Update on biomarkers for amyloid pathology in Alzheimer's disease

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Abstract

At the centre of Alzheimer's disease (AD) pathogenesis is the aberrant aggregation of amyloid-β (Aβ) into oligomers, fibrils, and plaques. Effective monitoring of Aβ deposition directly in patients is essential to assist anti-Aβ therapeutics in target engagement and participant selection. In the advent of approved anti-Aβ therapeutics, biomarkers will become of fundamental importance in initiating treatments having disease modifying effects at the earliest stage. Two well established Aβ biomarkers are widely utilised: Aβ-binding ligands for positron emission tomography (PET) and immunoassays to measure Aβ42 in cerebrospinal fluid (CSF). In this review, we will discuss the current clinical, diagnostic, and research state of biomarkers for Aβ pathology. Furthermore, we will explore the current application of blood-based markers to assess Aβ pathology.

1. Introduction

Alzheimer's disease (AD) is the predominant cause of dementia, characterised by memory impairment and cognitive decline which severely impacts on daily living. AD is an age-related, progressive and irreversible neurodegenerative disorder that eventually leads to synaptic dysfunction and neuronal damage in regions of the brain essential for higher cognitive function. The socioeconomic consequence of AD is of great concern. There are more than 40 million worldwide dementia sufferers, mostly older than 60 years of age, a number which is expected to exceed 115 million by 2050 [1]. Alarmingly, these projected increases in dementia prevalence are proportionally much higher for developing countries than for Western Europe and the USA, which already have much older populations [2]. The global economic burden of dementia care is currently estimated at $604 billion and in a similar fashion to prevalence rates this figure is set to rapidly increase, with a projected 85% rise in costs within the next 20 years.

Still to this date the diagnosis of AD can only be confirmed, with any certainty, by histological examination of brain tissue at autopsy. In addition to gross cortical atrophy, widespread cellular degeneration and neuronal loss, pathological inspection should demonstrate substantial evidence of the classical pathological hallmarks of AD; intracellular neurofibrillary tangles (NFT) consisting of hyperphosphorylated tau protein and extracellular amyloid plaques. Plaques consist of extracellular aggregates of the 42 amino acid-long amyloid-β peptide (Aβ42), as well as N-terminally truncated forms thereof, a consequence of proteolytic cleavage of amyloid precursor protein (APP) [3]. Thus far, genetic (reviewed in [4]), biochemical (reviewed in [5]), pathological (reviewed in [6]) and cellular (reviewed in [7]) studies strongly indicate that the disparity in the production and effective clearance of Aβ is the fundamental event that leads to dementia [8, 9]. Alternative hypotheses suggest that Aβ is a necessary but not sufficient event leading to dementia and its accumulation is in parallel with other pathological events [2, 10, 11]. As reviewed below in greater detail, some researchers describe diffusible Aβ aggregates as causative agents in a linear model of AD pathogenesis [5], whereas others argue for a more complex model in which Aβ deposition and racemization are a match that eventually induce critical downstream changes, including tau phosphorylation, microglial activation and cerebrovascular changes, that together interact to cause neurodegeneration [7]. According to this model, the direction of causality may vary, at least in the sporadic form of the disease; for example, cerebrovascular changes may induce amyloidogenic APP-processing and Aβ aggregation, opening the possibility for vicious feed-forward loops.

Although an age-related disease, usually affecting people over the age of 65, it has been widely shown that the accumulation of Aβ plaques begins 15-20 years prior to clinical presentation [12] and have reached a plateau when the onset of cognitive, functional and behavioural decline occurs [13] making an early diagnosis problematic. The clinical diagnosis for probable AD is still largely based upon progressive memory impairment and a decline in cognitive state. Recent modifications to the diagnostic criteria have recognised the significant role of biomarkers assessing Aβ by *in vivo* positron emission tomography (PET) and cerebrospinal fluid (CSF) analysis for preclinical, prodromal and phenotypical AD [14] . In the same manner, Aβ imaging and fluid biomarkers are fundamental inclusion criterion to support disease-specific trials, targeting the amyloid hypothesis, in participant selection and proof of target engagement [15]. In this review, we will discuss the current state of biomarkers for Aβ pathology, including their diagnostic performance and how they can be used in clinical diagnostic and research settings to support or exclude a diagnosis of AD.

2. Aβ in Alzheimer's disease

Research advances during the past two decades have produced detailed knowledge on the underlying disease mechanism of AD, which is centred around the proteolytic cleavage of APP. Typically, the cleavage of APP occurs by the sequential action of α-secretase and γ-secretase to produce non-amyloidogenic products (the non-amyloidogenic pathway). The initial cleavage of α-secretase releases a large soluble ectodomain of APP (sAPPα) and a carboxyl terminal fragment (αCTF) [16] which is further cleaved by γ-secretase to produce the APP intracellular cytoplasmic domain (AICD) and soluble peptide P3 [17]. However, a proportion of APP is cleaved by β-secretase (BACE1) to produce sAPPβ and βCTF. The subsequent cleavage by γ-secretase produces Aβ fragments and AICD which are transported, via exocytosis, to the extracellular space [16, 18, 19]. The predominant forms of Aβ produced by the amyloidogenic pathway are Aβ38, Aβ40 and Aβ42, of which the latter has a tendency to aggregate, working as a seed for plaque deposition [20] but also Aβ-approximate peptides exist as neurotoxic dimers, oligomers, protofibrils and fibrils [21, 22]. Subsequent diffuse Aβ plaque formation leads to localised microglial activation, cytokine release, astrocytosis and a multi-protein inflammatory response [23, 24]. The "amyloid cascade hypothesis", has been the prevailing theory to explain AD pathogenesis [9]. This model suggests that Aβ aggregation acts as a pathological trigger leading to neuronal injury, NFT formation and cell death which underpin neurodegeneration and cognitive decline in AD [8]. Recent evidence suggests that the failure

or imbalance of Aβ clearance, rather than APP mismetabolism, is the major contributor to toxic deposition in the sporadic form of the disease [25, 26]. This would attribute mechanisms implicated in the degradation of Aβ (*i.e.*, neprilysin, insulin degrading enzyme (IDE), intracellular lysosomal degradation and microglia activation) of central importance in the development of Aβ pathology [18, 27].

In recent years, with the amyloid cascade hypothesis as the driving force, the development of disease modifying AD therapeutics has focused on targeting the production or clearance of Aβ with the aim to inhibit its toxicity. These include; secretase inhibitors to reduce the production of Aβ fragment by APP mismetabolism, Aβ aggregation inhibitors to prevent fibrillization and finally, active and passive immunotherapy which aim to capture soluble or aggregated Aβ for clearance from the brain (reviewed in [28, 29]). Disappointingly, findings from Phase III passive immunotherapy trials targeting Aβ have so far reported no cognitive benefit in mild-to-moderate sporadic AD. These worrying results have challenged the Aβ-centric theory, questioning if Aβ is simply a consequence and not a cause of AD pathogenesis. Nonetheless, these failures could also be attributed to logistical shortcomings; i) such trials recruited participants with established dementia, who are likely to be too far advanced in disease state to have a positive clinical benefit using this type of therapeutic; ii) recruitment criteria were purely based upon non-specific clinical assessments and therefore participants without the target pathology were likely to be included. In fact, the estimated contribution of Aβ-negative participants recruited into Aβ-targeted trials was estimated to be >25%, demonstrated by retrospective Aβ PET imaging [30]. This highlights the importance for a biomarker-driven participant selection which will also have major impact on clinical decisions when prescribing Aβ-targeting drugs once these are available. At this time, on-going Phase III passive immunotherapy trials (Aducanumab, Crenezumab, Gantenerumab and Solanezumab) are targeting preclinical and/or mild AD with all participants recruited with supporting Aβ biomarker evidence – these trials reach completion during 2019 and 2020. An additional application of Aβ biomarkers would be their implementation into proof-of-principle Phase I trials [31] and/or proof-of-concept Phase II clinical trials [32] to assist in the triage of potential drug candidates. Some researchers would point towards the premature acceleration of poor drug candidates as a significant contributor to the number of failures in AD therapeutics. It has been commonplace to examine the capabilities of an Aβ reducing molecule in transgenic AD mice models, and if successful, examine said drug in large and expensive clinical trials without

sufficient evidence of target engagement in humans. Consequently, biomarkers that detect and monitor biochemical effects of the drug are common in the early development of an AD drug [30].

