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REVIEW

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Cerebrospinal Fluid Aβ_{42} Levels: When Physiological Become Pathological State

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SUMMARY

Impaired amyloid beta (Aβ) metabolism is currently considered central to understand the pathophysiology of Alzheimer's disease (AD). Measurements of cerebrospinal fluid Aβ levels remain the most useful marker for diagnostic purposes and to individuate people at risk for AD. Despite recent advances criticized the direct role in neurodegeneration of cortical neurons, Aβ is considered responsible for synaptopathy and impairment of neurotransmission and therefore remains the major trigger of AD and future pharmacological treatment remain Aβ oriented. However, experimental and clinical findings showed that Aβ peptides could have a wider range of action responsible for cell dysfunction and for appearance of clinico-pathological entities different from AD. Such findings may induce misunderstanding of the real role played by Aβ in AD and therefore strengthen criticism on its centrality and need for CSF measurements. Aim of this review is to discuss the role of CSF Aβ levels in light of experimental, clinical pathologic, and electrophysiological results in AD and other pathological entities to put in a correct frame the value of Aβ changes.

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Introduction

Alzheimer's disease (AD) is the most frequent form of cognitive decline leading to dementia worldwide. Clinical, pathologic, and pharmacologic approach to AD changed in last two decades, and several new technologies have been employed to reveal the real essence of AD. New strategies focused on development of reliable biomarkers to obtain early diagnosis and adequate pharmacological treatment. To date, amyloid biomarkers have been extensively used to validate AD diagnosis and consequently to develop new drugs [1–3]. However, the results are controversial and conflicting so that the so-called amyloid cascade hypothesis is now challenged [4]. Here, we will discuss the meaning of CSF Aβ levels in light of experimental, clinical, pathologic, and electrophysiological results in AD pathology.

Aβ42 as a Marker of Experimentally Induced Impaired Neuronal Transmission

Neuropathological findings of AD, namely the extracellular senile plaques and the intracellular neurofibrillary tangles, although diffused along the cortex, appear insufficient to explain entirely the cognitive decline symptoms [5–7]. It is now accepted that long before cognitive decline appears, several changes in the neuronal plasticity machinery occur that could be responsible for the neuronal degeneration [8]. In particular, it has been experimentally documented in glutamatergic excitatory terminals an impairment of long-term potentiation (LTP) mechanisms, useful for memory formation, and persistence of long-term depression (LTD) a mechanism with opposite effects [9]. These changes, when prolonged, are supposed to be responsible for dendritic spine shrinkage, neuronal disarray, cell degeneration, and death. Among the main responsibles for these changes, there are some peptides derived from the metabolism of the amyloid precursor protein (APP) [10]. APP is a membrane glycoprotein located at synaptic level, with a largely unknown function. *In vitro* evidence showed that APP has a broad physiological role during neuronal development and maturation, while in adults it seems to be more involved in the stabilization of glutamatergic synapses [11,12]. In particular, it seems to be useful in stabilizing synaptic membranes during sustained neurotransmission. APP catabolism is regulated by a group of peptidases called secretases that are distinct in two main pathways. Secretases cleave the APP producing catabolic soluble (nonamyloidogenic pathway) and unsoluble (amyloidogenic pathway) peptides [13]. Both pathways are in balance, being the nonamyloidogenic pathway favored in physiological condition. It is

