Towards Early Diagnosis of Alzheimer’s Disease

Geratology deals with the many clinical problems that are common in the elderly population, and many of these follow the orthodox pattern of clinical practice: symptoms begin; they consult a doctor who examines and orders appropriate investigations; and this identifies an underlying pathology which is well understood, and a treatment is instigated which cures or at least controls the problem for the long term. Tragically, Alzheimer’s disease does not follow this pattern. Patients characteristically have poor insight, and often attribute their early symptoms of amnesia to normal ageing. Consequently they present late, often only on the urging of their distressed spouse or children. A detailed history may reveal which other cognitive domains are affected, but there are no routine investigations which can help diagnose Alzheimer’s, and the emphasis is on excluding other causes of cognitive decline*. Having made a probable diagnosis of Alzheimer’s, the clinician can offer little comfort to the patient or their family, as current treatment options do not alter the natural history of the disease, and offer only modest objective improvements in cognition as measured by the mini-mental state examination, although they may provide bigger improvements in individualised functional outcomes.¹

However, the most devastating part of the interview is when the prognosis is discussed. The future holds an inexorable decline in cognition and ability to self-care, compounded by highly distressing changes in personality (suspiciousness and apathy), behaviour (dissimulation, sleep disturbance, aggression) and mood, with the possibility of persecutory delusions and hallucinations. The rate of this progression from diagnosis depends on the severity of disease at diagnosis, but of those with mild disease, 35% are in nursing homes by 2 years,² and while the range is large, most studies found a median survival of 5 to 6 years.³ Unfortunately many of these community studies do not account for length bias, which is responsible for cross-sectional studies over-representing those patients with slowly progressive disease. A recent community study from Canada found a median survival corrected for length bias of only 3 years.⁴ The misery this disease causes individual families cannot be overstated, but the economic costs to society are also huge, and the United Kingdom’s current strategies are inadequate to the task. This was highlighted by the collapse in June 2011 of Southern

*Other irreversible dementias: a history of cerebrovascular disease or ‘step-wise’ decline may point towards vascular dementia; visual hallucinations, parkinsonism, fluctuating awareness, REM sleep behaviour disorder and neuroleptic sensitivity suggest Dementia with Lewy Bodies. Psychiatric disease, particularly depression, may present as cognitive decline. Investigations generally seek to exclude the rare reversible causes and exacerbating factors: vitamin B12 deficiency, hypothyroidism and other metabolic derangements, chronic subdural haemorrhage, normal-pressure hydrocephalus, and alcohol abuse (which may not be reversible)
Cross Healthcare, the largest nursing home operator in the UK. More effective medical interventions for Alzheimer’s disease are desperately needed.

There have been many disappointments in the search for drugs and vaccines for Alzheimer’s, and this reflects the poverty of our knowledge about the underlying pathogenesis. Today Alzheimer’s disease has a very public profile, but 30 years ago, the disease known as senile dementia was regarded as almost a normal part of ageing, and it was only after the prion diseases emerged that Alzheimer’s stepped into the limelight, helped by high profile cases such as Rita Hayworth, Iris Murdoch and Ronald Reagan. However, despite the volume of work undertaken on the subject, the relationship between the various histological manifestations of Alzheimer’s disease and the clinical picture remain unclear.

The brains of Alzheimer’s patients are characterized by extracellular deposits of aggregated β-amyloid protein (‘plaques’, fig. 1) and accumulations of tau protein in cell bodies (neurofibrillary tangles, NFTs). These changes are also seen to some extent in the normal ageing brain, but the key differences are in the degree and location of these deposits. In comparison with normal controls, Alzheimer’s brains have much more extensive deposits of plaques, often associated with tau-containing neuritic halos, and found particularly in the cerebral cortex, hippocampus and various subcortical nuclei. NFTs in Alzheimer’s brains may be extracellular, probably due to the death of the neurons in which they formed, and are most common in the hippocampus and transentorhinal cortex. This may explain the correlation between degree of amnesia and NFT burden at autopsy, as the hippocampus is thought to be responsible for formation of new memories. However, in late disease both plaques and NFTs are found throughout the brain, and the correlation between NFTs and symptoms does not necessarily imply that tangle formation is the key event in disease progression.
Population studies of the genetics of Alzheimer’s strongly implicate β-amyloid as the primary cause, as several genes responsible for early onset familial cases of Alzheimer’s are intimately involved with the processing of the endogenous amyloid precursor protein (APP): the APP gene itself and the pre-senilin genes. These encode parts of the α-secretase complex which cleaves APP, and mutations increase the proportion of β-amyloid_{1-42} monomers, which aggregate more readily than β-amyloid_{1-40} monomers, and are therefore more pathogenic.

