Review

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# Amyloid biomarkers in Alzheimer's disease

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**Aggregation of amyloid-β (Aβ) into oligomers, fibrils, and plaques is central in the molecular pathogenesis of Alzheimer's disease (AD), and is the main focus of AD drug development. Biomarkers to monitor Aβ metabolism and aggregation directly in patients are important for further detailed study of the involvement of Aβ in disease pathogenesis and to monitor the biochemical effect of drugs targeting Aβ in clinical trials. Furthermore, if anti-Aβ disease-modifying drugs prove to be effective clinically, amyloid biomarkers will be of special value in the clinic to identify patients with brain amyloid deposition at risk for progression to AD dementia, to enable initiation of treatment before neurodegeneration is too severe, and to monitor drug effects on Aβ metabolism or pathology to guide dosage. Two types of amyloid biomarker have been developed: Aβ-binding ligands for use in positron emission tomography (PET) and assays to measure Aβ42 in cerebrospinal fluid (CSF). In this review, we present the rationales behind these biomarkers and compare their ability to measure Aβ plaque load in the brain. We also review possible shortcomings and the need of standardization of both biomarkers, as well as their implementation in the clinic.**

## Amyloid in Alzheimer's disease

'Amyloid' is the term used for proteins that are misfolded into a cross β-sheet structure and thereby bind dyes such as Congo Red and Thioflavin T [1]. AD is one of the major amyloidoses, with two types of amyloid deposited in the brain: (i) Aβ forming aggregates in the form of plaques and cerebrovascular amyloid angiopathy (CAA); and (ii) tau protein, which forms neurofibrillary tangles, dystrophic neurites, and neuropil threads (reviewed in [2]). When not specified otherwise, we use the term 'amyloid' here to refer to Aβ pathology rather than tau pathology.

Research advances during the past two decades have resulted in detailed knowledge on disease mechanisms. Aβ is produced by the sequential cleavage of amyloid precursor protein (APP) by two enzymes, β-site APP-cleaving enzyme 1 (BACE1), also called β-secretase, and the γ-secretase complex (Figure 1). The prevailing hypothesis for AD pathogenesis is called the amyloid cascade hypothesis (Figure 2), posing that Aβ aggregation is the initiating mechanistic event, in which the different stages of aggregates, from soluble oligomers to insoluble fibrils in plaques, are believed to impair synaptic function and ultimately damage neurons, resulting in chronic neurodegeneration leading to cognitive impairment and finally dementia [3]. AD research advances have also generated a large number of drug candidates with potential disease-modifying effects. Based on the strong belief in the amyloid cascade hypothesis, researchers have placed an overwhelming focus on molecules targeting Aβ production and aggregation in AD drug development, and most drug candidates tested aim to inhibit Aβ toxicity by reducing further Aβ aggregation and plaque formation. These drug candidates include secretase inhibitors to lower Aβ production from APP, Aβ aggregation inhibitors to inhibit Aβ oligomerization or fibrillization, as well as active and passive Aβ immunotherapies designed to capture either soluble or aggregated Aβ, or both, which will be either degraded or cleared from the brain (reviewed in [4]).

Alarmingly, an increasing number of large Phase III clinical trials on Aβ targeting drugs have reported no beneficial effects on cognitive symptoms in patients with sporadic AD [5–7]. These discouraging reports have caused increasing concern in the AD research community that the amyloid cascade hypothesis eventually will be falsified, that is, that Aβ aggregation is just a bystander, and not the cause, of neurodegeneration in AD [8]. A more optimistic viewpoint is that there are several logical explanations for the trial 'failures', including that the trials enrolled

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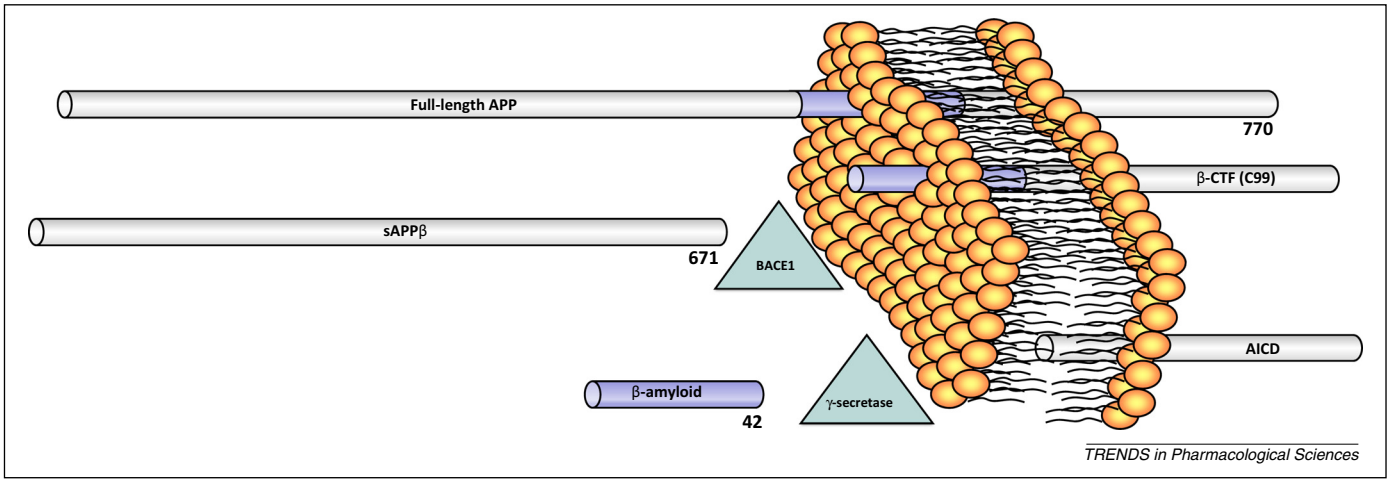