3. Imaging Biomarkers for Aβ pathology

The distinctive strength of Aβ-imaging across a clinical spectrum is the spatial nature of the quantitative information it provides, which fluid biomarkers simply cannot offer [33]. The development of Pittsburgh Compound B (PiB), a derivate of thioflavin-T, for the *in vivo* assessment of Aβ pathology, transformed the approach to AD in both a clinical and research setting allowing for significantly more advanced investigations. With a high affinity to fibrillar Aβ plaques, carbon-11 (^{11}C) -labelled PiB has long been the most utilised Aβ tracer [34, 35]. The major disadvantage of an [11]C -labelled tracer, however, is the 20-minute half-life of the positron emitting isotope, limiting its use to centres with an onsite cyclotron. The advancement of "*second generation*" fluorine-18 (^{18}F) -labelled ligands, with a half-life of ^{18}F of 110 minutes, has fostered wider availability for Aβ PET, allowing for centralised synthesis and regional distribution. Encouragingly, Aβ specific ^{18}F tracers have replicated findings from ^{11}C-PiB studies in AD [36-39]. To date, three [18]F Aβ specific radiotracers have been approved by the FDA and EMA for clinical use; ^{18}F-flutemetamol (Vizamyl®), ^{18}F-florbetapir (Amyvid®) and ^{18}F-florbetapen (Neuraceq®). Criticisms of the ^{18}F tracers are an apparent inferior cortical-to-white matter ratio compared with ^{11}C-PiB and this has been attributed to a reduced overall cortical retention and higher non-specific white matter binding [40]. In preliminary studies, another ("*third generation*") Aβ PET tracer, ^{18}F-NAV4694 (formerly known as AZD-4694), has demonstrated the ability to visualise small Aβ cortical deposits at the early stage of the disease due to reduced white matter binding, comparable to ^{11}C-PiB, but with the advantage of a longer half-life. Furthermore, ^{18}F-NAV4694 and ^{11}C-PiB have been shown to have identical retention ranges and binding kinetics with an excellent correlation in cortical retention [40].

Aβ imaging has evolved from being exclusively a research tool to be integrated into clinical criteria for AD [26, 41], mild cognitive impairment (MCI) [42] and preclinical AD [26] plus at the forefront of anti-Aβ trials. Assistance from Aβ PET has ensured superior subject selection, assessing target binding and evaluating treatment response of participants [30]. Inevitably, the use of several different Aβ tracers across trials, clinics and research centres has highlighted a

multi-centre issue. The "Centiloid-scale" for the conversion of semi-quantitative estimates in Aβ PET imaging has been developed to produce a common quantitative output value to improve the application and comparison of Aβ imaging across different tracers. Currently, all ^{18}F tracers are being cross calibrated against ^{11}C-PiB [43].

Aβ imaging in Alzheimer's disease

Visually assessed and quantitative PET studies that use diverse Aβ tracers have consistently verified an increase in retention between AD and elderly controls, with the binding typically elevated in the frontal, cingulate, precuneus, striatum, parietal and lateral temporal cortices [12, 44-46]. Often the occipital, sensorimotor and mesial temporal cortices have a tendency to be less effected [45]. This regional distribution visualised by Aβ PET retention closely follows the sequence of Aβ burden highlighted by *post mortem* studies [47-49]. However, owing to the long delay between the onset of disease pathophysiology and clinical manifestation, it has been difficult to ascertain the earliest regions of Aβ deposition. Neuropathological *post mortem* staging of Aβ phases demonstrate the frontal, parietal, temporal and occipital lobes occupying the preliminary stages of deposition in AD [50]. On the other hand, studies employing PET have demonstrated that the temporo-parietal junction and the precuneus are regions of early Aβ accumulation, while the lateral and inferior temporal cortex are proposed for monitoring Aβ deposition in the symptomatic stages [45]. More recently, hierarchical regional staging of Aβ deposition further suggested that the temporobasal and frontomedial areas are first affected [51]. To address this long-standing question, Palmqvist and colleagues [52] identified regions of early accumulation by the novel stratification of individuals by both Aβ PET and CSF biomarkers. Cross-sectional studies had previously indicated that CSF Aβ42 changes precede changes in Aβ PET standard uptake value ratios (SUVR) [53-56] with a recent longitudinal data adding further confirmation [57]. This signifies that individuals with AD may be initially classified as "CSF+/PET-" before converting to "CSF+/PET+" at a later stage. Using this methodology, a significant increase in Aβ accumulation was observed in the precuneus, posterior cingulate cortex and orbitofrontal cortex when examining individuals with early accumulation (CSF+/PET-) against no measurable Aβ accumulation (CSF-/PET-) [52]. Furthermore, increased retention in the medial orbitofrontal and posterior cingulate cortex at an even earlier stage was observed in individuals who converted from CSF-/PET- to CSF+/PET- within two years in comparison to stable CSF-/PET-. This study specifically highlights regions within default mode network (DMN) and to a lesser extent the frontoparietal

network as being associated with the very earliest signs of Aβ accumulation [52] but more generally centres in the brain with higher levels of connectivity. Aβ accumulation within the DMN has been previously reported in cross-sectional studies [58, 59] and it is suggested that enhanced activity or the metabolic demand of neurones located within the DMN exacerbate Aβ deposition [52]. Longitudinal studies of asymptomatic mutation carriers (*PSEN1* and *APP*) have consistently demonstrated initial Aβ depositions occur distinctly in the striatum, which is not observed in sporadic AD [60-62].

There are rare exceptions to the general rule of increased Aβ PET retention in AD (*e.g.* Arctic APP [*APP*arc] mutation carriers). When considering autosomal-dominant AD (ADAD) mutation carriers, most cortical regions demonstrate increased PET retention except for the sensorimotor cortex [60, 61, 63-66]. It has been revealed that carriers of *APP*arc mutation do not have ligand retention as shown by low ^{11}C-PiB binding [67, 68] despite having Aβ pathology and positive AD-typical biomarkers (glucose metabolism, medial temporal lobe atrophy, and CSF Aβ and tau changes) are all present in the same subjects [68]. The absence of cortical ^{11}C-PiB retention is suggested to be caused by the amplified promotion of protofibril production due to a glutamic acid substitution in the Aβ peptide [69]. Consequently, neuropathological assessment of *APP*arc mutation carriers demonstrated a lack of Aβ dense-core plaques which would preclude positive Aβ-imaging [68]. These studies further support the notion that Aβ oligomers, not Aβ fibrils, are the pathogens that inflict synaptic damage and cognitive decline. The presence of the apolipoprotein E (*APOE*) ε4 allele is the most common genetic risk connected to sporadic AD and has been linked to earlier age of onset and a gene dose-dependent higher risk of developing AD [70, 71]. ApoE plays a role in Aβ metabolism and independent of clinical diagnosis *APOE* ε4 carriers present substantially higher Aβ deposition than non-carriers at an earlier age [72, 73]. Despite this relationship, *APOE* ε4 dosage does not affect the rate Aβ deposition over-time [13].