noteworthy that the amyloidogenic pathway is under control of neurotransmitters such as acetylcholine, dopamine, serotonin and norepinephrine. Unsoluble amyloid peptides are protein fragments of 40 or 42 amino acids (Aβ1-40 and 1-42), here indicated as Aβ. In general, these peptides are degraded by peptidases, without specific function, such as insulin degrading enzyme, tissue plasmin activator, neprilysin, and so on [14–16]. Aβ peptides may have physiological and pathological function upon a bell-shaped curve. Aβ monomers, produced in physiological conditions, are useful to increase the strength of glutamatergic excitatory transmission and have protective effects on synapse [17]. Conversely, excessive accumulation of Aβ has the opposite effects, leading to the production of peptides able to aggregate in fibrils and then to accumulate in senile plaques. However, intermediate species lead to the formation of highly toxic forms such as dimmers, trimers, or tetramers, commonly identified as oligomers. These are soluble structures able to interfere with neurotransmission at synaptic level, leading to the above mentioned impairment of neural plasticity machinery and neuronal degeneration [18,19]. Therefore, increased levels of Aβ peptides aggregate in soluble products, oligomers, able to interfere with neurotransmission. The prolonged exposure to these peptides induces irreversible changes at synaptic level responsible for breakdown of memory formation mechanism. Oligomers have also a direct toxic effect on cholinergic, serotoninergic, noradrenergic, and dopaminergic neurons inducing their degeneration, and reducing their control on nonamyloidogenic pathway favoring the accumulation of unsoluble peptides [20,21]. Pathologic aggregation of Aβ peptides was suggested to be responsible for AD onset (amyloid hypothesis) [10]. In addition to the mechanisms related to AD, Aβ peptides were involved in several other pathological conditions all indicative of a clear perturbation of neural transmission on one hand and of toxic protein–protein interaction on the other. During mood disorders, changes of glutamatergic transmission, with pathologic dendritic spine remodeling [22] exposed to stress, in brain areas, such as hippocampus, orbito-frontal cortex, and amygdala, have been indicated causative of symptoms onset [23–25]. In all these cases and in the same areas, increased accumulation of Aβ was suggested to be related to AD onset in old individuals. Changes of sleep-wake cycle were associated to accumulation of Aβ peptides and AD pathogenesis [4], likely related to perturbed neurotransmission and catabolic mechanisms, like orexins system [26–28], a condition that resulted altered in CSF of patients with AD. Melatoninergic transmission in pineal gland has also been shown to be associated to Aβ [29,30]. Moreover, diabetes mellitus (DM) is considered a potential risk factor for AD. In DM, experimental models have been demonstrated that Aβ oligomers were capable of changing hypothalamic peripheral control of glucose levels, strengthening the relationship between AD and DM [31–33]. Even experimental models of traumatic injuries have been associated to Aβ pathology [34]. Aβ peptides can also interact with membrane proteins forming pores capable of increasing intracellular Ca^{2+} and inducing deleterious changes of cell activity. This is the case of Aβ and α-synuclein interaction in substantia nigra neurons responsible for cell degeneration [35,36] or of platelets in which the interaction with Aβ increase platelets adhesion increasing prothrombotic activity in small arteries [37–39]. The latter demonstration could be of importance to understand white matter changes formation considered an important factor for disease progression. In addition to these data are recent observations of the potential atherogenic mechanism promoted by Aβ. Both APP and Aβ were found in microvasculature surrounding advanced human carotid artery plaque [40], where platelet APP is proteolytically degraded to Aβ, a condition able to activate inducible nitric oxide synthase and macrophage activity, mechanisms involved in proinflammatory cascade of atherosclerosis [41,42]. In this case, Aβ would be responsible for induction of the brain-endothelial damage often observed in several studies [43,44]. These mechanisms adjunct to the role played by major cerebrovascular risk factors, such as hypertension, atherosclerosis, diabetes, and hyperlipemia, strengthen the potential link between atherosclerosis and Aβ metabolism as mechanisms mutually involved in neuronal synaptopathy of AD [42,45,46]. Finally, Aβ is also able to induce cytokine release from microglia, in particular interleukin 1 [47,48], speeding up mechanisms of neurodegeneration. Indeed, all of the above-described mechanisms and changes clearly indicate that Aβ accumulation and aggregation have toxic effects, perturbing neurotransmission and changing ionic intracellular influx even decades before cognitive decline onset. However, it is still unclear what triggers peptides accumulation and how or even whether this correlates with cognitive symptoms.

Notably, recent experimental findings demonstrated that other mechanisms independent from Aβ could be responsible for cognitive decline of AD. In particular, it has been proposed that inducing hyperphosphorylation of tau could be involved in mechanisms of neurodegeneration [49–54]. Unexpectedly, other substances like formaldehyde or mechanisms of D-ribosylation, not involved in Aβ metabolism could trigger neuronal degeneration. Further studies however are needed to understand if these mechanisms have a substantial role in the pathophysiology of AD. Nonetheless, a question remains unsolved over all: what does Aβ CSF level indicate?

