The use of CSF biomarkers to measure change in neurodegeneration in Alzheimer's disease clinical trials

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Abstract

Introduction

All recent phase 3 trials of potentially disease-modifying therapies for Alzheimer's disease (AD) have so far failed. Potential reasons include enrolling subjects whose disease is too advanced or who do not have AD pathology, or simply incorrect drug targets. The goal of disease-modifying AD trials is to halt the progress of neuronal damage and death and this can be assessed *in vivo* is using CSF biomarkers.

Area covered

We conducted a literature search of the use of CSF biomarkers in disease-modifying AD clinical trials using PubMed. We show that CSF biomarkers have only sparsely been used as outcome measures, and where they have, only in small subsets of patients. No clinical trials have yet showed any substantial effects on CSF biomarkers of neurodegeneration.

Expert commentary

In future trials, we advocate that CSF biomarkers be used more extensively to optimize the chance of detecting positive drug effects. This includes the identification of potential AD patients - already in the early prodromal stage – for inclusion, for stratification, as readout i.e. proximity markers for changes in axonal/neurodegeneration between treatment and placebo groups – this also enables proof of principle verification in the discovery/dose finding phase, and for monitoring of side effects.

Introduction

Alzheimer's disease (AD) is typically characterized by progressive memory loss, difficulty in planning and execution of everyday activities, language problems, disorientation and changes

in personality. AD is the most common neurodegenerative disease, accounting for 60-80% of all cases with dementia. The prevalence of AD in populations older than 65 years is approximately 11%, rises with age, with a lifetime risk of 10–20% [1-3]. The pathological hallmarks of AD, first described by Alois Alzheimer in 1907, are the presence of senile plaques now known to contain the 42 amino acid form of amyloid-β (Aβ42) and neurofibrillary tangles shown to be formed from the aggregation of a hyperphosphorylated form of the microtubule-associated protein tau [1,4,5]. The dominant hypothesis of AD pathogenesies – the amyloid hypothesis – suggests that the disease process is initiated by β-amyloid deposition, which leads to tau phosphorylation and tangle formation and subsequently neuronal cell death [6]. This hypothesis has guided the development of most drugs aiming to modify the pathology of AD in clinical trials. In this review, we give an updated account and considered critique on how CSF biomarkers have been used in attempts to detect beneficial effects of disease-modifying drug candidates on the neurodegenerative process in AD.

CSF biomarkers for core pathological changes in AD

Total tau

Total tau (T-tau), measured using assays with antibodies against mid-domain non-phosphorylated epitopes of tau, can be used as a general marker of neuroaxonal degeneration/injury in AD. AD patients have increased CSF T-tau concentrations [7], and the higher the increase, the more intense neurodegenerative process [8]. However, CSF T-tau increase is not specific for AD, and is for example seen in Creutzfeldt-Jakob disease (CJD) [9] and following stroke [10].

Phosphorylated tau

Phosphorylated tau (P-tau) is the main component of neurofibrillary tangles [11]. Tau can be phosphorylated at many different epitopes but the most commonly used assays are those specific for tau phosphorylated at amino acid 181 or 231. CSF levels of P-tau are increased in AD [7] and correlate with cognitive decline in patients with mild cognitive impairment (MCI) and with neocortical neurofibrillary pathology in AD [12]. Increased CSF levels of P-tau are associated with rapid progression of MCI to AD [13] and also the intensity of AD [14].

Amyloid β

Several lines of evidence link the 4 kDa form of beta-amyloid (Aβ42) to AD. First, Aβ42, generated by the sequential cleavage of the amyloid precursor protein (APP) by BACE1 and γ-secretase, is highly aggregation prone and is the main component of senile plaques which are characteristic for AD [15,16]. Second, several mutations in the *APP* gene result in familial AD [17]. Third, a number of mutations in the presenilin 1 and 2 genes that are part of the γ-secretase complex also results in familial AD [18,19]. Fourth, patients with Down syndrome due to trisomy 21 where the *APP* gene is localized develop AD at an early age [20]. AD is associated with low CSF levels of Aβ42, which is thought to reflect deposition of the protein in the brain parenchyma [21]; low levels of Aβ42 can be used not only to aid in AD diagnosis [22], but also to predict conversion from MCI to AD [7,23].