In the MCI population, Aβ imaging has shown that 50-70% display AD pathology [74, 75]. In several studies, a non-amnestic MCI diagnosis has consistently low Aβ binding and therefore a non-AD phenotype [12]. While there is a general agreement that Aβ burden only displays a weak correlation with memory impairment or disease severity, MCI patients demonstrate a consistent link between elevated Aβ retention and poorer episodic memory performance [76, 77].

In the healthy ageing population, it has been repeatedly shown that 25-35% exhibit Aβ PET SUVRs considered to be elevated Aβ burden, predominately in the prefrontal and posterior cingulate regions [78-80]. This figure is similar to what was previously reported by earlier *post mortem* studies [81, 82]. However, despite being cognitively normal, the presence of elevated Aβ burden put this group at far greater risk to disease conversion than individuals with low Aβ PET SUVRs [83, 84]. In fact, longitudinal Aβ imaging studies have shown that the probability of cognitively unimpaired individuals with low Aβ burden developing AD is exceptionally low [12, 85].

Aβ imaging has added conclusive evidence to indicate that Aβ deposition occurs decades before the clinical onset of AD [6, 13] and that cognitively healthy individuals with high Aβ burden are at significant risk to cognitive decline [40, 86]. It is this group of individuals that are the most likely to benefit from on-going therapies targeting Aβ clearance or production to halt the progression of AD [26]. Yet, the significant minority who exhibit Aβ burden who are absent of cognitive impairment, together with lack of strong relationship between Aβ burden (by PET) with cognition and atrophy in AD suggests that Aβ burden alone cannot cause neurodegeneration but indirectly via other processes such as the propagation of tau outside of the medial temporal lobe [87]. In addition to this, Aβ imaging which measures the fibrillar deposits may not correlate with cognitive decline as it has been suggested that soluble Aβ exists as the neurotoxic form.

Aβ imaging in other dementias

Aβ imaging has also been applied to a wide range of dementia-related conditions. While frontotemporolobar degeneration (FTLD) and Creutzfeldt-Jakob disease (CJD) exhibit no Aβ burden, a large number of dementia with Lewy bodies (DLB) patients (50-80%) demonstrate a cortical Aβ deposition distribution pattern similar to AD in both *post mortem* [88] and *in vivo* Aβ PET studies [89]. DLB patients with negative Aβ scans have been neuropathologically confirmed to have low Aβ burden [90]. Commonality in early cognitive signs with AD makes a differential diagnosis of DLB difficult and with a mixed DLB/AD pathology commonly found at *post mortem*, Aβ PET may be only useful in identifying the relatively rare pure DLB cases [91]. CAA is distinguished by localised Aβ deposition in small arterioles of the cerebral

cortex. Aβ ^{11}C-PiB has demonstrated a posterior retention in CAA individuals that is distinct from a pattern typically observed in sporadic AD [92]. Further to this, focal Aβ deposition has been shown to be helpful in highlighting new posterior microhaemorrhages that are usefully attributed to CAA patients [93]. Cortical Aβ is not usually consistent with non-dementia Parkinson's (PD), with no Aβ PET retention observed; conversely when dementia is present with PD (PDD) both vascular and cortical Aβ deposition are present. Aβ PET scans have been shown to determine which idiopathic normal-pressure hydrocephalus (iNPH) patients will benefit from shunt surgery by discriminating concomitant AD [94]. With Aβ not being a pathological trait in FTLD and the clinical onset of the disease being similar to other dementias, Aβ PET has been useful in the differential diagnosis between FTLD, different FTLD aphasias and AD [95]. Furthermore, the diagnostic performance of Aβ imaging has proven to be more accurate than FDG when diagnosing FTLD [96].

4. Cerebrospinal Fluid (CSF) Biomarkers for Aβ pathology, including Aβ response markers

The best-established fluid biomarkers for AD are CSF concentrations of total tau (T-tau), phosphorylated-tau (P-tau) and Aβ42 [97]. Consistently, a marked increase in both T-tau and P-tau in AD, accompanied by a decrease in CSF Aβ42 (reflecting deposition of the protein in the brain parenchyma) has been found [97]. In the heterogenous MCI population (where approximately 50% have AD) it has been shown that CSF Aβ42 changes (coupled with T-tau and P-tau changes) have already occurred in those individuals who progress to clinical AD [98]. Importantly, CSF Aβ is stable at follow-up when patients have reached dementia stage, indicating that Aβ levels do not change during the clinical phases of the disease. Hansson and colleagues [99], demonstrated that cognitively stable MCI patients do not have the typical AD biomarker profile, while progressive MCI patients could be identified with 95% sensitivity and 92% specificity against elderly controls and 83% specificity against stable MCI cases. Further to this, longitudinal studies in presymptomatic individuals have demonstrated that CSF Aβ42 changes are first detectable in the middle aged (45-54 years) and this is associated with later Aβ PET positivity and cognitive decline [100].During the past five years, it has been confirmed that CSF Aβ42 indeed is a reliable marker of Aβ (senile plaque) pathology in the brain (as determined at autopsy or through Aβ PET studies), especially when measured in a ratio with CSF Aβ40 [101]. The CSF Aβ42/Aβ40 ratio has been shown to have improved diagnostic

accuracy for Aβ pathology in AD as Aβ40 acts as a normalization factor for total Aβ production, which otherwise could produce false-positives or false-negatives if not compensated for [102].

In addition, several byproducts of the proteolytic cleavage of APP, generating a variety of Aβ species, can be measured in CSF [103-107]. However, these markers are still at preliminary investigation or show no association with AD while others have been shown to reflect target engagement in clinical trials [108, 109]. For CSF T-tau and P-tau, the interpretation is less clear; tau markers are robustly increased in AD CSF [97], but the exact mechanism remains unclear [110]. Some data suggest that neurons exposed to Alzheimer-associated factors such as Aβ may increase their secretion of both tau proteins [111]. Neurons who respond in this way may eventually accumulate tangle pathology and degenerate. In spite of these uncertainties, the diagnostic performance and clinical utility of CSF T-tau, P-tau and Aβ42 are undisputed: new diagnostic algorithms including CSF biomarkers have been formulated [112], automated routine clinical chemistry assays for the markers are now becoming available [113] and standardization efforts to harmonize assays are well underway; reference methods for Aβ42 have been formally certified by the Joint Committee for Traceability in Laboratory Medicine (JCTLM database accession numbers C11RMP9 and C12RMP1) [114, 115] and validated against amyloid PET [116], and a reference material for CSF Aβ42 was recently released (ERM®-DA480/IFCC, ERM®-DA481/IFCC and ERM®-DA482/IFCC). Similar work is ongoing for CSF tau biomarkers.

The concordance of CSF Aβ and Aβ PET

There is now conclusive evidence as assessed in large cohorts with disease and age-matched controls that a good inverse correlation between Aβ PET and CSF Aβ42 exists (reviewed in [101]), while no correlation between Aβ PET and CSF T-tau or P-tau has been reported [91]. Overall there is an agreement of ~88% in classifying Aβ+/- individuals, which is increased when assessing only clinically defined dementia (94%). A positive CSF investigation in the absence of a negative Aβ PET is more likely than the opposing. This is likely due to CSF changes occurring earliest and the disparity in measuring insoluble fibrillar Aβ versus soluble diffusible Aβ species [100, 117].