Aβ as a Biomarker of Cognitive Decline

Pathological hallmarks of AD are widespread deposits of plaques constituted mainly of Aβ peptides and neurofibrillary tangles constituted by hyperphosphorylated tau protein. These changes are mirrored in CSF of patients with AD and are currently the earliest signs of AD process. Decreased levels of Aβ in association to increased levels of total tau (T-tau) and phosphorylated tau (p-tau) proteins reflect the concomitant neurodegenerative process. These biomarkers are reliably used to detect incipient AD in patients with MCI defined individuals [1]. Aβ is considered a marker expressing the potentiality of an individual to develop cognitive decline symptoms [1,3]. Aβ levels lower in a very slowly fashion and can occur two or more decades to reach a pathological threshold. Of note is the observation that below a threshold considered pathological, there is not a real Aβ gradient that parallels the gravity of cognitive symptoms [55,56]. In addition, several other recent observations demonstrated that neuronal degeneration as well as cognitive decline symptoms are more adherent to variations of t-tau CSF levels than that of Aβ42 [57–60]. In other words, the more t-tau increases, the more rapidly cognitive decline appears, and high levels of t-tau do correspond to cognitive decline severity. These findings highlighted tau protein

metabolism as a more crucial marker in the neurodegenerative events of AD. Such evidences lead to revisit the amyloid hypothesis cascade as the main responsible for AD. To date, Aβ is still central in AD pathophysiology and currently is considered a sort of accelerator/initiator and tau an executor of the pathogenic process, being the interaction crucial for triggering AD [4,61]. As Aβ is crucially involved in AD pathogenesis, recent strategic intervention focused on the development of antiamyloid drugs (vaccines). Several trials performed so far failed unfortunately. Therefore, new strategy of intervention targeted patients in prodromal or even in preclinical phases of the disease, and some of them are still en course. These studies led to study also cognitively normal individuals, to identify the real candidate for antiamyloid therapy. Recently published research showed controversial results. Some of these studies showed that even in cognitively normal subjects Aβ could be low, a finding that led to the conclusion that individuals with low Aβ levels has to be considered potential future AD [62,63]. Others, more recently, showed that cognitive decline and neurodegeneration in cognitively normal individual is not related to Aβ burden [64,65]. Given these findings, a question about the real meaning of low CSF Aβ level in an individual arise, particularly because a direct link between Aβ and neurodegeneration is still difficult to find. Moreover, what kind of relationship might exist between Aβ levels and age would be interesting to clarify. Finally, the link between Aβ and tau pathology, which is considered the real marker of ongoing degeneration, is far from being elucidated, although several models have been proposed to explain such a gap. So far, available data on normal individuals, that currently represent the most important source of data, indicate that Aβ pathology might represent the marker of cortical neurotransmission dysfunction, being a mix of local network disruption, compensatory reorganization, and impaired control network function [66,67]. Such impairment would involve different neurotransmission systems, such as acetylcholine, serotonin, dopamine, and glutamate [21,68–70], and would be independent from AD degeneration. Thus, Aβ might represent a reliable "thermometer" of the cortical neurotransmission network status. In particular, Aβ seems to represent a marker of cortical synaptic health, in a way that an increased Aβ burden (low CSF levels) might reflect a diffuse synaptic impairment of cortical network. Such mechanism is likely to start and progress silently years before cognitive decline onset and to involve several different neurotransmission systems (acetylcholine, glutamate, serotonin, dopamine, etc.). The largest the transmission network impairment is, the easier cognitive decline appears. In this view, the pathophysiological link between AD and Aβ pathology appear to be related more to the synaptic impairment at the cortical levels of the main neurotransmitter systems in the brain than to neurodegeneration of neurons.

In vivo Electrophysiological Recordings and Aβ

Recently developed electrophysiological recordings, like Transcranial Magnetic Stimulation, allowed to the possibility to investigate cortical related events *in vivo*, like the cortico-cortical connectivity and synaptic plasticity-like mechanisms of human brain. These techniques were applied in both physiological and pathological conditions. Intermittent and continuous theta burst stimulation paradigm in particular can be reliably used to record LTP- and LTD-like activity in humans in a safe and easily reproducible manner. iTBS and cTBS were recently used to investigate synaptic plasticity mechanisms also in patients with AD. Results of these studies showed impaired LTP and pathological LTD [71,72], confirming experimental data obtained in laboratory animals [9].

To evaluate the impact of CSF biomarkers such as Aβ, t-tau and p-tau on neurophysiological features of synaptic transmission, our group recently coupled CSF biomarker levels to different electrophysiological recording results in individuals with AD. In particular, we tested to what extent the electrophysiological changes observed in patients with AD would be compatible with amyloid hypothesis. For instance, central cholinergic transmission can be evaluated *in vivo* using a neurophysiological tool called Short Latency Afferent Inhibition (SLAI) [73]. SLAI can be easily measured by applying an electric conditioning pulse on the median nerve at wrist that precedes the TMS test pulse applied over the contralateral primary motor cortex (M1) by 20–25 ms. With this method, the resulting Motor Evoked Potentials (MEPs) are generally inhibited. It was suggested that these inhibitory interactions are mediate by cholinergic projections over the primary motor cortex. SLAI is considered as a putative marker of central cholinergic activity because it is abolished by scopolamine, a potent muscarinic antagonist. Therefore, SLAI is currently considered a noninvasive way to test cholinergic activity in the cerebral cortex in healthy subjects.