Another form of beta-amyloid is Aβ40 which is the predominant form of beta-amyloid in CSF. Aβ40 is identical to Aβ42 except for two less amino acids at the C-terminal end. There are no differences in CSF Aβ40 levels between AD and controls [7] but Aβ40 can be used as an estimate of Aβ production in an individual. Thus, the ratio between Aβ42/Aβ40 is more informative in AD since Aβ42 can be naturally low in low producers without amyloid pathology, which would then be reflected in a normal Aβ42/Aβ40 ratio [24].

CSF biomarkers of axonal and synaptic degeneration in AD

Neurofilament light protein

Another CSF biomarker for axonal degeneration is neurofilament light protein (NFL), a structural protein in long axons [25]. CSF NFL concentrations are increased in AD [7] and especially in patients with rapid disease progress [26]. NFL is however not specific for AD; the highest concentrations are seen in FTD and vascular dementia (VaD) [27-29] as recently confirmed in a large retrospective analysis of data from the Swedish Dementia Registry [30]; and NFL is also elevated in atypical parkinsonian disorders [31,32] and motor neuron disease [33]. As with T-tau, the highest CSF concentrations of NFL are seen in CJD [34,35].

Neurogranin

Neurogranin (Ng) is a neural-enriched dendritic protein involved in long-term potentiation of synapses, particularly so in the hippocampus and basal forebrain. Recently, several independent studies have shown that the CSF concentrations of Ng are increased in AD [36-40], but not in other neurodegenerative disorders [41], and that Ng predicts future cognitive decline, brain atrophy and reduction in glucose metabolism in prodromal disease stages [42,43]. Currently, CSF Ng is the most evaluated CSF biomarker for synapse loss or dysfunction in AD, although there are other promising markers (e.g. SNAP-25, Rab3A) in various stages of development [44,45].

Clinical trials with CSF biomarker data

AN1792

The first clinical trial of active Aβ immunization was with AN1792 consisting of full length aggregated Aβ42 combined with the QS-21 adjuvant. In this phase 2 trial, 372 AD patients with AD (mini-mental state examination (MMSE) score 15-26) were included. Patients were

randomized (4:1) to receive an intramuscular injection of 225 μg AN1792 or placebo at day 0, month 1 and month 3. The study was designed to include injections at month 6, 9 and 12 but was discontinued due to adverse events, with a 6% rate of meningoencephalitis [46]. There were no effects on memory or cognition in this shortened trial. CSF biomarkers were analyzed in a subset of patients; 11 antibody responders (anti-AN1792 IgG titer ≥1:2,200) and 10 in the placebo group. There were no significant changes in CSF Aβ42 between groups, but there was a significant decrease in CSF T-tau towards normal levels in antibody responders vs. placebo.

Bapineuzumab

Bapineuzumab is the humanized form of the mouse monoclonal antibody 3D6 that recognizes the first five amino acids of Aβ42 [47]. Two multicenter phase 2 studies of bapineuzumab (201 and 202) were performed. The 201 study included 234 AD patients with an MMSE score of 16-26 randomised to bapineuzumab (n=124) or placebo (n=110). Bapineuzumab was dosed at 0.15, 0.5, 1.0 or 2.0 mg/kg at six occasions, 13 weeks apart, and patients were followed for 78 weeks [48]. The 202 study comprised 28 AD patients with an MMSE score of 18-26; 20 patients received 0.5, 1.0 or 2.0 mg/kg of bapineuzumab and 8 patients received placebo [49]. There was no treatment effect on cognition in either of these studies. CSF biomarkers were analyzed in a combined subset of these two studies. In total, 46 patients had paired CSF data at baseline and at week 54; 27 had received bapineuzumab and 19 placebo [50]. T-tau decreased by 72.3 pg/ml (from 811.3±466.3 to 739±336.8) in the bapineuzumb group compared to 5.6 pg/ml (from 1077.9±935.1 to 1072.4±972.8 pg/ml) in the placebo group (P=0.03). A significant difference in decrease in P-tau was also observed between the bapineuzumab group (baseline: 97.0±41.3 pg/ml, week 54: 87.1±31.0 pg/ml) compared to the placebo (baseline: 105.3±49.8 pg/ml, week 54: 102.7±47.8 pg/ml), e.g. -9.9 vs -2.6 pg/ml