5. Blood-based biomarkers for Aβ pathology

Both imaging biomarkers and CSF measurement of Aβ work well to identify AD pathophysiology. Nonetheless, PET imaging is costly, and access is limited to specialised centres. Therefore, it is seemingly unlikely to be implemented widely in a general routine assessment for cognitive complaints. CSF sampling is becoming routine in neurology clinics and the cost for the AD CSF assays are much lower per patient than for PET scans. For some clinicians, a lumbar puncture may be regarded as complicated, time-consuming and/or invasive. Therefore, a blood-based measure for AD or AD pathophysiology would have significant practical advantages for clinicians as well as being more attainable to patients.

It has been difficult to establish robust blood biomarkers for Aβ pathology in AD. Aβ peptides can be measured in plasma but historically the correlation with AD and/or cerebral β-amyloidosis has been absent or weak (statistically significant but clinically meaningless) [97]. Plasma Aβ concentrations have been interpreted as potentially influenced by production in platelets and other extra-cerebral tissues and the measurements have been confounded by matrix effects from plasma proteins [118]. Furthermore, the vast majority of peripheral Aβ studies have concentrated on plasma/serum which do not take into account the substantial amounts of peptides bound to plasma proteins and blood cells [119]. However, this view is now starting to change. Recent mass spectrometric studies suggest that a ratio of a certain APP fragment (APP669-711) to Aβ42 or Aβ42/Aβ40 identifies Aβ-positive individuals with high sensitivity and specificity [120-122]. Nakamura and colleagues have described the accuracy of predicting ^{11}C-PiB in the AD/MCI and cognitively normal population at 91% and 87%, respectively [122]. These results are in line with earlier data obtained using the ultrasensitive Simoa technology by which the sample can be diluted to remove confounding matrix effects in the Aβ measurement [123]. In this study it was also shown that plasma Aβ42 was decreased in AD compared with MCI, subjective cognitive decline (SCD) and controls, whereas, the Aβ42/Aβ40 ratio could distinguish MCI from controls (please add ref here as well to make it very clear).

The most promising blood candidate for neurodegeneration to date is neurofilament light (NFL), which has shown to be reflective of CSF NFL [124, 125] and to be elevated in AD [125]. In addition, plasma NFL has been shown to be highest in AD and MCI cases with positive Aβ PET scans and reduced Aβ42 CSF biomarkers changes [125]. However, as NFL

concentration also correlates with Aβ-independent conditions (poor cognition, MRI atrophy and NFT pathology) and is increased in non-AD neurodegenerative diseases [126, 127], it is likely that plasma NFL is a measure of generalised and on-going neurodegeneration, not of Aβ pathology *per se*. Nonetheless, as the amyloid cascade hypothesis suggests that Aβ deposition is the main initiator behind events that result in neurodegeneration, therefore a clear link between elevations of NFL in response to Aβ burden, if present, can be made.

In the advent of large well characterized research cohorts that now include Aβ PET and/or CSF Aβ measures as routine, the opportunity to use an "endophenotype" approach to discover peripheral markers of Aβ pathology is ever increasing. Pilot data suggest associations of the concentrations of a number of plasma proteins (e.g., pancreatic polypeptide Y, IgM, chemokine ligand 13, interleukin 17, vascular cell adhesion protein 1, α2-macroglobulin, apolipoprotein A1, fibrinogen gamma chain, interleukins and complement proteins) and metabolites with Aβ burden in the brain [128-134]. However, these data should be interpreted with some caution, as they are derived from multi-marker panels and as a mechanistic understanding of the associations is currently lacking. The majority of these endophenotype studies have been confounded by the imbalance of AD/MCI individuals in the Aβ+ group. Only a small number of studies have focused purely on cognitively normal participants with Aβ burden [128, 131].

6. Future perspectives

Utilising the advantages Aβ imaging – CSF measures of Aβ accumulation seem to precede global Aβ PET changes (CSF+/PET-). However, spatial information of Aβ imaging has recently shown to highlight regions of early accumulation that later demonstrate CSF biomarkers changes (CSF-/PET-) [52]. This finding should have practical implications for the enrolment of participants to anti-Aβ trials where there is evidence of Aβ accumulation (early CSF changes or even earlier regional PET changes) but before neurodegeneration has set in. At the present time ^{11}C-PiB, and analogues thereof, detect Aβ plaques, mainly consisting of insoluble fibrils of Aβ. Insoluble Aβ does not correlate well with disease progression and soluble Aβ is a better marker of disease status. Therefore, aggregates rather than Aβ plaques have been the focus in recent diagnostics and therapeutics. This highlights the pressing need for an imaging agent that can visualise soluble Aβ aggregates. Recently, Sehlin and colleagues [135] have successfully demonstrated, in two mouse models, the use of an

antibody-based (mAb158) PET ligand selective for Aβ protofibrils for brain imaging. This has a potential implication in a number of neurodegenerative disorders as it demonstrates the feasibility of antibody-based *in vivo* imaging of proteins aggregates for such as α-synuclein, tau or TDP-43.

New biomarkers for Aβ plaque pathology - whilst neurons secrete Aβ species starting at amino acid 1 (the BACE1 cleavage site at APP), plaques contain a lot of Aβ (most of which ends at amino acid 42) that is N-terminally truncated (e.g., Aβ4-42) and pyroGlu-modified (e.g., pyroGluAβ3-42 or 11-42). Potentially, these Aβ species form in the plaques and could serve as fluid-based biomarkers for cerebral Aβ pathology. Ultrasensitive assays could potentially trace such brain-derived Aβ forms in the blood, which would solve the problem with contamination of the signal by Aβ1-42 derived from extracerebral tissues. There are also many Aβ forms that are either being produced from APP or have been found in brain tissue that could be explored as potential Aβ pathology or toxicity markers. These include N-terminally extended Aβ forms that span the BACE1 cleavage site of APP [103, 136-138] and Aβ forms that extend C-terminally towards the δ- and ε-cleavage sites at amino acids 45, 46, 48 and 49 [139]. As no reliable assays presently exist to measure these Aβ forms to assess their pathophysiological relevance and relation to disease state, they were jokingly referred to as "the dark matter of Aβ" at the Clinical Trials in Alzheimer's disease (CTAD) conference in 2017.

Blood-based challenges and approaches – There are considerable challenges ahead in developing a blood-based marker that can accurately reflect Aβ pathology and working groups are taking steps, taking lessons from the CSF community, to standardize pre-analytical variables [140]. Without a doubt, blood measures of tau and NFL are the leading blood-based candidates for AD but the view-point on peripheral Aβ species is changing and should be re-visited. The constant evolution of sensitive instrumentation targeting single putative candidates, guided by unbiased discovery methodologies, mean the prospect of clinically meaningful blood-based biomarker for Aβ pathology and/or AD is a genuine possibility. Novel targets that associate with Aβ plaque pathology have been suggested in abundance and should be taken with caution whilst a mechanistic link can be established. To date, blood-based studies using an Aβ pathology endophenotype design have almost exclusively utilised global

measures of Aβ-imaging as a surrogate marker. With recent evidence demonstrating the earliest signs of Aβ accumulation to be visible in the CSF [57] or regional specific PET [52], such studies should now be adopting these modalities as more accurate endophenotypes of early Aβ accumulation.