In patients with AD, SLAI is reduced at various degrees depending on the severity of the disease so that the decreased inhibitory effect of SLAI is thought to reflect the cholinergic dysfunction in AD [74,75]. We recently investigated whether in patients with AD the levels of CSF biological markers of AD such as Aβ, total tau, and phosphorylated tau proteins could influence the cortical cholinergic activity assessed trough SLAI. The results showed that in patients with AD the amount of central cholinergic function, measured by SLAI recordings, is associated with the levels of Aβ and p-tau detected in the CSF. In particular, higher dysfunction of cholinergic transmission is significantly associated to lower levels of Aβ and to higher levels of p-tau. These data provided a novel *in vivo* demonstration that the intensity of central cholinergic dysfunction is strictly dependent on the underlying pathology of Aβ and tau [76]. In another study, we investigated the correlation between motor cortical plasticity, measured with 1 Hz repetitive transcranial magnetic stimulation (rTMS), and the levels of Aβ total tau (t-tau), and phosphorylated tau (p-tau) detected in CSF of patients with AD. Measures of cortical plasticity can be obtained noninvasively with repetitive transcranial magnetic stimulation (rTMS) using protocols that are considered to induce plastic changes resembling the LTP and LTD mechanisms described in animal models [72]. When rTMS is given at low frequencies of stimulation, in the range of 1 Hz, it can induce inhibition of cortical excitability for several minutes. Altered mechanisms of cortical plasticity have been recently described in patients with AD using these protocols [71,72,77]. However, whether changes of such synaptic plasticity mechanisms could be related to levels of CSF biomarker in patients with AD has scarcely been explored. We found that the overall rTMS after effects were milder in patients with AD in comparison with controls. In patients with AD, the amount of rTMS-induced inhibition correlated with CSF t-tau,

but not with Aβ CSF levels. Surprisingly, higher CSF t-tau levels were associated to a stronger inhibition of the motor evoked potentials, implying that the expected effects of the 1 Hz rTMS protocol were more evident in patients with more pathological t-tau CSF levels. These data could be interpreted as the consequence of CSF t-tau-mediated abnormal excitatory activity and could suggest that CSF t-tau may effectively impact mechanisms of cortical plasticity [78]. Taken together, these findings indicate that in CSF of patients with AD Aβ levels appear to be related rather to a dysfunction of cholinergic activity, then with synaptic plasticity mechanisms. Conversely, CSF tau proteins appear to be more involved in dysregulation of synaptic plasticity mechanisms. This hypothesis finds support in recent evidence showing that tau oligomers derived from brains of postmortem patients with AD are potent inhibitors of long-term potentiation in hippocampal brain slices [79]. These evidences do not exclude the involvement of Aβ in neurodegeneration, but highlight the need to better understand molecular mechanisms responsible for AD. In this view, Aβ appears more related to repeated synaptopathic events likely expression of cortical transmission system impairment. Each event would be associated to oligomers production further sustaining synaptopathy. Enduring synaptic dysfunction would likely induce shrinkage, inflammation, oxidative stress, and eventually senile plaques deposits and atrophy [80]. Such view could also explain the reason why Aβ levels can be low in other dementias like Lewy body dementia (DLB) and fronto-temporal lobe degeneration (FTD). In the case of AD, the sum of these mechanisms and the progressive impairment of neurotransmitters system would induce tau-mediated pathology responsible for disease onset and progression [21,81]. Neurotransmitters system that instead is not so heavily compromised in DLB [82] or FTD [83–85]. Therefore, it is conceivable to suppose that the synaptic dysfunction observed in our sample of patients with AD could involve a more complex interplay between tau and Aβ not fully investigated.

