Blood-based biomarkers for Alzheimer’s disease - an update

Henrik Zetterberg¹,²,³,⁴*

¹Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
²Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
³Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK
⁴UK Dementia Research Institute at UCL, London, UK

*Correspondence: henrik.zetterberg@clinchem.gu.se; h.zetterberg@ucl.ac.uk

Abstract
Cerebrospinal fluid (CSF) biomarkers for Alzheimer’s disease (AD) are in clinical use in many parts of the world and show good to excellent diagnostic accuracy in regards to identifying cerebral amyloid β (Aβ) and tau pathology irrespective of the clinical stage of the disease. However, CSF sampling is more difficult than a blood draw and a procedure only rarely performed by general practitioners. Since AD is such a common disease and since intense research on novel treatments that hopefully will be directed against underlying pathologies is moving forward, it would be excellent if the CSF tests for AD could be transformed into blood tests, as well as if novel blood biomarkers could be discovered. Brain-derived molecules are, however, present at much lower concentrations in blood than in CSF, which poses an analytical challenge. There are also additional issues with blood as a biofluid in which to measure biomarkers for central nervous system disease. Nevertheless, the past few years have seen an enormous development in the field of ultrasensitive measurement techniques. There is also much better availability of deeply phenotyped clinical cohorts for biomarker discovery and validation. This review gives an updated account of the current state of research on blood biomarkers for AD and related neurodegenerative dementias with special emphasis on findings that have been replicated by more than one research group.
1. Introduction
Alzheimer’s disease (AD) is an age-related neurodegenerative disease characterized by the accumulation of extracellular amyloid β (Aβ) plaques and intraneuronal inclusions (neurofibrillary tangles) composed of truncated and phosphorylated forms of the microtubule-stabilizing protein tau (Scheltens et al., 2016). AD is very common in the elderly and in spite of recent clinical trial failures, there are continued intense research efforts to develop Aβ- or tau-directed treatments to replace or supplement the existing symptomatic drugs that only counterbalance the neurotransmitter disturbance without affecting the underlying disease process (Winblad et al., 2016). Two phase II clinical trials have renewed the hope that it might be possible to reduce Aβ burden in the brain and thereby stabilize cognition using intravenously infused humanized monoclonal antibodies against Aβ (aducanumab and BAN2401). However, we still wait for conclusive results from large scale clinical trials.

2. Biomarkers
Broadly speaking, a biomarker is a measurable indicator of a biological state or pathological condition. In AD, we have validated cerebrospinal fluid (CSF) biomarkers for Aβ plaque pathology (a reduced CSF Aβ42 concentration or CSF Aβ42/40 ratio, because of selective retention of the 42 amino acid form of Aβ in plaque-laden brain tissue), as well as neuronal mismetabolism of tau that is associated with neurodegeneration and tangle pathology (increased secretion of both total tau [T-tau] and phosphorylated tau [P-tau] into the CSF) (Zetterberg, 2017a). In combination, these biomarkers are 85-95% sensitive and specific for AD in both mild cognitive impairment (MCI) and dementia stages of the disease. There are now fully automated clinical chemistry tests for these proteins and a global external quality control program (the Alzheimer’s Association QC program for CSF biomarkers) is in place. There are also certified reference methods and materials for CSF Aβ42 and similar work is ongoing for CSF Aβ40, T-tau and P-tau, which eventually will allow for fully standardized assays and the implementation of global reference and decision limits (Kuhlmann et al., 2017).

Although CSF biomarkers have been made part of the clinical criteria for AD (Dubois et al., 2014) and standardized operating procedures for CSF sampling, processing and interpretation have been published (Herukka et al., 2017; Simonsen et al., 2017), the CSF sampling per se (through lumbar puncture) may be regarded as a barrier towards large scale clinical implementation; undoubtedly, a blood test would be easier.
3. Blood biomarkers – general considerations

A few years ago, there was a lot of scepticism against blood-based biomarkers for central nervous system (CNS) disorders with a lot of irreproducible data being published. A major reason for the difficulties is the low concentration of CNS-derived proteins in the blood. In addition, if the biomarker is not CNS-enriched but also expressed in peripheral tissues, it may be challenging to determine if an altered concentration actually reflects what is happening in the brain or if it is secondary to systemic changes. This is relevant to several AD-related biomarkers, *e.g.*, Aβ that is also expressed in blood platelets and several other tissues of the body. Further, the high amount of other proteins in blood (*e.g*., albumin and immunoglobulins) may interfere in the assays (Apweiler et al., 2009). Blood may also contain endogenous antibodies directed against the non-human monoclonal antibodies of the assay, which may cause flawed results (Bolstad et al., 2013). Finally, the analyte of interest may undergo proteolytic degradation by various proteases in plasma (Yoshimura et al., 2008). This seems to be a problem for tau, which is stable in CSF but has a very short (~10 hours) half-life in blood (Zetterberg, 2017b).