7. Executive summary

Aβ in Alzheimer's disease

- The "amyloid cascade hypothesis", has been the prevailing theory suggesting that Aβ aggregation acts as a pathological trigger leading to neurodegeneration.
- Development of AD therapeutics have focused on targeting the production or clearance of Aβ with the aim to inhibit its toxicity, prevent plaque build-up and/or plaque clearance.
- Accurate Aβ imaging and fluid biomarkers are critical to support disease-specific trials in participant selection and proof of target engagement

Imaging Biomarkers for Aβ pathology

- Aβ (^{11}C and ^{18}F) PET imaging has added conclusive evidence that Aβ deposition occurs decades before the clinical onset of AD. Aβ PET studies have consistently found an increase in retention between AD, MCI and elderly controls.
- Regions within default mode network (DMN) have been shown to be the very earliest cortical area of Aβ accumulation, even preceding CSF Aβ changes.

Cerebrospinal Fluid (CSF) Biomarkers for Aβ pathology, including Aβ response markers

- A decreased CSF Aβ42 is a reliable marker of Aβ (senile plaque) pathology and the CSF Aβ42/Aβ40 further increases diagnostic accuracy for Aβ pathology in AD.
- CSF Aβ42 positive biomarkers precede a positive global PET SUVR signal.
- Validated reference material for CSF Aβ42 has been certified and is now available.

Blood-based biomarkers for Aβ pathology

- Aβ species in blood have been widely shown to be not clinically relevant however; the measurement of the APP669-711/Aβ42 ratio has been shown to be predictive of Aβ plaque pathology with high accuracy.

Future perspectives

- Fluid-based ultrasensitive assays that target novel Aβ species found in plaques (N-terminally truncated or post-translational modified) could serve as more accurate markers Aβ pathology (particularly in blood).
- Better endophenotype stratification (regional PET or CSF Aβ42) may foster improved and early peripheral panels for Aβ burden.

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9. References

1. Prince, M., et al., *The global prevalence of dementia: a systematic review and metaanalysis.* Alzheimers Dement, 2013. **9**(1): p. 63-75 e2.
2. Scheltens, P., et al., *Alzheimer's disease.* Lancet, 2016. **388**(10043): p. 505-17.
3. Masters, C.L., et al., *Amyloid plaque core protein in Alzheimer disease and Down syndrome.* Proc Natl Acad Sci U S A, 1985. **82**(12): p. 4245-9.
4. Van Cauwenberghe, C., C. Van Broeckhoven, and K. Sleegers, *The genetic landscape of Alzheimer disease: clinical implications and perspectives.* Genet Med, 2016. **18**(5): p. 421-30.
5. Selkoe, D.J., *Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior.* Behav Brain Res, 2008. **192**(1): p. 106-13.
6. Jack, C.R., Jr., et al., *Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers.* Lancet Neurol, 2013. **12**(2): p. 207-16.
7. De Strooper, B. and E. Karran, *The Cellular Phase of Alzheimer's Disease.* Cell, 2016. **164**(4): p. 603-15.
8. Hardy, J.A. and G.A. Higgins, *Alzheimer's disease: the amyloid cascade hypothesis.* Science, 1992. **256**(5054): p. 184-5.
9. Selkoe, D.J. and J. Hardy, *The amyloid hypothesis of Alzheimer's disease at 25 years.* EMBO Mol Med, 2016. **8**(6): p. 595-608.
10. Chetelat, G., *Reply: The amyloid cascade is not the only pathway to AD.* Nat Rev Neurol, 2013. **9**(6): p. 356.
11. Pimplikar, S.W., *Reassessing the amyloid cascade hypothesis of Alzheimer's disease.* Int J Biochem Cell Biol, 2009. **41**(6): p. 1261-8.
12. Villemagne, V.L., et al., *Longitudinal assessment of Abeta and cognition in aging and Alzheimer disease.* Ann Neurol, 2011. **69**(1): p. 181-92.

13. Villemagne, V.L., et al., *Amyloid beta deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study.* Lancet Neurol, 2013. **12**(4): p. 357-67.

14. Dubois, B., et al., *Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria.* Lancet Neurol, 2014. **13**(6): p. 614-29.

15. Villemagne, V.L., et al., *Abeta-amyloid and Tau Imaging in Dementia.* Semin Nucl Med, 2017. **47**(1): p. 75-88.

16. Zhang, Y.W., et al., *APP processing in Alzheimer's disease.* Mol Brain, 2011. **4**: p. 3.

17. Chow, V.W., et al., *An overview of APP processing enzymes and products.* Neuromolecular Med, 2010. **12**(1): p. 1-12.

18. Murphy, M.P. and H. LeVine, 3rd, *Alzheimer's disease and the amyloid-beta peptide.* J Alzheimers Dis, 2010. **19**(1): p. 311-23.

19. Masters, C.L., et al., *Molecular mechanisms for Alzheimer's disease: implications for neuroimaging and therapeutics.* J Neurochem, 2006. **97**(6): p. 1700-25.

20. Iwatsubo, T., et al., *Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43).* Neuron, 1994. **13**(1): p. 45-53.

21. Walsh, D.M. and D.J. Selkoe, *A beta oligomers - a decade of discovery.* J Neurochem, 2007. **101**(5): p. 1172-84.

22. Ballard, C., et al., *Alzheimer's disease.* Lancet, 2011. **377**(9770): p. 1019-31.

23. Eikelenboom, P., et al., *Inflammatory mechanisms in Alzheimer's disease.* Trends Pharmacol Sci, 1994. **15**(12): p. 447-50.

24. McGeer, P.L. and E.G. McGeer, *The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases.* Brain Res Brain Res Rev, 1995. **21**(2): p. 195-218.

25. Mawuenyega, K.G., et al., *Decreased clearance of CNS beta-amyloid in Alzheimer's disease.* Science, 2010. **330**(6012): p. 1774.

26. Sperling, R.A., et al., *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.* Alzheimers Dement, 2011. **7**(3): p. 280-92.

27. Agostinho, P., R.A. Cunha, and C. Oliveira, *Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease.* Curr Pharm Des, 2010. **16**(25): p. 2766-78.

28. Jindal, H., et al., *Alzheimer disease immunotherapeutics: then and now.* Hum Vaccin Immunother, 2014. **10**(9): p. 2741-3.

29. Folch, J., et al., *Current Research Therapeutic Strategies for Alzheimer's Disease Treatment.* Neural Plast, 2016. **2016**: p. 8501693.

30. Salloway, S., et al., *Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease.* N Engl J Med, 2014. **370**(4): p. 322-33.

31. Blennow, K., H. Hampel, and H. Zetterberg, *Biomarkers in amyloid-beta immunotherapy trials in Alzheimer's disease.* Neuropsychopharmacology, 2014. **39**(1): p. 189-201.

32. Lannfelt, L., et al., *Safety, efficacy, and biomarker findings of PBT2 in targeting Abeta as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomised, placebo-controlled trial.* Lancet Neurol, 2008. **7**(9): p. 779-86.

33. Johnson, K.A., et al., *Brain imaging in Alzheimer disease.* Cold Spring Harb Perspect Med, 2012. **2**(4): p. a006213.

34. Cohen, A.D., et al., *Using Pittsburgh Compound B for in vivo PET imaging of fibrillar amyloid-beta.* Adv Pharmacol, 2012. **64**: p. 27-81.

