Blood Biomarkers for Alzheimer's Disease

a report by
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Alzheimer's disease (AD) is a multifactorial and heterogeneous disease in both its clinical and histopathological appearance. In more than 99% of cases the cause of the disease is not understood. Independent of its cause, AD is clinically characterised by a developing dementia and histopathologically characterised by neuronal degeneration. Although the presence of neurofibrillary tangles (NFTs) and neurotic (senile) plaques are the characteristic hallmarks in the AD brain, AD histopathology shows considerable qualitative and quantitative heterogeneity. A definitive diagnosis has to await a *post mortem* biopsy, when a histopathological examination can be performed. Therefore, the clinical diagnosis today is made primarily by excluding other causes of dementia.[1]

AD is becoming a major health problem in the developed world as life expectancy increases, and the disease affects about 15 million people worldwide today.[2,3] The prevalence of AD is expected to rise dramatically in the next few decades and it is estimated that 20–30 million people just in the US will be living with the disease by 2030.[4] Concentrated efforts are under way to identify reliable cures or preventative measures for the disease. To facilitate these investigations, biomarkers are critically needed that can reliably detect the disease at the earliest possible stage.[5]

In AD, the main cause of dementia is assumed to result from the progressive loss of synaptic function and neurological degeneration.[6] The disease is associated with profound biochemical and pathological alterations in the brain, including aberrant amyloid precursor protein (APP), amyloid β-protein (Aβ) metabolism, tau protein phosphorylation, oxidative stress, inflammation and lipid dysregulation. NFTs and senile plaques are the neuropathological hallmarks of AD and were described by Alois Alzheimer as early as 1906. Senile plaques and NFTs, although not individually unique to AD, have a characteristic spreading and density in the diseased brain.[7] The plaque is an extracellular lesion composed mainly of amyloid peptides with 40 or 42 amino acids, designated as Aβ40 or Aβ42. Aβ42 is the initial and more toxic species deposited in the brain and is also fibrillogenic *in vitro*.[8–10] Conversely, NFTs are intracellular lesions composed mainly of paired helical fragments of highly phosphorylated and aggregated tau protein.[11] The tau protein is a normal and essential component of neurons and it is the incorporation of excess phosphate groups that leads to the formation of the aggregated tau protein.[11]

The development of biomarkers for AD is challenging as it is complicated by several factors. In addition to the variability in clinical features and multiple molecular aetiologies, the development of AD biomarkers is burdened with a diagnostic imprecision as confirmation of the disease preferentially has to await a *post mortem* histopathological examination. The long asymptomatic prodromal stages, rates of progression and complex disease genetics complicate the situation further. In this article we will review the current developments in the field of biomarkers for the detection of AD in blood.

Single-component Biomarkers

The physiology of the blood–brain barrier limits potential biomarkers that are closely associated to brain pathophysiology to small molecules, lipophilic molecules and molecules with specific transporters.[12] Brain-derived proteins and metabolites that pass into the plasma will also become markedly diluted in a biochemically complex medium.[12] Moreover, it is not known whether there are any direct patho-physiological processes associated with AD in blood cells. The traditional approach of using one or a few closely related molecules as a biomarker, a single-component biomarker, in plasma, serum or blood has been utilised since the late 1990s.[13–15] However, their usefulness has been limited, mainly due to discrepant results between studies.

Amyloid β-protein

Aβ can be detected in plasma and is thus a compelling candidate biomarker for AD. The plasma total Aβ or Aβ42 was increased in familial AD with presenilin or APP mutations[16,17] and in Down's syndrome with APP triplication,[18] which raises the possibility that sporadic AD may also be associated with detectable and diagnostic changes in Aβ plasma levels. Animal models suggest that Aβ can pass between cerebrospinal fluid (CSF) and plasma compartments,[19,20] but this has yet to be confirmed in humans. APP is also produced by platelets and is, therefore, an alternative source for the APP and Aβ pools found in plasma.

