

Spotlight:

Blood Biomarkers: Democratizing Alzheimer's Diagnostics

Henrik Zetterberg^{1,2,3,4*} & Kaj Blennow^{1,2}

¹*Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden*

²*Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden*

³*Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK*

⁴*UK Dementia Research Institute at UCL, London, UK*

*Correspondence: henrik.zetterberg@clinchem.gu.se

Summary

Currently available biomarkers for Alzheimer's disease are of limited availability due to high costs and perceived invasiveness. New studies reveal that Alzheimer's pathologies now can be reliably quantified *in vivo* using regular blood tests.

Main text

Alzheimer's disease (AD) is the most common form of neurodegenerative dementia. The disease process typically starts in mid-life with clinically silent build-up of amyloid β ($A\beta$) pathology in extracellular amyloid plaques in the brain tissue, followed by accumulation of aggregated phosphorylated tau (P-tau) in the form of paired helical filaments in dystrophic neurites surrounding the plaques and in intra-neuronal tangles (Scheltens et al., 2016). Whilst $A\beta$ pathology often is widespread at the symptomatic stage, the neuroanatomic distribution of tangles correlates with both the location at which neurons degenerate and the cognitive domains affected in patients with AD dementia.

Historically, AD has been diagnosed based on clinical criteria capturing symptoms of AD-type dementia with a misdiagnosis rate of around 30% when compared with neuropathology (the gold standard way of making a molecularly based definitive diagnosis of AD). However, now $A\beta$ pathology can be detected *in vivo* with amyloid positron emission tomography (PET)

or by using the ratio of cerebrospinal fluid (CSF) concentrations of 42 (aggregation-prone and selectively reduced in the presence of A β plaques) to 40 (soluble and unchanged in AD) amino acid-long A β (CSF A β 42/A β 40 ratio). Tau pathology can be visualized by tau PET. Its underlying molecular aberration (A β -induced tau hyperphosphorylation) can be quantified using CSF phosphorylated tau (P-tau) concentration. Neurodegeneration can be visualized and staged using magnetic resonance imaging of the brain, and the intensity of the process can be quantified using CSF concentrations of total-tau (T-tau) and neurofilament light (NfL), both of which are intra-axonal proteins that are secreted or released from degenerating neurons. It is believed that CSF T-tau primarily detects cortical neurodegeneration (typical of AD and some other disorders, *e.g.*, Creutzfeldt-Jakob's disease), whilst CSF NfL is a more general neurodegeneration biomarker that is also indicative of subcortical and spinal cord neuronal injury or degeneration (Zetterberg and Bendlin, 2020).

PET and CSF biomarkers are expensive, may be regarded invasive and are not available outside specialized centres. At the same time, AD is a very common disease. Currently available treatments are symptomatic but promising results from clinical trials of disease-modifying drug candidates (mainly immunotherapies targeting A β pathology) have been presented, and it is not unlikely that we may see conditional fast-track approvals of such treatments in the next few years. To facilitate clinical implementation of these drugs, as well as to speed up clinical trials of additional drug candidates, easily accessible low-cost biomarkers would be of great advantage, especially in the first screening of potentially eligible patients.

Measuring biomarkers for CNS pathologies in blood requires assays with exquisite analytical sensitivity and specificity, as well as adequate protection from heterophilic antibodies and other blood molecules that might interfere in the measurement. Ultrasensitive assays allow for dilution of complex samples to reduce the risk of molecular interference, and blockers against heterophilic antibodies in the sample diluent are also helpful to achieve reliable quantification. The first ultrasensitive blood test for neurodegeneration was NfL, quantified using Single molecule array (Simoa) digital enzyme-linked immunosorbent assay (ELISA) technology, and a few years later high-precision assays for plasma A β 42/A β 40 ratio were described (Zetterberg and Bendlin, 2020). These blood biomarkers detect neurodegeneration and A β pathology, respectively, although it should be noted that the A β 42/A β 40 ratio is reduced by only 14-20% in plasma, compared with 50% in CSF (Schindler et al., 2019), due to extra-

cerebral expression of A β peptides, which may make this test challenging (but not impossible) to implement in clinical laboratory practice.

