Biomarkers of Mild Cognitive Impairment and Alzheimer’s Disease
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Abstract
Alzheimer’s disease (AD) is currently diagnosed only via clinical assessments and confirmed by postmortem brain pathology. Biochemical and neuroimaging markers could facilitate diagnosis, predict AD progression from a pre-AD state of mild cognitive impairment (MCI), and be used to monitor efficacies of disease-modifying therapies. It is now clear that cerebrospinal fluid (CSF) levels of Aβ40, Aβ42, total tau and phosphorylated tau have diagnostic values in AD. Measurements of the above CSF markers in combination are useful in predicting the risk of progression from MCI to AD. Recent advances further support a notion that plasma Aβ levels, expressed as an Aβ42/Aβ40 ratio, could also be of value. New potential biomarkers are emerging, and CSF or plasma marker profiles may eventually become part of the clinician’s toolkit for accurate AD diagnosis and management. These biomarkers, along with clinical assessment, neuropsychological testing and neuroimaging could achieve a much higher diagnostic accuracy for AD and related disorders in the future.

Key words: Alzheimer’s disease, b-amyloid (Ab), Biomarkers, Mild cognitive impairment (MCI), Tau

Introduction
Alzheimer’s disease (AD) is the most prevalent form of age-related dementia in the modern society. Other than symptomatic treatment with acetylcholinesterase inhibitors at its earlier stages, no disease-modifying strategies are currently known. AD diagnosis relies on clinical assessments and is often possible only when full dementia has set in. Reliable confirmation of AD diagnosis further depends on unhelpful postmortem brain pathology. Full AD dementia is usually preceded by a stage of cognitive decline, particularly with amnesia. This preclinical or prodromal AD state has been conceptualised as mild cognitive impairment (MCI), which has gained much attention as an ideal target for prevention and early intervention.

The term MCI is defined as a transitional clinical state between normal ageing and very mild AD. The diagnosis of MCI as a stable and valid concept in the community settings has been challenged by other researchers. Not all individuals with MCI progress to AD, and the ability to predict which ones would see such a progression greatly facilitate the diagnosis of AD over normal ageing-associated cognitive impairment as well as other forms of dementia. It is clear that the discovery of clinically useful and robust biomarkers for AD and pre-AD are necessary for clinicians to accurately diagnose AD or predict conversion of a preclinical state of AD.

Alzheimer’s Disease Pathology and Biomarker Candidates
Age-related, late-onset AD is largely idiopathic, and has 2 distinct pathological features – extracellular amyloid plaques and intracellular neurofibrillary tangles. The amyloid cascade hypothesis’ posits that the extracellular amyloid plaque, consisting of aggregated beta-amyloid (Aβ) peptide generated from proteolytic cleavages of the amyloid precursor protein (APP), damages brain regions and precipitates AD symptoms. An extension of this hypothesis is now necessary, as new findings indicate that both intracellular and extracellular soluble oligomeric forms of Aβ could result in synaptic malfunctions and the onset of AD.
Aβ generation from APP occurs when the β-site APP-cleaving enzyme (BACE-1) cleaves the ectodomain of APP to first generate a membrane-bound C-terminal fragment. Another subsequent cleavage by γ-secretase activity further generates peptides, mainly of 40 or 42 amino acids in length, termed Aβ40 and Aβ42. Both species could be found in amyloid plaques, but the latter is more directly neurotoxic and has a greater propensity to aggregate, while the former may actually be neuroprotective. Known genetic predispositions to early-onset AD include mutations in APP and the presenilins (part of the γ-secretase complex), and all of which increase Aβ generation or Aβ42/Aβ40 ratio. Other than APP and presenilin mutations, the e4 allele of apolipoprotein E (ApoE) also constitute a risk factor for late-onset AD.

NFT, on the other hand, are intracellular filamentous aggregates of the microtubule binding protein tau. In AD and other tauopathies tau becomes hyperphosphorylated and accumulates into insoluble paired helical filaments, which aggregate into NFT. Like Aβ, NFT levels correlate well with neuron loss and cognitive impairment in AD patients. The trigger for tau hyperphosphorylation and aggregation in AD is yet unclear, although it would presumably involve an aberrant imbalance of tau phosphorylation and dephosphorylation, and which could be facilitated by Aβ.

