Editorial

Amyloid blood biomarker detects Alzheimer's disease prior to clinical onset

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Alzheimer's disease (AD) is the principal cause of dementia and impacts nearly 50 million people worldwide (1). This neurodegenerative disorder of the brain manifests as progressive loss of memory and decline in cognitive function, which may span three to twenty years. Currently diagnosis is based on clinical assessment using a standardized Mini-Mental State Examination (MMSE) (2), which may be supported by further tests, when available, including analysis of cerebrospinal fluid (CSF) and/or imaging of the brain by positron-emission tomography (PET) to quantify alterations in particular biomarkers (3). These latter procedures are costly, and may induce pain and/or stress to patients. Moreover they are not applicable as a routine preventative screen to detect people at risk of developing the illness and who could most likely benefit from lifestyle changes and trials of novel disease-modifying therapies. Recent studies have revealed a preclinical phase of ten years, when changes can be observed in the brain before the appearance of serious memory complaints, which is followed by a prodromal phase of another ten years when cognition begins to decline and which is referred to as mild cognitive impairment (MCI), and then, the final phase when AD symptoms become incapacitating (4). It is therefore postulated that early therapeutic intervention may halt disease progression. To this end, a simple and cost-effective biomarker is crucial to identify patients at the pre-symptomatic stage. A new study by Nabers *et al.* reports a blood-based biomarker that can detect AD eight years before clinical onset (5). Before discussing this finding, we will review the current AD diagnostic tools.

Today's research into AD biomarkers draws from the molecular constituents of the amyloid plaques and neurofibrillary tangles (NFT), which were identified as the pathological hallmarks of the disease by Alois Alzheimer over a century ago. The main components of amyloid plaques are peptide fragments, termed Aβ, which are proteolytically derived from the amyloid precursor protein (APP)—a receptor-like protein involved in neuronal development, maintenance and repair (6). The primary Aβ peptide that is deposited in amyloid plaques is comprised of 42 amino acids (Aβ42), compared to another peptide, Aβ40 that is more abundantly produced but less prone to aggregation and accumulation (7). NFT consist of twisted paired filaments of tau proteins, which are scaffolding proteins that consolidate microtubules, the major elements of the neuron's skeleton (8). In AD, tau undergoes hyperphosphorylation, which causes it to unravel from the microtubules, and thereby destabilize axonal structures. Furthermore, hyperphosphorylated tau self-associates and forms tangles that disrupt axonal transport and block synapses, ultimately leading to neuronal death.

PET using tracers such as the Pittsburgh Compound-B [PIB] has allowed visualization of the Aβ load in the brains of people with AD, and has helped to establish a cut-off value to separate patients from healthy matched controls (9). Longitudinal studies on aging have demonstrated that amyloid accumulation in frontal and parietal cortices occurs two decades prior to the observation of a marked cognitive decline, and therefore a PIB-PET positive score is considered to be a preclinical marker of AD (10). Tau imaging has also been recently developed, and has shown that the accumulation of this protein in the temporal lobe provides a

reliable marker of dementia progression (11). However, PET imaging remains too expensive for routine testing.

Quantitative analyses of Aβ and tau in CSF have offered alternative and complementary diagnostic tools. The accumulation and deposition of Aβ42 in the brain is exemplified by a decrease in Aβ42, and in its ratio to Aβ40 in the CSF (12). Indeed, an inverse correlation has been established between CSF Aβ42/Aβ40 and PET analysis of Aβ load in the brain (13). Changes in tau and 181-phospho tau have also been widely investigated as CSF biomarkers (14). An increase in the ratio of phosphorylated tau (P-tau) versus total tau (T-tau) is observed in AD sufferers, but is also associated with other types of dementia. The best diagnostic value is obtained when combining Aβ42, T-tau and P-tau, which have been termed the core AD biomarkers in CSF (15). CSF T-tau/Aβ1-42 and P-tau/Aβ1-42 data are in high concordance with PET analysis (16). Positive CSF core biomarkers can also predict a greater 2-year cognitive decline in patients with MCI (16). Adding Aβ42/Aβ40 ratio to the core biomarkers can help to distinguish AD from other types of dementia (17). But although CSF analysis can achieve excellent diagnostic performance, it is not routinely used because it requires a lumbar puncture, which is an invasive procedure and necessitates trained medical professionals.

