News & Views

Biomarkers for Alzheimer's disease – beyond amyloid and tau

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Amyloid β (Aβ) plaque and tau tangle pathology are hallmarks of Alzheimer's disease (AD), the commonest dementia, and each can be measured *in vivo* using both fluid and imaging biomarkers. In recent years it has become clear that many additional pathological changes occur in AD which may better predict disease onset and progression, and that non-AD pathologies also contribute to cognitive decline. In two separate studies in this issue of *Nature Medicine*, novel biomarkers of neurodegeneration and blood-brain barrier dysfunction (Figure) are examined in relation to the onset and progression of cognitive decline and AD.[1,2]

Combining longitudinal clinical and population-based cohorts with robust biomarkers validated against neuropathology has led to major advances in our understanding of the pathological sequence that underpins AD. Accumulation of Aβ in the brain is a very early event, starting at least a decade (and probably longer) before symptoms.[3] Aβ can measured using two broadly interchangeable biomarkers: cerebrospinal fluid (CSF) Aβ42/Aβ40 ratio and amyloid positron emission tomography (PET).[4] Recently, the CSF measure has been translated into promising blood tests[5-7] likely to find utility for screening to reduce the numbers of invasive tests required to determine Aβ positivity for clinical trials. Whilst Aβ is necessary for an AD diagnosis, it is not sufficient to cause cognitive decline. In parallel with Aβ accumulation, CSF concentrations of total and phosphorylated tau increase,[8] likely indicating an Aβ-related change in tau metabolism resulting in secretion from affected neurons.[9,10] This tau dysfunction

eventually manifests itself as tangle pathology, which can be visualized using tau PET imaging,[11] and then to neuronal cell loss, *i.e.* neurodegeneration, which correlate more closely with cognitive decline.

The majority of clinical trials attempting to modify the course of AD have targeted Aβ; whilst several promising studies are still underway, those completed to date have universally failed to meet their primary cognitive outcomes. Some have shown effects on Aβ pathology, but none has shown robust evidence of an effect on neurodegeneration.[12] A major challenge has been to identify biomarkers that reflect neurodegeneration, and can predict an individual's proximity to developing cognitive decline. One such candidate is neurofilament light (NfL), a protein intrinsic to the axonal cytoskeleton. When neurons die, NfL is released into brain interstitial fluid which communicates freely with the CSF, as well as with the blood through arachnoid villi and paravascular drainage systems.[13] CSF and blood NfL concentrations increase across a range of neurodegenerative and neuroinflammatory diseases associated with neuroaxonal injury;[14] and studies in multiple sclerosis show that NfL concentration normalizes within 6-12 months of start of immunomodulatory treatments.[15,16] CSF and blood levels of NfL correlate closely and show the same broad dynamics following acute injury both peaking around 40-70 days post-injury and normalizing within 6 months.[17]

Preische *et al*. report the most extensive study to date on serum NfL dynamics in relation to the onset and progression of AD.[1] Using the multi-centre Dominantly Inherited Alzheimer Network (DIAN) longitudinal cohort study, they found serum NfL concentration was elevated ~6.8 years prior to symptom onset in individuals carrying disease-causing mutations. Measuring within-subject rates of change, they show that mutation carriers have elevated rates of NfL increase even earlier, ~16 year before estimated disease onset. Rates of change in NfL were predictive of imaging measures of neurodegeneration and hypometabolism, and of change in cognitive scores. Taken together, these data suggest that blood NfL may have utility in clinical trials, providing a means of determining both when to initiate disease-modifying treatment, and a relatively non-invasive and cost-effective means of assessing effects on underlying neurodegeneration, noting that this should take into account an annual age-related increase in NfL concentration of ~3%.[18] Importantly, NfL appears to be a marker of neurodegeneration irrespective of underlying cause.[14] Whilst this means it is not useful as a diagnostic, it may be a proximity and outcome measure for trials of many neurodegenerative diseases, as well as in clinical trials using combinatorial therapies as are currently being considered for sporadic AD, the clinical phenotype of which may be modified by combination of tau, Aβ, TDP-43, α-synuclein and vascular pathologies.

Aside from deposition of misfolded proteins, a number of lines of evidence suggest that dysfunction at the interface between the neuron and its vascular supply – the so-called neurovascular unit – has an important role to play in late life cognitive impairment. Until recently, there have been a paucity of

biomarkers with which to investigate this *in vivo.* Nation *et al*. developed a CSF test for the shedded form of platelet-derived growth factor receptor-β (sPDGFRβ), a protein highly expressed in brain capillary pericytes[2]. The CSF concentration of this protein was measured in individuals who were cognitively normal or showed early cognitive dysfunction, and the results were related to regional blood-brain barrier permeability measured using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) [19]. CSF sPDGFRβ closely correlated with DCE-MRI evidence of blood-brain barrier dysfunction, particularly in the hippocampus, and was increased in individuals with incipient cognitive dysfunction independent of Aβ and tau. As well as a lack of association with classical AD markers, there was also no simple relationship to either increasing or conventional vascular risk factors, suggesting that predisposition to blood-brain barrier dysfunction – cause currently unknown – may be an independent risk factor for cognitive decline.

If blood-brain barrier breakdown is a risk for, or cause of, cognitive dysfunction, could this explain increased serum NfL concentration in Alzheimer's disease? The lack of association between AD pathology and CSF sPDGFRβ mitigates against this being the whole explanation, although sPDGFRβ concentration may reflect only some aspects of blood-brain-barrier dysfunction. Future studies combining measurement of NFL, Aβ, tau, and emerging biomarkers of blood-brain barrier dysfunction have the potential both to explain the mechanism by which NFL enters the blood, but also more fundamental questions concerning the relationship between, and relative contributions of, vascular dysfunction, AD pathology and neurodegeneration to late-life cognitive impairment. In the more immediate term, NfL is emerging as a dynamic fluid-based biomarker for neurodegeneration irrespective of primary cause(s) and is likely to find utility as an outcome measure (and possibly proximity marker) for a range of clinical trials aiming to slowing the neurodegenerative process.

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Conflicts of interest

Prof Zetterberg has served at scientific advisory boards of Roche Diagnostics, Wave, Samumed and CogRx and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. Prof Schott has received research funding and PET tracer from AVID Radiopharmaceuticals (a wholly owned subsidiary of Eli Lilly); has consulted for Roche, Eli Lilly, Biogen and Merck; received royalties from Oxford University Press and Henry Stewart Talks; given education lectures sponsored by Eli Lilly, Biogen and GE; and serves on a Data Safety Monitoring Committee for Axon Neuroscience SE.

Legend to figure

Schematic representation of a neuron and the capillary neurovascular unit. Capillary pericytes, capable of shedding PDGFβ, are located outside the endothelial cells and are separated from them and the parenchyma by a layer of basal lamina. In the parenchyma, astrocyte end-feet and NfL-rich neuronal terminals are closely associated with the capillary. DCE-MRI is an imaging technique in which the acquisition of a baseline image without contrast enhancement is followed by a series of images acquired over time after an intravenous bolus of contrast agent; the method can thereby monitor leakage across the BBB. Abbreviations: DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging. CSF, cerebrospinal fluid; sPDGFβ, soluble platelet-derived growth factor β; NfL, neurofilament light; BBB, blood-brain barrier. (Figure modified from https://doi.org/10.3389/fnene.2010.00005)

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