An AD biomarker should ideally have the following features. It should detect a fundamental feature of AD neuropathology, with results that could be validated in neurologically confirmed cases. It should have a sensitivity of >85% and a specificity of >75%, and should be precise, reliable, inexpensive, convenient and with low risk to patients. Such idealised biomarkers may never be discovered. However, even those that partially fulfilled the above criteria would aid both predictive AD diagnoses from MCI presentations, as well as the monitoring of efficacies of disease-modifying therapies on trial. Recent advances have reaffirmed that both cerebrospinal fluid (CSF) Aβ and tau could serve as biomarkers for AD, and that plasma Aβ profiles, if appropriately measured, could also be promising. We highlight some of these findings in the paragraphs below, and discuss the possibility of applying these biomarker measurements to both cross-sectional and longitudinal cohort studies in Singapore.

Cerebrospinal Fluid Aβ and Tau as Biomarkers of AD and Incipient AD

The Aβ40 and Aβ42 peptides mentioned above are found in amyloid plaques and could form synapse-damaging oligomers. In the CSF, Aβ40, Aβ42 and other minor forms of peptides generated from APP (e.g., Aβ37 and Aβ38) could be detected and measured by immunochemical methods (such as ELISA) or liquid chromatography-mass spectrometry. Likewise, tau (in its hyperphosphorylated forms), the principle protein in the neurofibrillary tangles, could be detected in CSF. A majority of findings have, however, indicated that CSF levels of Aβ40 exhibited no significant differences, and have a large degree of overlapping, between AD patients and controls. On the other hand, Aβ42 levels were generally noted to be decreased in AD patients, up to about half of that of controls. This parameter has both sensitivity and specificity in differentiating between AD patients and cognitively normal controls, but not between AD and other forms of dementia (e.g., vascular dementia, Creutzfeldt-Jakob disease and fronto-temporal dementia). This is likewise the case for total tau. More recent works have analysed specific phosphorylated forms of tau using phosphor-epitope-specific antibodies, and the results are also largely indicative of a high level of sensitivity and specificity.

Encouragingly, recent studies have also indicated that both Aβ42 and tau have significant predictive powers in cases of MCI progressing to AD. In particular, simultaneous measurement of CSF levels of Aβ peptides and tau, and expressing these levels as various ratios, help to increase the sensitivity and specificity of prediction. There are also studies with findings that indicate the ability of these markers to tell AD apart from other forms of dementia. CSF Aβ and tau could also be promising antecedent biomarkers that could predict the future development of dementia in cognitively normal older adults.

Plasma Aβ and AD

CSF collection is invasive and unlikely to become a routine procedure in geriatric clinics. Finding peripheral biomarkers for AD is therefore of great interest. However, the levels of tau in the plasma are too low for any useful analysis. Aβ levels, while detectable, are also at least a magnitude lower. Earlier studies did not reveal significant diagnostic values for plasma Aβ peptides. Unlike changes in the CSF, reports of changes in Aβ levels in AD and pre-AD are rather inconsistent, and plasma levels do not necessarily reflect that in the brain. In spite of these difficulties, several recent reports have now increased the confidence that plasma Aβ may be of diagnostic value.

The Rotterdam Study is one of the largest ongoing prospective population-based cohort studies on the incidence and risk factors for age-related diseases, unique both in terms of its size and long-term follow-up. Van Oijen and colleagues found in this cohort an association between high Aβ40 and low Aβ42 levels and risk for AD dementia. Another study, which compared plasma Aβ42 levels of 146 sporadic AD patients, 89 subjects with MCI and 89 age-matched controls found that a reduction in Aβ42 is predictive for AD, and specifically, a transition from a normal state of cognition or MCI to AD. A recent report
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Emerging Biomarkers for MCI and AD