The recent advent of highly sensitive and specific immuno- and mass spectrometry-based assays has infused the blood biomarker field with renewed enthusiasm. This has happened in parallel with better diagnostic work-up of patients included in the clinical cohorts in which biomarker discovery and validation is being made; in many of these, biomarker-supported diagnostics with CSF and/or amyloid PET are now used to ascertain that patients with clinical AD indeed have AD pathology and that the non-AD groups are AD pathology-free.

Reproducible AD-related biomarker changes in blood are now starting to emerge. It is hard to tell if the better reproducibility biomarker patterns are mainly due to technical improvements or improved diagnostic work-up; the fairest statement is probably that both factors likely have contributed. Below, I give an updated account of the current state of research on the most reproducible AD biomarkers in blood (summarized in Figure 1) and discuss the potential next steps in biomarker discovery, validation and clinical implementation.

4. Plasma Aβ

In the AlzBiomarker database (https://www.alzforum.org/alzbiomarker), there are plasma Aβ42 results from more than 2000 AD patients and 4000 controls, showing an increase, no change or decrease in 27 different studies, with an overall fold change between AD and
control groups of 1.031 (95% confidence interval 0.962 to 1.106) (Olsson et al., 2016). This negative result was for a long time interpreted to be inherent to the peripheral expression of Aβ; although brain expression is high, the Aβ precursor protein (APP) is produced and processed in most tissues of the body (https://www.proteinatlas.org/ENSG00000142192-APP/tissue). This notion was supported by the absence of a correlation of plasma with CSF Aβ concentrations (Hansson et al., 2010). However, it was also known that the spike-recovery and dilution linearity was low for plasma Aβ tests, suggesting matrix interferences in the measurements (Lachno et al., 2012; Okereke et al., 2009).

In 2011, an ultrasensitive digital enzyme-linked sandwich immunoassay (ELISA) for Aβ42 was published (Zetterberg et al., 2011). In this assay, the sample could be diluted to mitigate the matrix effects making the measurement more accurate. A correlation of plasma with CSF Aβ42 emerged and the improved analytical sensitivity clarified that the ratio of Aβ42 to Aβ40 in plasma was reduced in amyloid PET-positive individuals in a manner similar to CSF Aβ42/Aβ40, although with less good separation (Janelidze et al., 2016).

In parallel, research groups worked on developing mass spectrometry-based tests for total Aβ40 and Aβ42. A first immunoprecipitation mass spectrometry (IP-MS) assay was published in 2014 (Pannee et al., 2014). A non-significant trend towards a reduction in plasma Aβ42 could be seen in a small pilot study but the high volume (5 mL) required made it impossible to perform larger studies. The same year, promising results were published regarding the ratio of a certain APP fragment (APP669-711) to Aβ42 in plasma and its relation to Aβ pathology determined by amyloid PET (almost >90% diagnostic accuracy in 40 Aβ-positive and 22 Aβ-negative study participants) (Kaneko et al., 2014). In 2017, Ovod et al. showed a decrease in the plasma Aβ42/40 ratio determined using a more sensitive IP-MS method, optimised to extract total Aβ (Ovod et al., 2017). More recently, Nakamura and colleagues reported IP-MS results on the plasma Aβ42/40 ratio through which they could predict amyloid PET positivity in AD, MCI and cognitively normal populations at around 90% diagnostic accuracy (Nakamura et al., 2018).

In conclusion, we now have several independent studies suggesting that the plasma Aβ42/40 ratio, if determined using methods that measure Aβ with minimal matrix interferences, reflects Aβ pathology in the brain. The overall data suggest that the fold change in the ratio between Aβ-positive and –negative subjects might be slightly lower than for the
corresponding CSF ratio (Janelidze et al., 2017), but it may still work as a screening test, favouring sensitivity over specificity. Positive results could then be verified using amyloid PET or CSF Aβ42/40 ratio that are more specific. The next steps in the development of a reliable plasma test for Aβ pathology are head-to-head comparisons of the available assays, more replication studies in different clinical settings, as well as the development of reference methods and materials for assay standardization (provided the results continue to look promising). It will also be important to examine pre-analytical factors that might influence the performance of the marker.