To date, we have been lacking reliable blood tests for tau pathology. Whilst ultrasensitive plasma T-tau assays can detect neuronal injury in acute brain disorders, such as stroke and traumatic brain injury, they work relatively poorly in AD settings, and the correlation with CSF T-tau is weak (Pereira et al., 2017). Nevertheless, we are now seeing real breakthroughs in the plasma tau biomarker field. In 2017, Tatebe *et al.* published a modified version of the Simoa T-tau assay where one of the antibodies was replaced to specifically measure tau phosphorylated at threonine 181 (P-tau181) (Tatebe et al., 2017). With this assay, it was possible to quantify increased P-tau181 concentration in plasma from patients with AD and Down's syndrome but the assay was not sensitive enough to measure normal control levels. Using Meso Scale Discovery (MSD) technology with electrochemiluminescence detection and antibodies against N-terminal tau and a phosphorylated epitope around threonine 181, Mielke *et al.* created a sensitive assay that generated plasma P-tau181 concentrations that correlated with both amyloid and tau PET measures (Mielke et al., 2018). These findings were replicated by Palmqvist *et al.*, demonstrating that plasma P-tau181 associates with amyloid PET positivity and correlates strongly with CSF P-tau181 (Palmqvist et al., 2019). Recent validation studies using MSD-based P-tau181 quantification show very similar results (Janelidze et al., 2020; Thijssen et al., 2020), but with some samples measuring below the lower limit of quantification of the assay.

Using a sandwich immunoassay format on Simoa in which P-tau181 is captured between a magnetic bead-conjugated antibody against tau phosphorylated at threonine 181 (AT270) and an anti-tau antibody against N-terminal tau (Tau12) as detector, a very sensitive and specific P-tau181 assay for plasma and serum samples was developed (Karikari et al., 2020). The assay solved the problem with unmeasurable samples in the lower concentration range, and produced results that correlated strongly with CSF P-tau181 across the range of normal and pathologically increased concentrations. Plasma P-tau181 concentration differentiated AD from non-AD neurodegenerative diseases and cognitively normal controls, was increased in amyloid PET-positive individuals, and increased further in a step-wise manner with more advanced tau pathology, as visualized by tau PET (Karikari et al., 2020). Plasma P-tau181 is thus a test sensitive to both A β and tau pathology. All data generated using CSF P-tau181 assays have been replicated in blood using the Simoa P-tau181 test, corroborating plasma P-

tau181 as a robust blood biomarker for AD pathology that should be relatively easy to standardize and implement in clinical laboratory practice. It is also reassuring that different assay formats (MSD and Simoa with slightly different antibody combinations) generate similar results.

From a research standpoint, the obvious drawback with any fluid-based biomarker is the lack of anatomic information. This appears particularly relevant to biomarkers for tau pathology, the location of which correlates with how AD progresses over the brain, as reflected by region-specific neurodegeneration and cognitive worsening in a domain-related manner. For A β biomarkers, this appears less relevant, at least at symptomatic disease stages, as the pathology by then is widespread. From a clinical standpoint, we envision plasma P-tau181 to be used as an initial test in primary healthcare, potentially together with plasma NfL for non-AD neurodegenerative dementias, in patients who seek medical advice because of cognitive symptoms. Plasma P-tau-positive patients could be referred for more advanced examinations in memory clinics and started on a future disease-modifying drug against AD. Plasma P-tau-negative but NfL-positive patients could be examined for non-AD neurodegenerative disease, *e.g.*, frontotemporal dementia. Patients who are negative for both plasma P-tau and NfL are unlikely to suffer from progressive neurodegenerative disease (with Parkinson's disease as a notable exception). The blood tests could also be used to monitor treatment effects (successful treatment detoxifying A β pathology should normalise plasma P-tau181 and hopefully also reduce NfL, or slow its increase over time, as a sign of reduced neurodegeneration). If further validation studies in primary healthcare settings are successful, it might be enough to measure increased plasma P-tau181 (potentially in combination with reduced plasma A β 42/A β 40 ratio, when those assays become available as validated standard clinical chemistry tests) at baseline in patients with suspected mild symptoms of AD to start them on disease-modifying treatments, dose these treatments until plasma P-tau181 concentration has normalised, and then follow the patients clinically and with repeated blood testing to ensure that the disease is maintained in check. This would represent a significant step towards making AD diagnostics and treatment available and affordable for large patient populations around the globe. In the meantime, while we wait for approved treatments, the novel blood tests could be of immediate use as pre-screening tools in clinical trials to reduce the costs of unnecessary PET and CSF examinations with negative results, as well as in large-scale epidemiological and genetic studies to identify risk factors and polygenic risk scores for AD-related brain pathologies in addition to the clinical phenotype.

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