Several other candidates for AD biomarkers have shown some initial promise but have not received as much attention as Aβ and tau. Lipid peroxidation is known to be a rather early event in AD,⁵⁷ and the measurement of CSF F2-isoprostane levels may, in combination with other parameters, serve to predict AD.⁵⁸ A rather accessible peripheral marker is platelet amyloid precursor protein (APP) isoform. An altered pattern of platelet APP isoform, manifested by a reduced ratio of the upper (130 kDa) to the lower (110 to 106 kDa) APP band, is associated with AD.⁵⁹ Interestingly, this platelet APP isoform ratio has also shown some documented predictive power in terms of MCI to AD progression.⁶⁰,⁶¹

Advances in the techniques of whole proteome analysis have naturally prompted the search of AD biomarkers using this particular approach.⁶² Comparative proteomics analysis with the aim of discovering proteins whose levels might be altered in diseased compared to control states, have been largely focused on CSF.⁶³-⁶⁶ The outcome of such analysis could be the identification of not 1 or 2, but a panel of proteins whose levels may be altered between normal controls, MCI subjects and cases of clinically diagnosed AD dementia.⁶⁷,⁶⁸ This sort of profiling analysis could increase sensitivity and specificity, particularly in discriminating AD from other forms of dementia.

Although plasma biomarkers are more clinically useful, there are potentially confounding problems with biomarker mining. One prominent problem is that a small number of abundant proteins [such as albumin, α2-macroglobulin (α2-M) and immunoglobulins] could account for as much as 80% of the total protein in plasma. The large quantity of these proteins makes it difficult to identify low-abundance proteins in serum using traditional 2-dimensional electrophoresis. One way to go about this is to selectively deplete these abundant proteins, but there is a possibility that candidate biomarkers, already in low abundance, could be co-depleted. In view of the above difficulties, a recent report on novel possible plasma biomarkers discovered by a proteomics approach is of interest.⁶⁹ The authors reported that the levels of 2 proteins, complement factor H (CFH), and an abundant serum protein, α2-M, are elevated in AD plasma. Preliminary analysis suggests that these elevations may correlate with disease severity. Both proteins have at least some remote relationship with AD pathology. α2-M can be induced by inflammatory cytokines, is present in amyloid plaques, and competes with ApoE for the same receptor. CFH has also been previously shown to be present in plaques, and its encoding gene has been strongly associated with age-related macular degeneration, which shares some pathological features with AD. Although further studies will be necessary to fully validate the usefulness of α2-M and CFH as AD or pre-AD biomarkers, the fact that these could be analysed in plasma makes them attractive from a clinical sampling point.

While there is a growing interest in studying biomarkers, there is still a scarcity of data in this area. It is also important to recognise that at this stage, biomarkers can only be used as a research tool and the data from small clinical populations on these still cannot be generalised to the general population. A way to move forward is to study existing biomarkers as well as to develop newer ones and test them in large representative samples of AD subjects. In order to be used widely as a diagnostic or prognostic marker, a candidate marker has to be studied both in clinical as well as epidemiological settings. Ideally, such studies should not only be collaborative in nature but should also include excellent clinical and neuropsychological assessments, cutting-edge neuroimaging and neurophysiological techniques, and genetic and genomic studies. Ultimately, instead of relying heavily on solitary markers alone, only a combined multimodal approach is going to be effective.

Studying MCI and AD in Singapore’s Ageing Population

Singapore is entering into an exciting time of neuroscience research. Policy makers and international advisory panels have rightly identified neuroscience research as an important strategic area of development, and have encouraged both clinical as well as basic scientists to work hand-in-hand to tackle age-related neurodegenerative diseases. Singapore
has an ageing population that would facilitate community-based, cohort studies. One such ongoing ageing cohort study is the Singapore Longitudinal Ageing Study (SLAS). There are also much interest and expertise in longitudinal monitoring of the cognitive impairment after stroke and vascular dementia, as well as preclinical AD.

There is clearly a growing interest among clinicians and basic scientists to tap on each other’s expertise in the area of ageing neurobiology research. Such collaborations between geriatricians, neuroimaging specialists, neuropsychiatrists as well as molecular and cellular neurobiologists are being fostered. Streamlining research initiatives in a way that would maximise subject resources, data acquisition and multifaceted analyses should be of high priority. The prospect of seeing how the above CSF and plasma biomarkers correlate with the clinical findings, stratified ethnically, is an exciting one.

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REFERENCES