Conclusion

Aβ maintain its centrality in AD pathogenesis, although alone is not sufficient as predictor for AD. Measurements of its levels in CSF samples, associated to t-tau and p-tau, remain useful to diagnose AD cognitive decline and to differentiate AD from other forms of dementia. Low Aβ levels in CSF alone likely are the most adherent representation on synaptic impairment of an individual. In this view, lowering of Aβ may start long before cognitive decline occurs. Synaptic dysfunction due to Aβ levels has no clear electrophysiological correlate *in vivo*. Intense neurodegeneration, related to high t-tau levels, conversely is strictly associated to neurophysiological changes. Thus, Aβ levels in CSF to our opinion have to be considered by clinicians as a "thermometer" useful to measure synaptic dysfunction temperature.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Mattsson N, Zetterberg H, Hansson O, et al. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *JAMA* 2009;**302**:385–393.
2. Lewczuk P, Mroczko B, Fagan A, Kornhuber J. Biomarkers of Alzheimer's disease and mild cognitive impairment: a current perspective. *Adv Med Sci* 2015;**60**:76–82.
3. Blennow K, Mattsson N, Schöll M, Hansson O, Zetterberg H. Amyloid biomarkers in Alzheimer's disease. *Trends Pharmacol Sci* 2015;**36**:297–309.
4. Musiek ES, Holtzman DM. Three dimensions of the amyloid hypothesis: time, space and 'wingmen'. *Nat Neurosci* 2015;**18**:800–806.
5. Armstrong RA. The molecular biology of senile plaques and neurofibrillary tangles in Alzheimer's disease. *Folia Neuropathol* 2009;**47**:289–299.
6. Lesné SE, Sherman MA, Grant M, et al. Brain amyloid-β oligomers in ageing and Alzheimer's disease. *Brain* 2013;**136**:1383–1398.
7. Xekardaki A, Kövari E, Gold G, et al. Neuropathological changes in aging brain. *Adv Exp Med Biol* 2015;**821**:11–17.
8. Donohue MC, Sperling RA, Salmon DP, et al. The preclinical Alzheimer cognitive composite: measuring amyloid-related decline. *JAMA Neurol* 2014;**71**:961–970.
9. Palop JJ, Mucke L. Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. *Nat Neurosci* 2010;**13**:812–818.
10. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002;**297**:353–356.
11. Hoe HS, Lee HK, Pak DT. The upside of APP at synapses. *CNS Neurosci Ther* 2012;**18**:47–56.
12. Laßek M, Weingarten J, Einsfelder U, Brendel P, Müller U, Volknandt W. Amyloid precursor proteins are constituents of the presynaptic active zone. *J Neurochem* 2013;**127**:48–56.
13. Selkoe DJ. Amyloid beta-peptide is produced by cultured cells during normal metabolism: a reprise. *J Alzheimers Dis* 2006;**9**(3 Suppl):163–168.
14. Melchor JP, Pawlak R, Chen Z, Strickland S. The possible role of tissue-type plasminogen activator (tPA) and tPA blockers in the pathogenesis and treatment of Alzheimer's disease. *J Mol Neurosci* 2003;**20**:287–289.
15. Miners JS, Baig S, Palmer J, Palmer LE, Kehoe PG, Love S. Abeta-degrading enzymes in Alzheimer's disease. *Brain Pathol* 2008;**18**:240–252.