(P=0.001). However, there was no difference in change between baseline and week 54 for Aβ42 (-10.5 vs -6.1 pg/ml for bapineuzumab vs. placebo, P=0.44).

Bapineuzumab has since been tested in two large multicenter phase 3 studies – one of *APOE* ε4 positive individuals (carriers), and one of *APOE* ε4 non-carriers. In the carrier study, 1090 individuals with 1 or 2 copies of the ε4 allele received either 0.5 mg/kg bapineuzumab (n=658, MMSE=20.7±3.2) or placebo (n=432, MMSE=20.8±3.1) every 13 weeks up to week 78 [51]. In the non-carrier study of 1114 AD patients without an *APOE* ε4 allele, patients received either 0.5 mg/kg bapineuzumab (n=314, MMSE=21.2±3.4), 1.0 mg/kg bapineuzumab (n=307, MMSE=21.2±3.3) or placebo (n=493, MMSE=21.2±3.2) every 13 weeks up to week 78 [51]. There was no significant effect on cognition in either study. P-tau was the only CSF biomarker that was analyzed, in 212 patients in the carrier study and in 178 patients in the non-carrier study. In the carrier study, P-tau decreased from 108.9 ±38.7 to 103.4±37.3 pg/ml in the bapineuzumab group and increased from 119.9±50.7 to 121.1± 54.9 pg/ml in the placebo group (-5.8 vs. +0.9 pg/ml, P=0.005) from baseline to week 71. In the non-carrier study, P-tau decreased from 97.5±50.6 to 94.1±45.56 in the combined bapineuzumab group and from 104.2±47.8 to 101.3±47.4 in placebo, but this was not significant (-3.4 vs. -2.9, P=0.11).

Solanezumab

Solanezumab is a humanized version of the of the mouse monoclonal antibody m266 that recognizes amino acids 13-28 in Aβ42 [52]. In a 12 week phase 2 study, 52 AD patients received solanezumab at either 100 mg weekly, 100 mg every four weeks, 400 mg weekly, 400 mg every four weeks or placebo for a total of 12 weeks [53]. Repeated CSF sampling was only available in a subgroup of patients. The number of patients in each group was low and no clear effects were observed between baseline and after 12 weeks on CSF T-tau or P-tau levels between treatment and placebo groups. Thus the change in the placebo group (n=8) was -

4.1% for T-tau and -2.1% P-tau; in the 100 mg every fourth week group (n=8) +0.7% for T-tau and -2.1% for P-tau; in the 100 mg weekly group (n=8) -3.9% for T-tau and -4.9% P-tau; in the 400 mg every fourth week group (n=8) -2.7% for T-tau and -3.3% P-tau; and in the 400 mg weekly group (n=5) +2.8% for T-tau and +2.2% P-tau.