35. Klunk, W.E., et al., *Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B.* Ann Neurol, 2004. **55**(3): p. 306-19.

36. Nelissen, N., et al., *Phase 1 study of the Pittsburgh compound B derivative 18F-flutemetamol in healthy volunteers and patients with probable Alzheimer disease.* J Nucl Med, 2009. **50**(8): p. 1251-9.

37. Vandenberghe, R., et al., *Amyloid PET in clinical practice: Its place in the multidimensional space of Alzheimer's disease.* Neuroimage Clin, 2013. **2**: p. 497-511.

38. Choi, S.R., et al., *Preclinical properties of 18F-AV-45: a PET agent for Abeta plaques in the brain.* J Nucl Med, 2009. **50**(11): p. 1887-94.

39. Wolk, D.A., et al., *Amyloid imaging in Alzheimer's disease: comparison of florbetapir and Pittsburgh compound-B positron emission tomography.* J Neurol Neurosurg Psychiatry, 2012. **83**(9): p. 923-6.

40. Rowe, C.C., et al., *Head-to-head comparison of 11C-PiB and 18F-AZD4694 (NAV4694) for beta-amyloid imaging in aging and dementia.* J Nucl Med, 2013. **54**(6): p. 880-6.

41. Dubois, B., et al., *Revising the definition of Alzheimer's disease: a new lexicon.* Lancet Neurol, 2010. **9**(11): p. 1118-27.

42. Albert, M.S., et al., *The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.* Alzheimers Dement, 2011. **7**(3): p. 270-9.

43. Klunk, W.E., et al., *The Centiloid Project: standardizing quantitative amyloid plaque estimation by PET.* Alzheimers Dement, 2015. **11**(1): p. 1-15 e1-4.

44. Jack, C.R., Jr., J.R. Barrio, and V. Kepe, *Cerebral amyloid PET imaging in Alzheimer's disease.* Acta Neuropathol, 2013. **126**(5): p. 643-57.

45. Villain, N., et al., *Regional dynamics of amyloid-beta deposition in healthy elderly, mild cognitive impairment and Alzheimer's disease: a voxelwise PiB-PET longitudinal study.* Brain, 2012. **135**(Pt 7): p. 2126-39.

46. Scheinin, N.M., et al., *Follow-up of [11C]PIB uptake and brain volume in patients with Alzheimer disease and controls.* Neurology, 2009. **73**(15): p. 1186-92.

47. Braak, H. and E. Braak, *Frequency of stages of Alzheimer-related lesions in different age categories.* Neurobiol Aging, 1997. **18**(4): p. 351-7.

48. Ikonomovic, M.D., et al., *Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease.* Brain, 2008. **131**(Pt 6): p. 1630-45.

49. Kadir, A., et al., *Positron emission tomography imaging and clinical progression in relation to molecular pathology in the first Pittsburgh Compound B positron emission tomography patient with Alzheimer's disease.* Brain, 2011. **134**(Pt 1): p. 301-17.

50. Thal, D.R., et al., *Phases of A beta-deposition in the human brain and its relevance for the development of AD.* Neurology, 2002. **58**(12): p. 1791-800.

51. Grothe, M.J., et al., *In vivo staging of regional amyloid deposition.* Neurology, 2017. **89**(20): p. 2031-2038.

52. Palmqvist, S., et al., *Earliest accumulation of beta-amyloid occurs within the default-mode network and concurrently affects brain connectivity.* Nat Commun, 2017. **8**(1): p. 1214.

53. Bateman, R.J., et al., *Clinical and biomarker changes in dominantly inherited Alzheimer's disease.* N Engl J Med, 2012. **367**(9): p. 795-804.

54. Blennow, K., H. Zetterberg, and A.M. Fagan, *Fluid biomarkers in Alzheimer disease.* Cold Spring Harb Perspect Med, 2012. **2**(9): p. a006221.

55. Fagan, A.M., et al., *Cerebrospinal fluid tau and ptau(181) increase with cortical amyloid deposition in cognitively normal individuals: implications for future clinical trials of Alzheimer's disease.* EMBO Mol Med, 2009. **1**(8-9): p. 371-80.

56. Mattsson, N., et al., *Independent information from cerebrospinal fluid amyloid-beta and florbetapir imaging in Alzheimer's disease.* Brain, 2015. **138**(Pt 3): p. 772-83.

57. Palmqvist, S., N. Mattsson, and O. Hansson, *Cerebrospinal fluid analysis detects cerebral amyloid-beta accumulation earlier than positron emission tomography.* Brain, 2016. **139**(Pt 4): p. 1226-36.

58. Sperling, R.A., et al., *Amyloid deposition is associated with impaired default network function in older persons without dementia.* Neuron, 2009. **63**(2): p. 178-88.

59. Myers, N., et al., *Within-patient correspondence of amyloid-beta and intrinsic network connectivity in Alzheimer's disease.* Brain, 2014. **137**(Pt 7): p. 2052-64.

60. Klunk, W.E., et al., *Amyloid deposition begins in the striatum of presenilin-1 mutation carriers from two unrelated pedigrees.* J Neurosci, 2007. **27**(23): p. 6174-84.

61. Villemagne, V.L., et al., *High striatal amyloid beta-peptide deposition across different autosomal Alzheimer disease mutation types.* Arch Neurol, 2009. **66**(12): p. 1537-44.

62. Koivunen, J., et al., *PET amyloid ligand [11C]PIB uptake shows predominantly striatal increase in variant Alzheimer's disease.* Brain, 2008. **131**(Pt 7): p. 1845-53.

63. Benzinger, T.L., et al., *Regional variability of imaging biomarkers in autosomal dominant Alzheimer's disease.* Proc Natl Acad Sci U S A, 2013. **110**(47): p. E4502-9.

64. Knight, W.D., et al., *Carbon-11-Pittsburgh compound B positron emission tomography imaging of amyloid deposition in presenilin 1 mutation carriers.* Brain, 2011. **134**(Pt 1): p. 293-300.

65. Remes, A.M., et al., *Carbon 11-labeled pittsburgh compound B positron emission tomographic amyloid imaging in patients with APP locus duplication.* Arch Neurol, 2008. **65**(4): p. 540-4.

66. Scholl, M., et al., *Glucose metabolism and PIB binding in carriers of a His163Tyr presenilin 1 mutation.* Neurobiol Aging, 2011. **32**(8): p. 1388-99.

67. Tomiyama, T., et al., *A new amyloid beta variant favoring oligomerization in Alzheimer's-type dementia.* Ann Neurol, 2008. **63**(3): p. 377-87.

68. Scholl, M., et al., *Low PiB PET retention in presence of pathologic CSF biomarkers in Arctic APP mutation carriers.* Neurology, 2012. **79**(3): p. 229-36.

69. Nilsberth, C., et al., *The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation.* Nat Neurosci, 2001. **4**(9): p. 887-93.

70. Papassotiropoulos, A., et al., *Genetics, transcriptomics, and proteomics of Alzheimer's disease.* J Clin Psychiatry, 2006. **67**(4): p. 652-70.

71. Farrer, L.A., et al., *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, 1997. **278**(16): p. 1349-56.

72. Ossenkoppele, R., et al., *Prevalence of amyloid PET positivity in dementia syndromes: a meta-analysis.* JAMA, 2015. **313**(19): p. 1939-49.

73. Lautner, R., et al., *Preclinical effects of APOE epsilon4 on cerebrospinal fluid Abeta42 concentrations.* Alzheimers Res Ther, 2017. **9**(1): p. 87.