16. de Tullio MB, Castelletto V, Hamley IW. Martino Adami PV, Morelli L, Castaño EM. Proteolytically inactive insulin-degrading enzyme inhibits amyloid formation yielding non-neurotoxic aβ peptide aggregates. *PLoS One.* 2013;**8**:e59113.
17. Puzzo D, Privitera L, Leznik E, et al. Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. *J Neurosci* 2008;**28**:14537–14545.
18. Shankar GM, Li S, Mehta TH, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* 2008;**14**:837–842.
19. Li S, Jin M, Koeglsperger T, Shepardson NE, Shankar GM, Selkoe DJ. Soluble Aβ oligomers inhibit long-term potentiation through a mechanism involving excessive activation of extrasynaptic NR2B-containing NMDA receptors. *J Neurosci* 2011;**31**:6627–6638.
20. Trillo L, Das D, Hsieh W, et al. Ascending monoaminergic systems alterations in Alzheimer's disease. translating basic science into clinical care. *Neurosci Biobehav Rev* 2013;**37**:1363–1379.
21. Martorana A, Koch G. Is dopamine involved in Alzheimer's disease? *Front Aging Neurosci* 2014;**25**:252.
22. Licznerski P, Duman RS. Remodeling of axo-spinous synapses in the pathophysiology and treatment of depression. *Neuroscience* 2013;**22**:33–50.
23. Namekawa Y, Baba H, Maeshima H, et al. Heterogeneity of elderly depression: increased risk of Alzheimer's disease and Aβ protein metabolism. *Prog Neuropsychopharmacol Biol Psychiatry* 2013;**3**:203–208.
24. Chung JK, Plitman E, Nakajima S, et al. Lifetime history of depression predicts increased amyloid-β accumulation in patients with mild cognitive impairment. *J Alzheimer Dis* 2015;**45**:907–919.
25. Brendel M, Pogarell O, Xiong G, Delker A, Bartenstein P, Rominger A, Alzheimer's Disease Neuroimaging Initiative. Depressive symptoms accelerate cognitive decline in amyloid-positive MCI patients. *Eur J Nucl Med Mol Imaging* 2015;**42**:716–724.
26. Roh JH, Jiang H, Finn MB, et al. Potential role of orexin and sleep modulation in the pathogenesis of Alzheimer's disease. *J Exp Med* 2014;**211**:2487–2496.
27. Ju SJ, Zhao Y, Chang X, Guo L. Orexin A protects cells from apoptosis by regulating FoxO1 and mTORC1 through the OX1R/PI3K/AKT signaling pathway in hepatocytes. *Int J Mol Med* 2014;**34**:153–159.
28. Liguori C, Romigi A, Nuccetelli M, et al. Orexinergic system dysregulation, sleep impairment, and cognitive decline in Alzheimer disease. *JAMA Neurol* 2014;**71**:1498–1505.
29. Brunner P, Sözer-Topcular N, Jockers R, et al. Pineal and cortical melatonin receptors MT1 and MT2 are decreased in Alzheimer's disease. *Eur J Histochem* 2006;**50**:311–316.
30. Cecon E, Chen M, Marçola M, Fernandes PA, Jockers R, Markus RP. Amyloid β peptide directly impairs pineal gland melatonin synthesis and melatonin receptor signaling through the ERK pathway. *FASEB J* 2015;**29**:2566–2582.
31. de la Monte SM. Insulin resistance and Alzheimer's disease. *BMB Rep* 2009;**42**:475–481.
32. de la Monte SM. Brain insulin resistance and deficiency as therapeutic targets in Alzheimer's disease. *Curr Alzheimer Res* 2012;**9**:35–66.