Two additional plasma Aβ tests have recently been developed. One is an immunoprecipitation method that detects total Aβ conformation (not concentration) in plasma (Nabers et al., 2018). Using this method, amyloid PET-positive individuals have been reported to have a higher content of abnormally folded Aβ in their plasma than amyloid PET-negative individuals. The other method is based on quantification of plasma Aβ42 using a technique called immunomagnetic reduction (IMR). The method involves conjugation of an anti-Aβ antibody to magnetic particles, whereafter a change in the IMR upon addition of plasma and binding of Aβ42 to the antibodies can be sensed (Yang et al., 2011). Using the technique, an increase in plasma Aβ42 signal has been observed (Lue et al., 2017; Teunissen et al., 2018), i.e., a result that goes opposite to the mass spectrometry-based methods. One potential explanation for this result is that the technique might detect some sort of Aβ aggregate or Aβ bound to other proteins and that these may be present at increased levels in AD plasma. More replication studies are needed for both of these methods.

5. Plasma protein changes that relate to cerebral Aβ status

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 (Ashton et al., 2018). These changes may represent some kind systemic response to Aβ pathology but should be interpreted with caution, as they are derived from multi-marker panels and as a mechanistic understanding of the associations is currently lacking.
6. Plasma tau

CSF total tau (T-tau) and phosphorylated tau (P-tau) are well validated biomarkers for altered tau metabolism in AD (Olsson et al., 2016). They are stably increased already in the MCI stage of the disease and seem to reflect disease intensity rather than tau pathology for which the correlation is weak or absent (Zetterberg, 2017b). Recent tau kinetics data suggest that the concerted increase of CSF T-tau and P-tau concentrations in AD may be a neuronal response to Aβ (i.e., increased neuronal tau secretion in response to Aβ pathology) (Sato et al., 2018), rather than a direct reflection of neurodegeneration and tangle pathology. This interpretation resonates well with earlier mouse work showing increased CSF tau concentration in Aβ-positive animals in the absence of neuronal loss (Maia et al., 2013).

ELISAs for T-tau have been transferred onto the Single molecule array (Simoa) platform, which allows for the ultrasensitive measurement of tau in blood (Randall et al., 2013). More assays are in development with a lot of additional data expected within the next few years. Plasma T-tau concentrations, measured using currently available methods, correlate poorly with CSF (Zetterberg et al., 2013), but in acute hypoxic brain injury, a biphasic release of tau into the bloodstream has been observed with a first peak occurring during the first few hours post-injury and a second broad peak occurring after a few more days; these increases are predictive of outcome (Randall et al., 2013). Similar acute changes (within hours) have been seen in concussion (Shahim et al., 2014) and during anaesthesia (Evered et al., 2018).

In the dementia stage of AD, plasma tau concentrations are slightly increased compared with cognitively normal control individuals, but not as clearly as in CSF (Zetterberg et al., 2013), which is a well-replicated finding (Olsson et al., 2016). The findings in the MCI stage of the disease are less clear (Mattsson et al., 2016). Nevertheless, in a recent paper, Mielke and colleagues examined the relationship of plasma T-tau concentration, determined by Simoa, with cognitive decline in 458 participants from the Mayo Clinic Study on Aging (Mielke et al., 2017). High plasma T-tau predicted steeper decline in global cognition, memory, attention and visuospatial ability over three years in both the cognitively normal and MCI groups. High plasma T-tau also predicted progression to MCI or AD but the overlap in concentrations was large and the number of converters small.

In regards to P-tau, a semi-sensitive assay for tau phosphorylated at threonine 181 (similar to the most employed CSF test) with electrochemiluminescence detection has been developed
(Mielke et al., 2018). Using this assay, plasma P-tau concentration was higher in AD dementia patients than controls. Plasma P-tau concentration was associated with both Aβ and tau PET and more AD-associated than the corresponding plasma T-tau test, which are promising results in need of replication.

The expression of tau is brain-enriched, but tau is also detectable at both mRNA and protein level in salivary glands and kidney (http://www.proteinatlas.org/ENSG00000186868-MAPT/tissue). This is an important potential confounder that may help explain the poor correlation of plasma with CSF tau. The half-life of tau also appears to be much shorter (~10 hours) in plasma (Randall et al., 2013) than in CSF (weeks) (Sato et al., 2018), suggesting that the protein may be enzymatically degraded in blood.

Another novel way of exploring tau as a potential biomarker in blood is to enrich for neuronally derived exosomes that are then lysed and examined for tau protein content. Higher T-tau and P-tau protein concentrations in exosomal extracts were reported in a couple of papers (Fiandaca et al., 2015; Winston et al., 2016), but the results have been hard to replicate (Shi et al., 2016), and the approach requires more research before a conclusion on its potential usefulness can be made.