Doody et al presented two large phase 3 studies of solanezumab. EXPEDITION 1 included 506 AD patients receiving 400 mg iv solanezumab and 506 AD patients receiving placebo every four weeks for 18 months. EXPEDITION 2 included 521 AD patients receiving solanezumab and 519 AD patients receiving placebo in the same doses and time points as for the first study [54]. The MMSE score of all included patients was between 16-26. CSF levels of Aβ40 decreased by 1,902 (95% CI -6660 to +2856) pg/ml in the placebo group and increased by +1,325 (95% CI -3162 to +5813) pg/ml in the solanezumab group in EXPEDITION 1 between baseline and week 80 (P=0.002). In EXPEDITION 2 study Aβ40 decreased by -876 (95% CI -4342 to +2590) pg/ml in the placebo group and increased by +2157 (95% CI -1212 to +5525) pg/ml in the solanezumab group (P<0.001). For CSF Aβ42, in EXPEDITION 1 the placebo group declined by -242 (95% CI -1144 to +660) pg/ml whilst the solanezumab group increased by +471 (95% CI -436 to +1379) pg/ml (P<0.001); whilst in EXPEDITION 2 study the placebo group increased by +324 (95% CI +86 to +561) pg/ml and the solanezumab group by +727 (95% CI +489to +964) pg/ml (P<0.001). However, there were no significant effects on cognition or CSF levels of T-tau and P-tau between patients treated with solanezumab and placebo.

In a follow-up trial (EXPEDITION 3), 2100 patients with AD were recruited. As well as narrowing the entry criteria to milder disease (MMSE=20-26) the included patients had to have a positive 18F-florbetapir PET scan i.e. indicating amyloid pathology. This trial failed to reach its primary outcome measure i.e. a significant change in ADAS-Cog14 between the treatment (400 mg solanezumab) and placebo groups after 80 weeks (unpublished data;

http://www.alzforum.org/news/conference-coverage/ctad-solanezumab-seen-nudge-ad-ever-so-slightly). However, both secondary outcomes (MMSE and Clinical Dementia Rating – Sum of Boxes Score) declined less in the treatment group than in controls. An unexpected finding in this study was a statistically significant increase in P-Tau and borderline significant increase in T-Tau (P=0.06) in the treatment group compared to the placebo group. In all studies, solanezumab induced sharp increases in plasma Aβ concentrations. Recent data suggest that this may be due to blocking of enzymatic clearance of Aβ in blood by insulin-degrading enzyme [55].

Ponezumab

Ponezumab is a humanized monoclonal anti-Aβ antibody that binds to the C-terminal part of Aβ40 and only to the soluble form [56]. Ponezumab was tested in a phase 1 study where AD patients (MMSE 16-26), received a single infusion of the drug of either 1 mg/kg, 3 mg/kg, 5 mg/kg or 10 mg/kg [57]. There was a dose-dependent increase in plasma Aβ40. However, there were no baseline CSF measurements in this study and the only data were from 6 h after the infusion. Since CSF biomarkers only were analyzed in three patients receiving the dose 5 mg/kg and three receiving 10 mg/kg, no conclusions can be drawn.

Gantenerumab

Gantenerumab is a fully human IgG1 anti-Aβ antibody that was derived from a human phage display library and optimized for high-affinity binding with fibrillar Aβ [58]. In a phase 3 study of gantenerumab, 770 patients with prodromal AD with a MMSE score ≥24 received injections of 105 mg, 225 mg gantenerumab or placebo every fourth week until week 104. The CSF data from this trial was presented at the 2016 Springfield meeting (http://www.siumed.edu/cpd/alzheimer/pdf/abstractbook_2016.pdf). There was no effect on CSF Aβ42 levels. However a significant reduction in T-tau level was observed after 2 years in

the 225 mg group compared with placebo, accompanied by a dose-dependent decrease in P-tau and neurogranin.