33. Clarke JR, Lyra E, Silva NM, et al. Alzheimer-associated Aβ oligomers impact the central nervous system to induce peripheral metabolic deregulation. *EMBO Mol Med* 2015;**7**:190–210.
34. Gatson JW, Warren V, Abdelfattah K, et al. Detection of β-amyloid oligomers as a predictor of neurological outcome after brain injury. *J Neurosurg* 2013;**118**:1336–1342.
35. Mandal PK, Pettegrew JW, Masliah E, Hamilton RL, Mandal R. Interaction between Abeta peptide and alpha synuclein: molecular mechanisms in overlapping pathology of Alzheimer's and Parkinson's in dementia with Lewy body disease. *Neurochem Res* 2006;**31**:1153–1162.
36. Resende R, Marques SC, Ferreiro E, Simões I, Oliveira CR, Pereira CM. Effect of α-synuclein on amyloid β-induced toxicity: relevance to Lewy body variant of Alzheimer disease. *Neurochem Res* 2013;**38**:797–806.
37. Jarre A, Gowert NS, Donner L, et al. Pre-activated blood platelets and a pro-thrombotic phenotype in APP23 mice modeling Alzheimer's disease. *Cell Signal* 2014;**26**:2040–2050.
38. Gowert NS, Donner L, Chatterjee M, et al. Blood platelets in the progression of Alzheimer's disease. *PLoS ONE* 2014;**9**:e90523.
39. Canobbio I, Guidetti GF, Oliviero B, et al. Amyloid β-peptide-dependent activation of human platelets: essential role for Ca2+ and ADP in aggregation and thrombus formation. *Biochem J* 2014;**462**:513–523.
40. De MG, De CD, Cooper S, et al. Platelet phagocytosis and processing of beta-amyloid precursor protein as a mechanism of macrophage activation in atherosclerosis. *Circ Res* 2002;**90**:1197–1204.
41. Bell RD, Zlokovic BV. Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. *Acta Neuropathol* 2009;**118**:103–113.
42. Gupta A, Iadecola C. Impaired Aβ clearance: a potential link between atherosclerosis and Alzheimer's disease. *Front Aging Neurosci* 2015;**16**:115.
43. Kelleher RJ, Soiza RL. Evidence of endothelial dysfunction in the development of Alzheimer's disease: Is Alzheimer's a vascular disorder? *Am J Cardiovasc Dis* 2013;**3**:197–226.
44. de la Torre JC. Cerebral hemodynamics and vascular risk factors: setting the stage for Alzheimer's disease. *J Alzheimers Dis* 2012;**32**:553–567.
45. Luzzi S, Vella L, Bartolini M, Provinciali L, Silvestrini M. Atherosclerosis in the evolution of Alzheimer's disease: can treatment reduce cognitive decline? *J Alzheimers Dis* 2010;**20**:893–901.
46. Viticchi G, Falsetti L, Vernieri F, et al. Apolipoprotein E genotype and cerebrovascular alterations can influence conversion to dementia in patients with mild cognitive impairment. *J Alzheimers Dis* 2014;**41**:401–410.
47. López-González I, Schlüter A, Aso E, et al. Neuroinflammatory signals in Alzheimer disease and APP/PS1 transgenic mice: correlations with plaques, tangles, and oligomeric species. *J Neuropathol Exp Neurol* 2015;**74**:319–344.
48. Taneo J, Adachi T, Yoshida A, Takayasu K, Takahara K, Inaba K. Amyloid β oligomers induce interleukin-1β production in primary microglia in a cathepsin B-and reactive oxygen species-dependent manner. *Biochem Biophys Res Commun* 2015;**458**:561–567.
49. Yang M, Lu J, Miao J, et al. Alzheimer's disease and methanol toxicity (part 1): chronic methanol feeding led to memory impairments and tau hyperphosphorylation in mice. *J Alzheimers Dis* 2014;**41**:1117–1129.
50. Yang M, Miao J, Rizak J, et al. Alzheimer's disease and methanol toxicity (part 2): lessons from four rhesus macaques (*Macaca mulatta*) chronically fed methanol. *J Alzheimers Dis* 2014;**41**:1131–1147.
51. Su T, Monte WC, Hu X, He Y, He R. Formaldehyde as a trigger for protein aggregation and potential target for mitigation of age-related, progressive cognitive impairment. *Curr Top Med Chem* 2015. [Epub ahead of print]. PMID: 26268337.
52. Lu J, Miao J, Su T, Liu Y, He R. Formaldehyde induces hyperphosphorylation and polymerization of Tau protein both in vitro and in vivo. *Biochim Biophys Acta* 2013;**1830**:4102–4116.
53. Wei Y, Han C, Wang Y, et al. Ribosylation triggering Alzheimer's disease-like Tau hyperphosphorylation via activation of CaMKII. *Aging Cell* 2015;**14**:754–763.
54. Han C, Lu Y, Wei Y, Wu B, Liu Y, He R. D-ribosylation induces cognitive impairment through RAGE-dependent astrocytic inflammation. *Cell Death Dis* 2014;**5**:e1117.
55. Seppälä TT, Koivisto AM, Hartikainen P, Helisalmi S, Soininen H, Herukka SK. Longitudinal changes of CSF biomarkers in Alzheimer's disease. *J Alzheimers Dis* 2011;**25**:583–594.
56. Toledo JB, Xie SX, Trojanowski JQ, Shaw LM. Longitudinal change in CSF Tau and Aβ biomarkers for up to 48 months in ADNI. *Acta Neuropathol* 2013;**126**:659–670.
57. Wallin AK, Blennow K, Andreasen N, Minthon L. CSF biomarkers for Alzheimer's Disease: levels of beta-amyloid, tau, phosphorylated tau relate to clinical symptoms and survival. *Dement Geriatr Cogn Disord* 2006;**21**:131–138.