74. Forsberg, A., et al., *PET imaging of amyloid deposition in patients with mild cognitive impairment.* Neurobiol Aging, 2008. **29**(10): p. 1456-65.

75. Kemppainen, N.M., et al., *PET amyloid ligand [11C]PIB uptake is increased in mild cognitive impairment.* Neurology, 2007. **68**(19): p. 1603-6.

76. Mormino, E.C., et al., *Episodic memory loss is related to hippocampal-mediated beta-amyloid deposition in elderly subjects.* Brain, 2009. **132**(Pt 5): p. 1310-23.

77.   Ong, K., et al., *(18)F-florbetaben Abeta imaging in mild cognitive impairment.* Alzheimers Res Ther, 2013. **5**(1): p. 4.

78.   Villemagne, V.L., et al., *Abeta deposits in older non-demented individuals with cognitive decline are indicative of preclinical Alzheimer's disease.* Neuropsychologia, 2008. **46**(6): p. 1688-97.

79.   Rowe, C.C., et al., *Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging.* Neurobiol Aging, 2010. **31**(8): p. 1275-83.

80.   Aizenstein, H.J., et al., *Frequent amyloid deposition without significant cognitive impairment among the elderly.* Arch Neurol, 2008. **65**(11): p. 1509-17.

81.   Davies, L., et al., *A4 amyloid protein deposition and the diagnosis of Alzheimer's disease: prevalence in aged brains determined by immunocytochemistry compared with conventional neuropathologic techniques.* Neurology, 1988. **38**(11): p. 1688-93.

82.   Morris, J.C. and J.L. Price, *Pathologic correlates of nondemented aging, mild cognitive impairment, and early-stage Alzheimer's disease.* J Mol Neurosci, 2001. **17**(2): p. 101-18.

83.   Sperling, R.A., et al., *Amyloid deposition detected with florbetapir F 18 ((18)F-AV-45) is related to lower episodic memory performance in clinically normal older individuals.* Neurobiol Aging, 2013. **34**(3): p. 822-31.

84.   Jagust, W., *Is amyloid-beta harmful to the brain? Insights from human imaging studies.* Brain, 2016. **139**(Pt 1): p. 23-30.

85.   Rowe, C.C., et al., *Predicting Alzheimer disease with beta-amyloid imaging: results from the Australian imaging, biomarkers, and lifestyle study of ageing.* Ann Neurol, 2013. **74**(6): p. 905-13.

86.   Resnick, S.M., et al., *Longitudinal cognitive decline is associated with fibrillar amyloid-beta measured by [11C]PiB.* Neurology, 2010. **74**(10): p. 807-15.

87.   Stancu, I.C., et al., *Models of beta-amyloid induced Tau-pathology: the long and "folded" road to understand the mechanism.* Mol Neurodegener, 2014. **9**: p. 51.

88.   Lippa, C.F., et al., *DLB and PDD boundary issues: diagnosis, treatment, molecular pathology, and biomarkers.* Neurology, 2007. **68**(11): p. 812-9.

89.   Gomperts, S.N., et al., *Imaging amyloid deposition in Lewy body diseases.* Neurology, 2008. **71**(12): p. 903-10.

90.   Ikonomovic, M.D., et al., *Early AD pathology in a [C-11]PiB-negative case: a PiB-amyloid imaging, biochemical, and immunohistochemical study.* Acta Neuropathol, 2012. **123**(3): p. 433-47.

91.   Villemagne, V.L. and G. Chetelat, *Neuroimaging biomarkers in Alzheimer's disease and other dementias.* Ageing Res Rev, 2016. **30**: p. 4-16.

92.   Johnson, K.A., et al., *Imaging of amyloid burden and distribution in cerebral amyloid angiopathy.* Ann Neurol, 2007. **62**(3): p. 229-34.

93.   Farid, K., A. Charidimou, and J.C. Baron, *Amyloid positron emission tomography in sporadic cerebral amyloid angiopathy: A systematic critical update.* Neuroimage Clin, 2017. **15**: p. 247-263.

94.   Jang, H., et al., *Prognostic value of amyloid PET scan in normal pressure hydrocephalus.* J Neurol, 2017.

95.   Rabinovici, G.D., et al., *Abeta amyloid and glucose metabolism in three variants of primary progressive aphasia.* Ann Neurol, 2008. **64**(4): p. 388-401.

96.   Rabinovici, G.D., et al., *Amyloid vs FDG-PET in the differential diagnosis of AD and FTLD.* Neurology, 2011. **77**(23): p. 2034-42.

97.   Olsson, B., et al., *CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis.* Lancet Neurol, 2016. **15**(7): p. 673-684.

98. Blennow, K., *A Review of Fluid Biomarkers for Alzheimer's Disease: Moving from CSF to Blood.* Neurol Ther, 2017. **6**(Suppl 1): p. 15-24.

99. Hansson, O., et al., *Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study.* Lancet Neurol, 2006. **5**(3): p. 228-34.

100. Sutphen, C.L., et al., *Longitudinal Cerebrospinal Fluid Biomarker Changes in Preclinical Alzheimer Disease During Middle Age.* JAMA Neurol, 2015. **72**(9): p. 1029-42.

101. Blennow, K., et al., *Amyloid biomarkers in Alzheimer's disease.* Trends Pharmacol Sci, 2015. **36**(5): p. 297-309.

102. Lewczuk, P., et al., *Amyloid-beta 42/40 cerebrospinal fluid concentration ratio in the diagnostics of Alzheimer's disease: validation of two novel assays.* J Alzheimers Dis, 2015. **43**(1): p. 183-91.

103. Brinkmalm, G., et al., *An online nano-LC-ESI-FTICR-MS method for comprehensive characterization of endogenous fragments from amyloid beta and amyloid precursor protein in human and cat cerebrospinal fluid.* J Mass Spectrom, 2012. **47**(5): p. 591-603.

104. Portelius, E., et al., *Novel abeta isoforms in Alzheimer's disease - their role in diagnosis and treatment.* Curr Pharm Des, 2011. **17**(25): p. 2594-602.

105. Zetterberg, H., et al., *Elevated cerebrospinal fluid BACE1 activity in incipient Alzheimer disease.* Arch Neurol, 2008. **65**(8): p. 1102-7.

106. Holtta, M., et al., *Evaluating amyloid-beta oligomers in cerebrospinal fluid as a biomarker for Alzheimer's disease.* PLoS One, 2013. **8**(6): p. e66381.

107. Savage, M.J., et al., *A sensitive abeta oligomer assay discriminates Alzheimer's and aged control cerebrospinal fluid.* J Neurosci, 2014. **34**(8): p. 2884-97.

108. Portelius, E., et al., *beta-site amyloid precursor protein-cleaving enzyme 1(BACE1) inhibitor treatment induces Abeta5-X peptides through alternative amyloid precursor protein cleavage.* Alzheimers Res Ther, 2014. **6**(5-8): p. 75.

109. Portelius, E., et al., *A novel Abeta isoform pattern in CSF reflects gamma-secretase inhibition in Alzheimer disease.* Alzheimers Res Ther, 2010. **2**(2): p. 7.

110. Zetterberg, H., *Review: Tau in biofluids - relation to pathology, imaging and clinical features.* Neuropathol Appl Neurobiol, 2017.

111. Maia, L.F., et al., *Changes in Amyloid-beta and Tau in the Cerebrospinal Fluid of Transgenic Mice Overexpressing Amyloid Precursor Protein.* Sci Transl Med, 2013. **5**(194): p. 194re2.