Intravenous immunoglobulins

An alternative to using antibodies directed specifically against Aβ is to trial more generic immunomodulation using intravenous immunoglobulins (IvIg). In a phase 2 multicenter trial of 55 AD patients (MMSE 16-26), patients were randomized to receive a total of three doses; 0.1 g/kg every 2 weeks (n=6), 0.25 g/kg every 2 weeks (n=7), 0.4 g/kg every 2 weeks (n=7), 0.2 g/kg every 4 weeks (n=7), 0.5 g/kg every 4 weeks (n=8) or 0.8 g/kg IvIg every 4 weeks (n=7) or placebo [59]. There were neither significant effects on memory/cognition nor on CSF levels of T-tau, P-tau, Aβ40 or Aβ42 between the treatment groups and placebo at week 24. Plasma levels of Aβ42 were significantly decreased in individuals receiving 0.25 g/kg dosed every 2 weeks compared with placebo; this was not paralleled in plasma Aβ40 levels.

Semagacestat

Semagacestat is a γ-secretase inhibitor. It is not selective for APP but also targets Notch among other substrates [60]. In a clinical trial of 52 AD patients who received 100 mg, 140 mg semagacestat or placebo daily for 14 weeks, no difference in CSF T-tau and P-tau were found between baseline and at 14 weeks for any of the groups. The change in the placebo group (n=11) was +1.5% for T-tau and -0.8% for P-tau; for the 100 mg group (n=17), +4.7% for T-tau and +3.5% for P-tau; and for the 140 mg group (n=12) +7.7% for T-tau and +2.8% for P-tau [53]. In a large multicenter clinical trial, AD patients with MMSE between 16-26 were randomized to either 100 (n=482) or 140 mg (n=495) semagacestat daily or placebo (n=485) for 76 weeks [61]. There were no significant effects on cognition between the treatment groups and placebo groups. CSF data which were available on a very small sample were presented as annualized change: Aβ42 increased in the placebo group (n=10) by 11±88 pg/ml and decreased in both the 100 mg (n=19, -40±110 pg/ml) and also in and 140 mg

(n=18, -36±183 pg/ml) semagacestat groups. The same trend was observed for the annualized change in Aβ1-40: in the placebo group (n=10) there was an increase of 88±847 pg/ml, with decreases on -11±1151 pg/ml and -599±1786 pg/ml in the 100 (n=19) and 140 mg (n=18) semagacestat groups respectively. For T-tau there was an annualized increase of +95±117 pg/ml in the placebo group (n=10), a decrease of -44±219 pg/ml in the 100 mg semagacestat group (n=17) but an increase of +40±128 pg/ml in the 140 mg (n=18) semagacestat groups. The annualized change of P-tau in the placebo group (n=10) was +11±7 pg/ml, with decreases of -7±15 and -4±11 pg/ml, respectively, in the 100 (n=19) and 140 mg (n=18) semagacestat groups. Treatment with semagacestat has also been shown to result in a dose-dependent increase in the Aβ isoforms Aβ1-14, Aβ1-15 and Aβ1-16 [62].

Tramiprosate

Tramiprosate (3-amino-1-propanesulfonic acid, 3APS) is an amyloid-binding agent that in transgenic animal studies has been shown to reduce oligmeric and fibrillar (plaque) Aβ [63]. In a phase 2 study, 58 AD patients with an MMSE score of 13-25 were randomized to either 50, 100, 150 mg tramiprosate or placebo twice daily. Tramiprosate had no effect on memory or cognition or CSF levels of T-tau and Aβ40. However, there was a dose-dependent decrease in CSF Aβ42 levels [64].

Tideglusib

Tideglusib is a thiadiazolidinone which irreversibly inhibits GSK-3. GSK-3 has in preclinical studies been shown to phosphorylate tau [65] and regulate the production of Aβ [66]. In this study, AD patients with an MMSE score of 14-26 were randomized to 500 mg (n=50) or 1000 mg tideglusib once daily (n=81), 1000 mg tideglusib every other day (n=90) or placebo (n=85) for 26 weeks [67]. There was no clinical benefit of the treatment in this study. Only a few patients were sampled for CSF; 15 treated with tideglusib and six with placebo. There was no change in Aβ42 (tideglusib -7.1±132.2 vs. placebo +7.9±106.3pg/ml, P=0.81), T-tau (-

2.5±129.2 vs. -45.8±135.8 pg/ml, P=0.50), P-tau181 (+1.9±22.7 vs. +25.2±37.3 pg/ml, P=0.09) or P-tau396 (-0.18±0.64 vs. +0.18±0.49 pg/ml, P=0.23).