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Several studies have investigated plasma Aβ levels in AD.[13–15,21,22] Although one study showed an increase in Aβ levels,[22] the majority of studies have found no significant differences between AD and control cases.[13,14,16,17,23] Increased Aβ40 and sometimes Aβ42 correlate strongly with age.[15,23] A broad overlap in plasma Aβ levels between AD and control cases suggests that plasma Aβ cannot reliably differentiate sporadic AD from control cases. Although not useful for diagnosis, plasma Aβ measurement could be evaluated in the context of AD prediction, progression and therapeutic monitoring. Studies have suggested that high plasma Aβ levels are a risk factor for developing AD.[22,24,25] In one of the studies, plasma Aβ42 declined more rapidly over three years in individuals who developed AD.[21] In other studies, no correlation between plasma Aβ levels and disease progression or severity was observed.[22,24] Interestingly, the results from a recent study suggest that an increased level of Aβ42 is an indicator of increased risk of developing AD. However, conversion to AD was accompanied by a significant decline in Aβ42 and a decreased Aβ42/Aβ40 ratio.[25] A dynamic change with a peak level of Aβ42 ahead of conversion to AD followed by a decline can help explain some of the discrepant results observed between different studies.

Markers of Inflammation

Amyloid deposition in the AD brain elicits a range of reactive inflammatory responses.[26] Whether the accumulation of cytokines and acute-phase reactants within the brain is also reflected in serum or plasma is not straightforward because many of these proteins do not easily cross the blood–brain barrier. Alternatively, AD may be associated with a more widespread immune dysregulation that is detectable in plasma. There is some controversy in the literature regarding the measurement of immune mediators in AD serum or plasma. Inflammatory molecules including C-reactive protein (CRP), interleukin (IL)-1β, tumour necrosis factor (TNF)-β, IL6, IL-6 receptor complex, β1-antichymotrypsin and transforming growth factor (TGF)-β show inconsistent changes across studies, while other cytokines such as IL-12, interferon (INF)-α and INF-β remain unchanged.[27]

Multicomponent Biomarkers

Given the multiplicity of pathophysiological processes implicated in AD, the diagnostic accuracy may be further improved by combining several markers. The standard approach using only a single marker or a few related markers may not be enough to include all the variants of a heterogeneous disease such as AD.

Knowledge-based Approaches

Developing a multicomponent biomarker can be approached in two ways. It can be a 'knowledge-based' approach, incorporating known putative biomarkers, or it can be an unbiased survey of many hundreds or thousands of biomolecules. A few knowledge-based approaches have attempted to integrate data of selected molecules known to be involved in AD.[28] In one study, a panel of 29 serum biomarkers for inflammation, homocysteine metabolism, cholesterol metabolism and brain-specific proteins were evaluated. A model incorporating IL-6 receptor, cysteine, protein fraction β1 and cholesterol levels proved to be the best combination to discriminate AD from controls, although specificity to other cognitive disorders and Parkinson's disease was weaker.[28] In another study examining archived plasma samples, 120 different signalling proteins were evaluated. From these proteins, a model was generated that included 18 proteins, which predicted a test set of 42 AD and 39 non-demented controls with high accuracy (89%).[29] Although the number of samples tested was low, the

results indicate that the model may also be able to predict AD with a reasonable degree of specificity to other forms of dementia.[29] The model was also effective in predicting those mild cognitive impairment (MCI) patients who later converted to AD.[29]

Unbiased Approaches

Unbiased approaches have also been pursued to evaluate a broad range of proteins (proteomics), small-molecule metabolites (metabolomics) or transcripts (transcriptomics) in blood.

Proteins

A proteomic study in plasma identified more than 70 proteins using 2D electrophoresis (2D-PAGE).[30] The study included a limited number of samples, and further studies are needed to determine whether the identified proteins can be used as potential biomarkers for AD. Another study divided the 100 samples into two equal sets: a test set and a replication set. In the replication set, 27 proteins were present in different amounts in AD compared with control samples.[31] When including all identified proteins, 34 of the 50 samples were correctly predicted and gave a sensitivity of 56% and specificity of 80%.[31] The complexity of serum and plasma, imprecision in peak matching in mass spectroscopy and spot matching in 2D-PAGE and difficulties in assay standardisation make these approaches challenging, but advances in technology platforms and bioinformatics will allow broader applicability to diseases such as AD.[32]

RNA

The uniform chemical nature of RNA make transcriptome studies less of a challenge than both proteome and metabolome studies, and the potential use of blood-based gene expression profiling in the diagnosis of brain disorders has been described by several independent groups.[33–35] Extensive studies have shown that with careful control in the experimental design, the microarray data are reproducible both between labs and between experiments within a lab.[36] This is also true for realtime reverse transcriptase-polymerase chain reaction (RT-PCR).[37] A study by Sullivan et al.[38] evaluating the comparability of gene expression in blood suggested that whole blood shares significant gene expression similarities with multiple central nervous system (CNS) tissues. A supportive example of this is a recent study that showed that the Parkinson's-disease-linked β-synuclein gene was upregulated both in blood and in the substantia nigra of patients with Parkinson's disease.[39] Thus, there are studies supporting the idea that expression of a selected set of genes in blood has potential as multicomponent biomarkers for different brain diseases, including AD.