In attempt to find a simple, minimally invasive and inexpensive test for the detection of AD, research groups have examined the components of blood. The detection of Aβ in both platelets and plasma has spurred researchers to investigate if changes that occur in the brain of AD patients could be evaluated in blood (18,19). This has proved challenging and major hurdles were encountered in the quantification of Aβ peptides in plasma, due to their very low concentrations compared to the brain. Considerable technological advances over the last few years have finally led to breakthroughs, and three recent studies support that changes in Aβ are indeed detectable in the blood of AD patients, even at the preclinical and prodromal stages.

Dr Bateman and colleagues were the first to describe a blood biomarker based on Aβ detection, which could discriminate between AD patients and controls. They examined the turnover of Aβ peptides in plasma of 41 people aged over sixty, 18 of which were PIB-positive, and 23 were PIB-negative. They immunoprecipitated the Aβ peptides from plasma, and analysed them by limited proteolysis and mass spectrometry (MS). The results showed that the ratio of Aβ42/Aβ40 concentrations in plasma was very stable over twenty-four hours, and that this ratio was significantly lower in the group of participants who tested positive in Aβ imaging, compared to those who tested negative. A receiver-operating characteristic (ROC) curve was established, and a threshold value was set to separate the two groups, with an area under the curve (AUC) of 89%. Furthermore, the ratio of Aβ42/Aβ40 concentrations in plasma was strongly correlated with their ratio in the CSF (correlation coefficient =0.7). The exciting results of this pilot study supported that an Aβ-based blood test may be achievable to detect AD at the preclinical stage.

Following on from this, early this year Nakamura and colleagues reported a composite blood biomarker that could identify people with preclinical AD within two cohorts of older adults (20). Using a similar approach, they subjected plasma samples from individuals with AD, MCI, and cognitively normal controls to immunoprecipitation coupled with MS analysis (IP-MS) to quantify APP669-711 fragment, as well as the Aβ40 and Aβ42 peptides. This technology was applied to samples from the Japanese Centre for Geriatrics and Gerontology, named the NCGG discovery cohort (N=121), and samples from the Australian Imaging Biomarker and Lifestyle study, the AIBL validation cohort (N=251). The IP-MS results showed significant differences, in both cohorts, between individuals who tested positive or negative in Aβ-PET, with Aβ42 being significantly lower on average in patients who tested negative on PET, whilst ratios of APP669-711/Aβ42 and Aβ40/Aβ42 were higher. A composite biomarker of APP669-711/Aβ42 relative to Aβ40/Aβ42 was optimal at discriminating between Aβ-PET positive and Aβ-PET negative individuals, with more than 90% accuracy. The composite biomarker showed an excellent performance in ROC analysis, with an AUC of ~95%.

A more recent study published by Nabers, Perna, and colleagues demonstrates the predictive value of an Aβ-blood biomarker in preclinical individuals (5). The researchers developed a new technology that takes advantage of the conformational change undergone by amyloid peptides in AD, from α-helix and random coil structures to a β-sheet; a rearrangement that is known to mediate Aβ aggregation as toxic oligomers, followed by deposition in the brain parenchyma to form amyloid plaques (21,22). As subtle differences in the vibration of the carbonyl groups of amide bonds between peptides displayed in α-helix or β-sheet structures can be picked up by a narrow spectrum of infra-red (IR) rays (23), the authors applied IR spectroscopy to record structural changes of Aβ peptides in the blood of patients with AD (24). Their approach consisted of using a sensor matrix functionalized with an antibody to capture soluble Aβ peptides from biological fluids—including