Resveratrol

Resveratrol is an activator of sirtuin 1 (SIRT1), a histone deacetylase involved in aging and longevity [68]. This multicenter phase 2 study included 119 AD patients with MMSE scores of 14-26. The patients were randomized to either resveratrol in 500 mg increments every 13 weeks i.e. 500 mg every morning, 500 mg twice daily, 1000 mg every morning and 500 every afternoon and finally 1000 mg twice daily (n=64) or placebo (n=55) [69]. There was no clinical benefit of the treatment in this study and no significant effects on either CSF levels of Aβ42, T-tau or P-tau between the groups (data not presented in the paper).

Avagacestat

Avagacestat is a γ-secretase inhibitor evaluated in a multicenter phase 2 study. 209 AD patients (MMSE 16-26), were randomized to receive either 25, 50, 100 or 125 mg avagacestat or placebo once daily for 24 weeks [70]. There was no effect on cognition in the 25 and 50 mg groups compared with placebo but a trend for worsening in the 100 and 125 mg groups. No significant changes of CSF T-tau or P-tau were observed between the treatment groups and placebo. For Aβ40 and Aβ42 a significant decrease was observed in the 125 mg group only compared to placebo.

Antioxidants

In a three arm study of AD (MMSE 16-30), 26 patients received 800 IU vitamin E, 500 mg vitamin C and 900 mg α-lipoic acid daily, 26 patients received 400 mg coenzyme Q three times a day, and 26 patients received placebo [71]. No significant effects on memory and cognition or levels of Aβ42, T-tau or P-tau were observed between the groups.

Expert commentary

None of the clinical trials for AD described above achieved any significant effect on memory or cognition. There are several likely reasons for this. First of all, many of the studies included patients with low MMSE scores, and in some cases down to 14, indicating already advanced cognitive and memory decline. At this stage of disease, it is likely to be too late to halt the spread of pathology and influence the already advanced neurodegeneration that is present in AD at this stage. Furthermore, the diagnosis of AD has often been based on the clinical McKhann criteria from 1984 [72] which did not require biomarker evidence of AD pathology. Data from some studies have indicated that around 15-20% of the included patients did not have Alzheimer pathology [73,74]. Having a significant proportion of patients with other diagnoses in an AD trial not only exposes patients who are very unlikely to benefit to potential side-effects, but also considerably reduces the power to identify a positive effect of a drug if one is present. New criteria for AD take biomarkers – both PET and CSF based – into account [22] but very few clinical trials have finished since these criteria were launched. If the correct individuals were targeted, it is of course possible that the drugs tested in these trials were not targeting the correct pathology. For example, of the two major immunotherapy drugs that failed at phase 3 – solanezumab only binds soluble monomeric Aβ whereas bapineuzumab binds both soluble and fibrillar Aβ. By contrast, the drug that has shown hints of efficacy in terms of an effect on cognition – albeit only in a phase 1b trial – is aducanumab which is an antibody that only binds aggregated Aβ, and especially so in the parenchyma [75]. Therefore, it might be important which Aβ form that is targeted, and CSF studies with the potential to delineate different Aβ moieties may become very important as entry inclusion or for stratification. In terms of outcome measures, whilst alteration in Aβ load may be an indicator of target engagement, and potentially a correlate of efficacy in primary or secondary prevention studies, demonstration of an effect on neurodegeneration is likely to require

evidence of a change in other markers – perhaps particularly T-tau, P-tau, or NFL; or perhaps synaptic function, as might be indicated by Ng. With the exception of an effect with bapineuzumab, such changes have not been demonstrated in trials to date. Whilst this is likely to simply reflect a lack of effect on downstream neurodegeneration which may in turn reflect one or more of the wrong drug being given at the wrong dose at the wrong time, it is notable that in most cases only subsets of patients had serial CSF examination, limiting power to detect change.