Several gene expression studies have been performed for AD biomarker discovery using blood as the clinical sample. A pilot study of 16 AD patients and controls using a complementary DNA (cDNA) microarray, including probes for 3,200 genes, identified a set of 20 candidate probes that showed an altered expression in AD.[40] Screening a set of 6,424 cDNA clones representing unique genes with RNA isolated from blood mononuclear cells from 14 AD and 14 controls, 19 upregulated and 136 downregulated genes common to both males and females were identified.[41] Clear gender differences were seen and many genes were differentially expressed in either males or females. No model for AD prediction was generated using these genes. In another pilot study including 19 AD patients and 24 healthy age-matched controls using 663 randomly picked cDNA clones, a set of 33 clones was able to generate a model that correctly predicted 34 out of 37 samples.[42] This study, with few samples and also few cDNA clones, should

be treated with caution; however, it indicates that a blood-based gene expression test for AD can be developed. The study provided the basis for initiating a more extensive whole-genome array analysis using 94 AD patients and a similar number of healthy controls in a training set. From this training set, a model was generated that was used to predict the diagnosis in an independent test set of 80 samples including 31 AD patients. The model predicted the disease with an accuracy of 87%, a specificity of 91% and a sensitivity of 84%. Of 27 samples with Parkinson's disease, 24 were correctly predicted as non-AD.[43] In this model, more than 1,200 gene probes were used. A selection of these gene probes has been converted to gene assays and used in studies using RT-PCR instead of microarray hybridisation. In these studies using independent sample cohorts, the gene assays retain the diagnostic information found with the gene probes. The number of gene assays in the model has been reduced to fit within a 96-assay format without significantly reducing the accuracy (81%).[43] This approach, using the expression pattern from many informative genes, has the potential to cover more of the multifactorial nature of AD than existing single or double biomarkers.

Biomarkers for the Future

In the field of biomarkers, there may be particular merit for the use of approaches that simultaneously assay multiple biological markers and their interactions.[41,44,45] These approaches have the potential to take into account the fact that AD is a multifactorial disease and that it is both clinically and histopathologically heterogeneous. Many of the biological characteristics of AD are also shared by other neurological diseases and neither the senile plaques and the NFTs are unique to AD, although they have a characteristic spreading and density in the diseased brain.[7] Biomarkers that include only one or a few of these processes are less likely to be AD-specific and sensitive enough to detect all subgroups of the disease.

The multicomponent biomarkers using blood samples, such as the proteome[29] and transcriptome[43] approaches, show promise to function as biomarkers for AD.[30,46] The use of a set of 18 polypeptides or a set of fewer than 96 gene-expression assays show both high specificity and sensitivity and have the potential to simultaneously measure changes in several biological processes associated with AD.

Although most biomarkers for AD use CSF as the clinical sample, it has to be remembered that, except for a few European countries, CSF is not routinely collected in the evaluation of AD. A biomarker in blood would clearly be more widely applicable and reduce the need for invasive, expensive or time-consuming testing. Not surprisingly, the two latest approaches for multicomponent biomarker discovery and development[29,43] have chosen blood as the clinical sample, and it is expected that future development of clinically useful biomarkers for AD will focus on this strategy.

Concluding Remarks

With the introduction of acetylcholinesterase inhibitors and an N-methyl-D-aspartic acid (NMDA) antagonist for the symptomatic treatment of AD, the importance of diagnostic markers for AD has been highlighted. Increased awareness of possible treatment options has also made patients seek medical advice at an earlier stage of the disease. As there is no clinical method that can either accurately identify AD in the early stages or identify at-risk cases, it presents the physician with a greater challenge and, therefore, diagnostic tools to aid the diagnosis of early AD would be of great importance. Such diagnostic markers will be of even greater significance when new drugs, with the promise of disease-arresting effects, show clinical effects. It is likely that these drugs will be more effective in the earlier stages of the disease, before neurodegeneration becomes too severe and widespread. The various forms of dementia are likely to respond differently to treatment, while new AD drugs may not benefit all types of dementia. Moreover, these new drugs may also have significant side effects; therefore, it is desirable that a biomarker for AD is able to differentiate between the types of dementia. ■

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