### 7. Plasma neurofilament light

CSF neurofilament light (NfL) is a biomarker of neurodegeneration that is increased in most neurodegenerative diseases and correlate with longitudinal imaging findings of neurodegeneration (Khalil et al., 2018). In the AlzBiomarker database (Olsson et al., 2016), it stands out as the second most AD-associated biomarker, but it is important to remember that it is not disease-specific; increased CSF NfL concentration is seen in all neurodegenerative diseases (Khalil et al., 2018), which contrasts CSF T-tau that is surprisingly AD-specific.

Serum or plasma NF-L concentration (either sample matrix works well) correlates with CSF (correlation coefficients of 0.75 to 0.97) and most CSF findings (increased NF-L concentrations in AD, FTD, VaD and atypical parkinsonian disorders) have been replicated in blood (Zetterberg, 2016). Recent data show that serum NfL effectively identifies onset of neurodegeneration in familial AD (Weston et al., 2017) and Huntington’s disease (Byrne et al., 2017) and correlates with longitudinal measures of disease intensity/neurodegeneration in AD (Mattsson et al., 2017), frontotemporal dementia (Meeter et al., 2016), progressive
supranuclear palsy (Donker Kaat et al., 2018; Rojas et al., 2016) and Huntington’s disease (Johnson et al., 2018). Plasma NF-L concentration is increased in patients with Charcot-Marie-Tooth disease and correlates with disease severity, suggesting that peripheral nerves may also release NF-L (Sandelius et al., 2018). This could potentially smear the association of plasma NF-L with central axonal degeneration, but the robust correlation of plasma-serum NF-L with CSF NF-L suggests that most of the NF-L signal in blood is CNS-derived (Gisslen et al., 2016; Kuhle et al., 2016; Ljungqvist et al., 2017; Rojas et al., 2016), at least in the absence of significant peripheral nerve disease. In multiple sclerosis, serum NfL normalizes in response to effective treatment (Disanto et al., 2017; Novakova et al., 2017), suggesting that the marker can be used to detect treatment effects of disease-modifying therapies across the neurodegenerative dementias; a treatment that does not lower serum/plasma NfL over 6-12 months has probably not had any beneficial impact on the neurodegenerative disease process.

8. Conclusions and future perspectives
The blood biomarker field has seen a virtual explosion of intriguing results during the past few years. The progress is probably due to both technological advances in the field of ultrasensitive assays and better characterized patient cohorts with molecular endophenotypes reflecting AD pathology. The progress is timely, as we now have greater hope to see disease-modifying treatment breakthroughs in the near future after years of disappointing results. Disease-modifying treatments are likely to be most effective if initiated as early as possible and the development of blood biomarkers for AD may find clinical utility quite rapidly. The most robust blood-based biomarker at the moment is plasma-serum NfL that reliably reflects neurodegeneration in AD and other neurodegenerative dementias. It is not a disease-specific biomarker but could be used in combination with other biomarkers, e.g., a reliable blood test for Aβ pathology. Also here, the results look promising with a consensus emerging that a reduced plasma Aβ42/40 ratio reflects cerebral Aβ pathology with quite good diagnostic accuracy, although more studies are needed on the topic. Also for plasma T-tau and P-tau, results look reasonably promising. The rapid turnover of tau in the bloodstream may, however, be a problem and we should continue to look for tau fragments or proteoforms that more reliably reflect what is going on in the brain.

A potential upcoming scenario (depending on the mechanism of action, effectiveness, costs and side effects of future drugs) could be individualized risk stratification using polygenic risk scores; high risk individuals could then be followed using biomarkers to detect onset of
pathology (preferably using a blood test and, if needed, CSF and imaging). Onset of pathology should then lead to personalized treatment and biomarker-based verification of a treatment-induced reduction of the pathology using biomarkers. In this context, reliable blood tests would save costs for the society and make it much easier for both patients and the healthcare professionals to monitor the efficacy of the selected treatment.

**Acknowledgements**

HZ is a Wallenberg Academy Fellow supported by grants from the Swedish Research Council, the European Research Council, the Wellcome Trust, the Olav Thon Foundation, the Swedish Brain Foundation and the UK Dementia Research Institute at UCL.

**Conflicts of Interest**

HZ has served at scientific advisory boards for Eli Lilly, Roche Diagnostics, Wave, Samumed and CogRx, has received travel support from Teva and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg.

**References**


Figure legend

Schematic illustration of a neurofibrillary tangle-bearing neuron with para-synaptic amyloid β (Aβ) plaques. Arrows indicate candidate plasma biomarkers for neurodegeneration (neurofilament light [NfL] and total tau [T-tau]), Aβ plaque pathology (Aβ42/40 ratio) and tau phosphorylation/tangle pathology (phospho-tau [P-tau]).