So what can CSF biomarkers aid with in clinical trials for AD?

(1) Inclusion criteria. For an AD trial, is it vital to demonstrate that the individual in question has AD pathology. A biomarker of amyloid deposition is of utmost importance, and currently, amyloid PET or analysis of CSF levels of Aβ42 can be used for this purpose. (2) Going earlier. It is increasingly evident that it will be necessary to start treatment as early as possible, before neuronal damage becomes irreversible – it becomes ever more important to use biomarkers to demonstrate the underlying pathology the earlier in the disease process the trial is targeting. There are a number of reasons why CSF may be preferred to PET for the demonstration of Aβ pathology. Several studies have shown that CSF levels of Aβ42 drop before amyloid PET becomes positive; CSF allows for various forms of Aβ pathology – not just fibrillary forms – to be demonstrated. Measurement of CSF Aβ42 is also considerably cheaper than amyloid PET – and may be easier to roll out to the general population as/when such drugs enter the market. (3) A robust measure of neurodegeneration is required to demonstrate an effect on neurodegeneration. Whilst MR measures of atrophy or change in tau PET may provide an alternative, the multiplicity of CSF measures of different aspects of neurodegeneration potentially provides invaluable insights into the disease process. (4) Proximity markers. To improve the turnaround for AD clinical trials a proximity marker of upcoming memory problems and cognition would be desirable. Since memory and cognition

problems in AD are linked to neuronal cell death it is likely that biomarkers of neuronal cell death may provide the link between amyloid deposition and the onset of neurodegeneration. Tau PET is unlikely to be widely available or cost effective enough to be used in this context, whilst measures of T-tau, P-Tau, NFL and measures of synaptic density (Ng) can be measured in parallel from the same sample. Whilst no disease-modifying trial has so far shown any clinical effect, and so it is not surprising that no clear effects on T-tau have been found, neurogranin data from the gantenerumab trial has not yet been published and NFL and has not been evaluated in any trials at all. A detailed understanding of the longitudinal stability of these biomarkers and changes in both health and disease will be vital for these purposes; as will a much larger update of CSF sampling in clinical trials rather than the typically ad hoc subgroup sampling that currently often occurs.

Five year view

It will be increasingly important to use biomarkers to enrich the study populations for subjects with the pathologies against which the drug candidate is directed, and to stratify in terms of rate of neurodegeneration and timing in relation to symptom onset. This will determine that the drug is given only to individuals who may benefit from treatment, and allow for recruitment of individuals with presymptomatic disease, and for rationale trial design to demonstrate disease modification. Therefore, our prediction is that demonstration of amyloid pathology using either measures of CSF Aβ42 or amyloid PET will become a mandatory trial inclusion. Serial analysis of amyloid pathology is also likely to be used more in order to follow drug effect on amyloid pathology in the trials where that mechanism is targeted. We also foresee a more general shift from PET to CSF analyses given that several biomarkers can be analyzed on the CSF from a single lumbar puncture and samples stored for future analysis (future proofing) in contrast to PET which can only measure certain aspects of pathology and

requires several different scans. We predict the increasing use of CSF studies in very early (dose finding/phase 1 studies) to provide proof of concept that the drug in question reaches the brain at sufficient concentration. Newer methodologies that can measure the dynamics and turnover of proteins in the CSF will also increasingly be used to demonstrate in vivo effects of putative therapies in very small patient numbers [76]. When it comes to evaluation of the treatment effect on neuronal degeneration serial CSF measures of NFL and T-tau will be used more extensively. In addition to NFL, T-tau and Aβ42, it is likely that there will be an increasing availability of newer biomarkers that can probe other aspects of disease activity. Biomarkers already available or emerging include not only P-tau as a marker of tau phosphorylation and neurogranin as a marker of synapse and dendrite dysfunction but also YKL-40 and sTREM2, markers of astroglial activity (Figure 1). New multiplex CSF assays which allow for very large numbers of proteins to be measures simultaneously and at femtomolar concentrations, will allow for numerous aspects of pathology to be measured concurrently – and consequently for the effects of putative treatments to be evaluated with unprecedented detail. Inevitably therefore, CSF studies will enter the mainstream becoming a vital part of the early evaluation of putative drugs, as well as inclusion, safety and outcome measures for clinical trials.