**Figure 1**. Generation of β-amyloid (Aβ) by metabolism of amyloid precursor protein (APP). APP is a transmembrane protein with a large extracellular N terminus. The Aβ domain is partly embedded in the plasma membrane, with 28 amino acids outside the membrane and 14 amino acids embedded in the membrane. APP is cleaved by β-site APP-cleaving enzyme 1 (BACE1), also called β-secretase, and a large soluble part (sAPPβ) is released. The remaining C-terminal fragment, called β-CTF or C99, is then cleaved by γ-secretase, releasing soluble Aβ. γ-secretase is an intramembranous protease complex, with four components, the active enzyme presenilin, together with nicastrin, presenilin enhancer (Pen-2), and anterior pharynx-defective (Aph-1).

patients with AD and dementia, which is probably too advanced a stage of the disease to enable this type of drug to show any effect on clinical symptoms, and that trial patients have been diagnosed based on purely clinical criteria, which are too nonspecific; thus, trials will comprise a cohort with only approximately 80% of enrolled patients having genuine AD pathology [9]. Both of these shortcomings call for diagnostic biomarkers to aid clinicians in making an early and accurate diagnosis. In future clinical trials on Aβ-targeting drugs, amyloid biomarkers would be especially valuable to confirm that enrolled patients do have Aβ pathology and, thus, the disease for which the drug is intended, which would increase the possibility of identifying a positive clinical effect of the drug [10]. If drug effects will be seen only in subjects with biomarker evidence of pathology, such biomarkers would also be useful to guide clinical decisions on whether to prescribe Aβ-targeting drugs, once these are available.

Another possible explanation for some of the trial failures is that poor drug candidates have been taken to Phase II and III clinical trials based on promising, but misleading data from preclinical drug development [2]. It has been common in AD drug development to test whether novel Aβ drug candidates reduce the Aβ plaque load in AD transgenic mice and, if so, take the drug into large and expensive clinical trials without examining whether target engagement can be verified in humans. There are numerous examples of failed trials (e.g., tarenflurbil and phenserine), which probably are due to the poor predictive power of these disease models [2,9]. For this reason, it is becoming increasingly common to apply theragnostic biomarkers during the early stages of AD drug development [5,11–13]. In this context, amyloid biomarkers applied in short small-scale trials to prove target engagement in Phase I proof-of-principle studies on healthy volunteers [9] or Phase II proof-of-concept studies in patients with AD [11] may be valuable in the selection of drug candidates and may improve success rates in late-stage clinical trials.

In contrast to most other neurodegenerative brain disorders, a set of biomarkers has been developed for the different pathogenic processes in AD and examined in a large number of clinical studies. These AD biomarkers include magnetic resonance imaging (MRI) of hippocampal or whole-brain atrophy, PET evaluation of glucose metabolism in cortical neurons and glial cells, CSF assays to measure tau protein, reflecting the intensity of the neuronal degeneration, and phosphorylated tau, reflecting the presence of tangles, and the two amyloid biomarkers amyloid PET and CSF Aβ42 (reviewed in [14]). In this review, we focus on the amyloid biomarkers, which have been much examined and reviewed individually, while an objective head-to-head comparison on their performance to measure Aβ plaque load or Aβ metabolism in the brain is lacking. We also discuss mechanistic differences between the amyloid biomarkers and their implementation in clinical trials and in the clinical routine management of patients with cognitive symptoms.

## Biomarkers for AD

According to the National Institutes of Health (NIH) Biomarkers Definitions Working Group, a biomarker is defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention' [15]. While the National Cancer Institute at the NIH defines a biomarker as 'a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease' (http://www.cancer.gov/dictionary?cdrid=45618), the World Health Organization (WHO) has a broader definition of biomarkers, which includes 'almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction.' (http://www.inchem.org/documents/ehc/ehc/ehc155.htm).