40336-013-0030-2
64. Herholz K (2014) The role of PET quantification in neurological imaging: fDG and amyloid imaging in dementia. Clin Transl Imaging 2:321–330. doi:10.1007/s40336-014-0073-z
65. Dubois B, Feldman HH, Jacova C, et al. (2010) Revising the definition of Alzheimer's disease: a new lexicon. In: Lancet Neurol. pp 1118–1127
66. Shaw LM, Vanderstichele H, Knapik-Czajka M et al (2009) Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol 65:403–413. doi:10.1002/ana.21610
67. Blennow K, Dubois B, Fagan AM et al (2015) Clinical utility of cerebrospinal fluid biomarkers in the diagnosis of early Alzheimer's disease. Alzheimers Dement 11:58–69. doi:10.1016/j.jalz.2014.02.004
68. Buchhave P, Minthon L, Zetterberg H et al (2012) Cerebrospinal fluid levels of β-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. Arch Gen Psychiatry 69:98–106. doi:10.1001/archgenpsychiatry.2011.155
69. Braak H, Zetterberg H, Del Tredici K, Blennow K (2013) Intraneuronal tau aggregation precedes diffuse plaque deposition, but amyloid-β changes occur before increases of tau in cerebrospinal fluid. Acta Neuropathol 126:631–641. doi:10.1007/s00401-013-1139-0
70. Tolboom N, van der Flier WM, Yaqub M et al (2009) Relationship of cerebrospinal fluid markers to 11C-PiB and 18F-FDDNP binding. J Nucl Med 50:1464–1470. doi:10.2967/jnumed.109.064360
71. Toledo JB, Bjerke M, Da X et al (2015) Nonlinear association between cerebrospinal fluid and florbetapir F-18 β-amyloid measures across the spectrum of alzheimer disease. JAMA Neurol 72:571–581. doi:10.1001/jamaneurol.2014.4829
72. Buerger K, Ewers M, Pirttilä T et al (2006) CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. Brain 129:3035–3041. doi:10.1093/brain/awl269
73. Sämgård K, Zetterberg H, Blennow K et al (2010) Cerebrospinal fluid total tau as a marker of Alzheimer's disease intensity. Int J Geriatr Psychiatry 25:403–410. doi:10.1002/gps.2353
74. Tarawneh R, Head D, Allison S et al (2015) Cerebrospinal fluid markers of neurodegeneration and rates of brain atrophy in early alzheimer disease. JAMA Neurol 72:656–665. doi:10.1001/jamaneurol.2015.0202
75. Jansen WJ, Ossenkoppele R, Knol DL et al (2015) Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. JAMA 313:1924–1938. doi:10.1001/jama.2015.4668
76. Ritchie C, Smailagic N, Noel-Storr AH et al (2014) Plasma and cerebrospinal fluid amyloid beta for the diagnosis of Alzheimer's disease dementia and other dementias in people with mild cognitive impairment (MCI). Cochrane Database Syst Rev 6:CD008782. doi:10.1002/14651858.CD008782.pub4
77. Mattsson N, Insel PS, Donohue M et al (2015) Predicting reduction of cerebrospinal fluid β-amyloid 42 in cognitively healthy controls. JAMA Neurol 72:554–560. doi:10.1001/jamaneurol.2014.4530
78. Schoonenboom NSM, Reesink FE, Verwey NA et al (2012) Cerebrospinal fluid markers for differential dementia diagnosis in a large memory clinic cohort. Neurology 78:47–54. doi:10.1212/WNL.0b013e31823ed0f0
79. Vos SJ, Xiong C, Visser PJ et al (2013) Preclinical Alzheimer's disease and its outcome: a longitudinal cohort study. Lancet Neurol 12:957–965. doi:10.1016/S1474-4422(13)70194-7
80. Slaets S, Le Bastard N, Theuns J et al (2013) Amyloid pathology influences aβ1-42 cerebrospinal fluid levels in dementia with lewy bodies. J Alzheimers Dis 35:137–146. doi:10.3233/JAD-122176
81. Koopman K, Le Bastard N, Martin J-J et al (2009) Improved discrimination of autopsy-confirmed Alzheimer's disease (AD) from non-AD dementias using CSF P-tau(181P). Neurochem Int 55:214–218. doi:10.1016/j.neuint.2009.02.017
82. Mattsson N, Andreasson U, Persson S et al (2013) CSF biomarker variability in the Alzheimer's Association quality control program. Alzheimers Dement 9:251–261. doi:10.1016/j.jalz.2013.01.010
83. Ferreira D, Rivero-Santana A, Perestelo-Pérez L et al (2014) Improving CSF biomarker' performance for predicting progression from mild cognitive impairment to Alzheimer's disease by considering different confounding factors: a meta-analysis. Front Aging Neurosci 6:287. doi:10.3389/fnagi.2014.00287
84. Yang Y, Cui M (2014) Radiolabeled bioactive benzoheterocycles for imaging β-amyloid plaques in Alzheimer's disease. Eur J Med Chem 87:703–721. doi:10.1016/j.ejmech.2014.10.012
85. Okamura N, Harada R, Furumoto S et al (2014) Tau PET imaging in Alzheimer's disease. Curr Neurol Neurosci Rep 14:500. doi:10.1007/s11910-014-0500-6
86. Harada R, Okamura N, Furumoto S, et al. (2015) 18F-THK5351: A Novel PET Radiotracer for Imaging Neurofibrillary Pathology in Alzheimer's Disease. J Nucl Med Nov 5. pii: jnumed.115.164848. [Epub ahead of print]
87. Castellani RJ, Perry G (2014) The complexities of the pathology–pathogenesis relationship in Alzheimer disease. Biochem Pharmacol 88:671–676. doi:10.1016/j.bcp.2014.01.009
88. Janssen B, Vugts DJ, Funke U et al (2015) Imaging of neuroinflammation in Alzheimer's disease, multiple sclerosis and stroke: recent developments in positron emission tomography. Biochim Biophys Acta. doi:10.1016/j.bbadis.2015.11.011