Figure legends

Figure 1. CSF biomarkers and their use in clinical trials.

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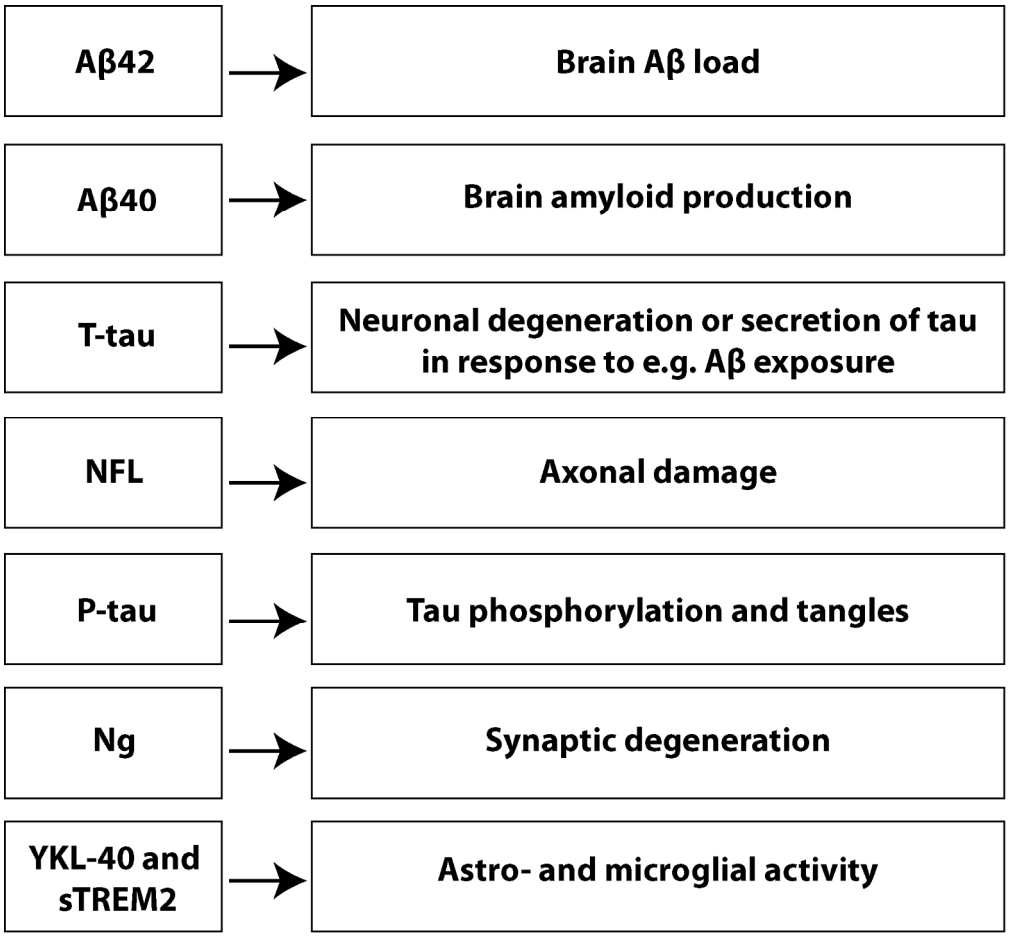

Figure 1. CSF biomarkers and their use in clinical trials.

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