both Aβ40 and Aβ42—and then applying attenuated total reflection Fourier Transform Infrared analysis (ATR-FTIR), which enables to study the conformation of peptides in their natural state in aqueous environment. The group led by Dr Gewaerts first employed this technology to analyse CSF and plasma samples from a German cohort comprised of three groups, including patients with MCI, patients with moderate Alzheimer's-type dementia (DAT), and cognitively normal controls (25). They observed a shift of the Aβ IR-absorbance curve in the samples from DAT patients compared to the controls, and showed that Aβ-IR shift measurements could be related to the CSF core biomarkers, with an accuracy of 90% in CSF, and 84% in plasma. The accuracy of the assay was lower when it came to distinguishing patients with MCI from the controls, reaching 79% in CSF, and 70% in plasma.

In their latest study, they have further investigated the diagnostic power and predictive value of the Aβ IR-shift assay as a blood biomarker in well-characterized cohorts (5). First they analysed plasma samples from the BioFINDER study, a well-characterized Swedish cohort, and compared data from 36 patients with MCI and 37 age-matched controls with no memory complaints. The results showed that Aβ-IR shift assay could discriminate between patient and control groups with a sensitivity of 69% and specificity of 86%, as well as a good predictive value for those who tested positive in the PET scan (AUC =0.78%). Significant correlations were established between data from the Aβ-IR shift assay and the measurements of Aβ42, Aβ42/Aβ40 ratio, as well as T-tau and P-tau in CSF. Furthermore, data from the plasma Aβ-IR shift were also correlated to the density of Aβ brain load, as determined by PET analysis.

Next they asked if the Aβ-IR shift assay might help to predict AD conversion in asymptomatic individuals. To this end, they analysed samples from the ESTHER longitudinal study. The ESTHER cohort consists of approximately 10,000 aged adult participants who were enrolled in Germany in 2000–2002, and had blood collected at baseline, and then every three years for an average follow-up of eight years. The authors chose samples from a nested study, which included 970 participants, among which 195 people had developed dementia during the course of the study. 70 of the latest were given a diagnosis of AD, 85 of vascular dementia (VD), and 40 of mixed dementia (MD). Participants who did not show any sign of cognitive impairment by the end of the study were assigned to each patient group, so as to optimize matching for age, gender, education and medical history. After excluding haemolytic samples and patients with a revised dementia diagnosis, the Aβ-IR shift assay was applied to baseline samples from 65 patients who developed AD during the course of the study and 247 well-matched controls. A threshold value was established, which could discriminate between the two groups, with 71% sensitivity, 91% selectivity, and a diagnostic accuracy of 86%. The assay was further validated by showing that prevalence of ApoEe4 carriers, among those who tested positive, was more than twice that of non-carriers, which is consistent with this ApoE allele being a risk factor in AD. Overall this study demonstrates the proof-of-principle that changes in Aβ conformation can be detected in the blood of AD patients before the onset of clinical symptoms. The Aβ immuno-IR shift assay could help to predict AD on average eight years prior to a clinical diagnosis, and also independently from VD and MD. The positive likelihood ratio was 7.9, indicating that those who had tested positive at baseline had a 7.9 times greater chance of developing AD than those who had tested negative. Technological adjustments will be required to improve both the sensitivity and accuracy of this assay. If these issues can be resolved, the IR-shift test may compete favourably with IP-MS assays, in regard to the cost and complexity of technology.

In conclusion, we are witnessing a leap forward in the development of blood biomarkers for AD. At present the highly technical skills, elaborate reagents and instrumentation required to assay these markers limit their broad application. But despite these limitations, the new Aβ blood tests offer useful tools to help researchers identify patients at the preclinical stage of the illness and who may be suitable candidates for clinical trials of novel disease-modifying therapies, such as Aβ and tau immunotherapies, or gamma-secretase modulators and BACE inhibitors. It is reasonable to expect that in the near future, blood tests will become available as a routine screen for people over 50, so that therapeutic interventions can be commenced early and contain AD progression.

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Footnote

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