112. Dubois, B., et al., *Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria.* Lancet Neurol, 2014. **13**(6): p. 614-629.

113. Bittner, T., et al., *Technical performance of a novel, fully automated electrochemiluminescence immunoassay for the quantitation of beta-amyloid (1-42) in human cerebrospinal fluid.* Alzheimers Dement, 2015.

114. Korecka, M., et al., *Qualification of a surrogate matrix-based absolute quantification method for amyloid-beta(4)(2) in human cerebrospinal fluid using 2D UPLC-tandem mass spectrometry.* J Alzheimers Dis, 2014. **41**(2): p. 441-51.

115. Leinenbach, A., et al., *Mass Spectrometry-Based Candidate Reference Measurement Procedure for Quantification of Amyloid-beta in Cerebrospinal Fluid.* Clin Chem, 2014.

116. Pannee, J., et al., *Reference measurement procedure for CSF amyloid beta (Abeta)1-42 and the CSF Abeta1-42 /Abeta1-40 ratio - a cross-validation study against amyloid PET.* J Neurochem, 2016. **139**(4): p. 651-658.

117. Toledo, J.B., et al., *Nonlinear Association Between Cerebrospinal Fluid and Florbetapir F-18 beta-Amyloid Measures Across the Spectrum of Alzheimer Disease.* JAMA Neurol, 2015. **72**(5): p. 571-81.

118. Zetterberg, H., *Plasma amyloid beta-quo vadis?* Neurobiol Aging, 2015. **36**(10): p. 2671-3.

119. Pesini, P., et al., *Reliable Measurements of the beta-Amyloid Pool in Blood Could Help in the Early Diagnosis of AD.* Int J Alzheimers Dis, 2012. **2012**: p. 604141.

120. Kaneko, N., et al., *Novel plasma biomarker surrogating cerebral amyloid deposition.* Proc Jpn Acad Ser B Phys Biol Sci, 2014. **90**(9): p. 353-64.

121. Ovod, V., et al., *Amyloid beta concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis.* Alzheimers Dement, 2017.

122. Nakamura, A., et al., *High performance plasma amyloid-beta biomarkers for Alzheimer's disease.* Nature, 2018. **554**(7691): p. 249-254.

123. Janelidze, S., et al., *Plasma beta-amyloid in Alzheimer's disease and vascular disease.* Sci Rep, 2016. **6**: p. 26801.

124. Gisslen, M., et al., *Plasma Concentration of the Neurofilament Light Protein (NFL) is a Biomarker of CNS Injury in HIV Infection: A Cross-Sectional Study.* EBioMedicine, 2016. **3**: p. 135-40.

125. Mattsson, N., et al., *Association of Plasma Neurofilament Light With Neurodegeneration in Patients With Alzheimer Disease.* JAMA Neurol, 2017. **74**(5): p. 557-566.

126. Rohrer, J.D., et al., *Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia.* Neurology, 2016. **87**(13): p. 1329-36.

127. Rojas, J., et al., *Plasma neurofilament light chain predicts disease progression in progressive supranuclear palsy.* Journal of Neurochemistry, 2016. **138**: p. 241-242.

128. Thambisetty, M., et al., *Proteome-based plasma markers of brain amyloid-beta deposition in non-demented older individuals.* J Alzheimers Dis, 2010. **22**(4): p. 1099-109.

129. Voyle, N., et al., *Blood Protein Markers of Neocortical Amyloid-beta Burden: A Candidate Study Using SOMAscan Technology.* J Alzheimers Dis, 2015. **46**(4): p. 947-61.

130. Burnham, S.C., et al., *Predicting Alzheimer disease from a blood-based biomarker profile: A 54-month follow-up.* Neurology, 2016. **87**(11): p. 1093-101.

131. Westwood, S., et al., *Blood-Based Biomarker Candidates of Cerebral Amyloid Using PiB PET in Non-Demented Elderly.* J Alzheimers Dis, 2016. **52**(2): p. 561-72.

132. Ashton, N.J., et al., *Blood protein predictors of brain amyloid for enrichment in clinical trials?* Alzheimers Dement (Amst), 2015. **1**(1): p. 48-60.

133. Pedrini, S., et al., *A blood-based biomarker panel indicates IL-10 and IL-12/23p40 are jointly associated as predictors of beta-amyloid load in an AD cohort.* Sci Rep, 2017. **7**(1): p. 14057.

134. Voyle, N., et al., *Blood metabolite markers of neocortical amyloid-beta burden: discovery and enrichment using candidate proteins.* Transl Psychiatry, 2016. **6**: p. e719.

135. Sehlin, D., et al., *Antibody-based PET imaging of amyloid beta in mouse models of Alzheimer's disease.* Nat Commun, 2016. **7**: p. 10759.

136. Portelius, E., et al., *Identification of novel APP/Abeta isoforms in human cerebrospinal fluid.* Neurodegener Dis, 2009. **6**(3): p. 87-94.

137. Portelius, E., et al., *Mass spectrometric characterization of amyloid-beta species in the 7PA2 cell model of Alzheimer's disease.* J Alzheimers Dis, 2013. **33**(1): p. 85-93.

138.   Willem, M., et al., *eta-Secretase processing of APP inhibits neuronal activity in the hippocampus.* Nature, 2015. **526**(7573): p. 443-7.
139.   Lessard, C.B., et al., *gamma-Secretase Modulators and APH1 Isoforms Modulate gamma-Secretase Cleavage but Not Position of epsilon-Cleavage of the Amyloid Precursor Protein (APP).* PLoS One, 2015. **10**(12): p. e0144758.
140.   O'Bryant, S.E., et al., *Guidelines for the standardization of preanalytic variables for blood-based biomarker studies in Alzheimer's disease research.* Alzheimers Dement, 2015. **11**(5): p. 549-60.

10. Reference annotations

** Palmqvist, S., N. Mattsson, and O. Hansson, *Cerebrospinal fluid analysis detects cerebral amyloid-beta accumulation earlier than positron emission tomography.* Brain, 2016. **139(Pt 4): p. 1226-36 – cerebrospinal fluid amyloid-β42 becomes abnormal in the earliest stages of Alzheimer's disease, before amyloid positron emission tomography and before neurodegeneration starts.

Palmqvist, S., et al., *Earliest accumulation of beta-amyloid occurs within the default-mode network and concurrently affects brain connectivity.* Nat Commun, 2017. **8(1): p. 1214 – Aβ fibrils start to accumulate within certain parts of the DMN in preclinical AD before CSF Aβ become present.

*Sutphen, C.L., et al., *Longitudinal Cerebrospinal Fluid Biomarker Changes in Preclinical Alzheimer Disease During Middle Age.* JAMA Neurol, 2015. **72**(9): p. 1029-42 – Longitudinal CSF biomarker patterns consistent with AD are first detectable during early middle age and are associated with later amyloid positivity and cognitive decline

* Janelidze, S., et al., *Plasma beta-amyloid in Alzheimer's disease and vascular disease.* Sci Rep, 2016. **6**: p. 26801 – Using the ultra senstive digital ELISA (Simoa) plasma levels of Aβ42 and Aβ40 were reduced in AD dementia compared with all other diagnostic groups

* Nakamura, A., et al., *High performance plasma amyloid-beta biomarkers for Alzheimer's disease.* Nature, 2018. **554**(7691): p. 249-254 – This articles demonstrates with high accuracy using a validated IP-MS method to predcit Aβ burden using APP699-711/Aβ42 ratio.