58. Kester MI, van der Vlies AE, Blankenstein MA, et al. CSF biomarkers predict rate of cognitive decline in Alzheimer disease. *Neurology* 2009;**73**:1353–1358.
59. van der Vlies AE, Verwey NA, Bouwman FH, et al. CSF biomarkers in relationship to cognitive profiles in Alzheimer disease. *Neurology* 2009;**72**:1056–1061.
60. Koch G, Belli L, Giudice TL, et al. Frailty among Alzheimer's disease patients. *CNS Neurol Disord Drug Targets* 2013;**12**:507–511.
61. Stancu IC, Vasconcelos B, Terwel D, Dewachter I. Models of β-amyloid induced Tau-pathology: the long and "folded" road to understand the mechanism. *Mol Neurodegener* 2014;**18**:51.
62. Mormino EC, Betensky RA, Hedden T, et al. Synergistic effect of β-amyloid and neurodegeneration on cognitive decline in clinically normal individuals. *JAMA Neurol* 2014;**71**:1379–1385.
63. Pietrzak RH, Lim YY, Neumeister A, et al. Amyloid-β, anxiety, and cognitive decline in preclinical Alzheimer disease: a multicenter, prospective cohort study. *JAMA Psychiatry* 2015;**72**:284–291.
64. Wirth M, Villeneuve S, Haase CM, et al. Associations between Alzheimer disease biomarkers, neurodegeneration, and cognition in cognitively normal older people. *JAMA Neurol* 2013;**70**:1512–1519.
65. Wirth M, Oh H, Mormino EC, Markley C, Landau SM, Jagust WJ. The effect of amyloid β on cognitive decline is modulated by neural integrity in cognitively normal elderly. *Alzheimers Dement* 2013;**9**:687–698.e1.
66. Song Z, Insel PS, Buckley S, et al. Brain amyloid-β burden is associated with disruption of intrinsic functional connectivity within the medial temporal lobe in cognitively normal elderly. *J Neurosci* 2015;**35**:3240–3247.
67. Elman JA, Oh H, Madison CM, et al. Neural compensation in older people with brain amyloid-β deposition. *Nat Neurosci* 2014;**17**:1316–1318.
68. Danysz W, Parsons CG. Alzheimer's disease, β-amyloid, glutamate, NMDA receptors and memantine–searching for the connections. *Br J Pharmacol* 2012;**167**:324–352.
69. Baker-Nigh A, Vahedi S, Davis EG, et al. Neuronal amyloid-β accumulation within cholinergic basal forebrain in ageing and Alzheimer's disease. *Brain* 2015;**138**:1722–1737.
70. Kar S, Slowikowski SP, Westaway D, Mount HT. Interactions between beta-amyloid and central cholinergic neurons: implications for Alzheimer's disease. *J Psychiatry Neurosci* 2004;**29**:427–441.
71. Koch G, Di Lorenzo F, Bonnì S, et al. Impaired LTD- but not LTD-like cortical plasticity in Alzheimer's disease patients. *J Alzheimers Dis* 2012;**31**:593–599.
72. Koch G, Di Lorenzo F, Bonnì S, et al. Dopaminergic modulation of cortical plasticity in Alzheimer's disease patients. *Neuropsychopharmacology* 2014;**39**:2654–2661.
73. Battaglia F, Wang HY, Ghilardi MF, et al. Cortical plasticity in Alzheimer's disease in humans and rodents. *Biol Psychiatry* 2007;**6**:1405–1412.
74. Tokimura H, Di Lazzaro V, Tokimura Y, et al. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. *J Physiol (Lond)* 2000;**523**:503–513.
75. Di Lazzaro V, Oliviero A, Tonali PA, et al. Noninvasive in vivo assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation. *Neurology* 2002;**13**:392–397.
76. Martorana A, Mori F, Esposito Z, et al. Dopamine modulates cholinergic cortical excitability in Alzheimer's disease patients. *Neuropsychopharmacology* 2009;**34**:2323–2328.
77. Martorana A, Esposito Z, Di Lorenzo F, et al. Cerebrospinal fluid levels of Aβ42 relationship with cholinergic cortical activity in Alzheimer's disease patients. *J Neural Transm* 2012;**119**:771–778.
78. Inghilleri M, Conte A, Frasca V, et al. Altered response to rTMS in patients with Alzheimer's disease. *Clin Neurophysiol* 2006;**117**:103–109.
79. Koch G, Esposito Z, Kusayanagi H, et al. CSF tau levels influence cortical plasticity in Alzheimer's disease patients. *J Alzheimers Dis* 2011;**26**:181–186.
80. Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, et al. Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. *Mol Neurodegener* 2011;**6**:39.
81. Godoy JA, Rios JA, Zolezzi JM, Braidy N, Inestrosa NC. Signaling pathway cross talk in Alzheimer's disease. *Cell Commun Signal* 2014;**28**:23.
82. Martorana A, Esposito Z, Koch G. Beyond the cholinergic hypothesis: do current drugs work in Alzheimer's disease? *CNS Neurosci Ther* 2010;**16**:235–245.
83. Duda JE. Pathology and neurotransmitter abnormalities of dementia with Lewy bodies. *Dement Geriatr Cogn Disord* 2004;**17**(Suppl 1):3–14.
84. Huey ED, Putnam KT, Grafman J. A systematic review of neurotransmitter deficits and treatments in frontotemporal dementia. *Neurology* 2006;**66**:17–22.
85. Mendez MF. Frontotemporal dementia: therapeutic interventions. *Front Neurol Neurosci* 2009;**24**:168–178.