These classical histological changes are accompanied by many other changes: gross atrophy (fig. 2), and histological loss of neurons with an increase in microglia and astrocytes, as well as changes in the balance of different neurotransmitters, particularly a reduction in acetylcholinesterase activity. Additionally, amyloid deposition occurs in blood vessel walls, and Hirano bodies appear in hippocampal neurons, associated with neuronal loss. The link between amyloid protein and these changes is not clear, but it is thought that oligomeric (2-12 monomers) β-amyloid_{1-42} is the neurotoxic agent, and the large plaques of β-amyloid may in fact be protective, by sequestering these oligomers. The situation is further complicated by the associations between vascular risk factors and Alzheimer’s disease. Clearly hypertension, diabetes and high cholesterol increase the risk of developing stroke-related and other vascular dementia. There is some evidence that diabetes increases the risk of Alzheimer’s disease, as does hypertension, but it is less clear whether or not treating hypertension and diabetes reduces the risk of Alzheimer’s.

![Figure 2.6 serially acquired T1-weighted MRI scans from initially asymptomatic patient destined to develop familial Alzheimer’s disease. Symptoms began between scans 4 and 5. Scans overlaid with the atrophic areas marked in red. From Fox et al. (2004) The Lancet 363(9406):392 doi:10.1016/S0140-6736(04)15441-X](image)

Just as the link between cardiovascular risk factors and amyloid disease is unclear, so is the link to clinical symptoms. The idea of ‘cognitive reserve’ has been posited to account for, among other
things, the protective effect of high pre-morbid intelligence, and may also underlie the brain’s ability to make full recoveries from focal lesions such as strokes and traumatic injuries. If both Alzheimer’s disease and vascular dementia have a long sub-clinical prodrome in which disease progression gradually ‘uses up’ this cognitive reserve, this could potentially account for the link between vascular risk factors and Alzheimer’s, as the former cause a subclinical vascular dementia which uses up the cognitive reserve and leads to a more aggressive presentation of Alzheimer’s. On the other hand, there is evidence that at post-mortem those with lower pre-morbid intelligence do have more neuropathology, and it has been suggested that deficient brain development in early life may account for both lower intelligence and heightened susceptibility to neuropathology.

The pathogenesis of Alzheimer’s thus remains largely a mystery, but there is increasing evidence that it begins long before symptoms appear. Furthermore, of all human tissues the brain is least capable of regeneration, so there is a strong imperative to develop treatments which arrest the disease process early. A reliable way to recognise those individuals undergoing early Alzheimer’s-type pathology is crucial if new treatments are to be developed and applied to the (rapidly expanding) elderly population. As well as facilitating clinical trials in the future, a specific diagnostic test would also be of immediate benefit: earlier diagnosis would enable families to plan for the future. A positive diagnosis of Alzheimer’s renders redundant the tests to exclude other causes of dementia, and enables accurate distinctions to be made between Alzheimer’s and benign age-related cognitive decline. This in turn will improve epidemiological studies by purifying the study populations and facilitate longer studies, through earlier diagnosis, of the natural history of Alzheimer’s. It also will help clarify whether risk factors which appear to increase the incidence of Alzheimer’s truly do so, or whether they just reduce the age at onset. This difficulty arises because death is common in the elderly, so many people who would develop Alzheimer’s late in life die before symptom onset, giving the illusion of a truly higher rate of disease in those with positive risk factors.

This essay will focus on various approaches to the earlier diagnosis of Alzheimer’s: clinical and psychological testing, plasma and CSF biomarkers, and neuroimaging are the three routes most thoroughly explored. With the increasing number of genetic associations, and continuous improvements in genome sequencing, it may soon be possible to add individualised genetic risk profiling to this array, and there may be other biological assays which merit exploration. On current evidence, a composite of several different tests may provide the best route to earlier diagnosis, and establishing a profession-wide consensus on this is an ongoing task, with several large multicentre investigations seeking to methodically compare all the possible combinations.
Mild Cognitive Impairment

A probable diagnosis of Alzheimer’s disease can only be made if the memory and cognitive impairments are sufficient to impair day to day function, according to both the DSM IV and ICD 10 classifications (a definite diagnosis can only be made with brain biopsy or autopsy). However, it is undoubtedly the case that many patients are aware of cognitive changes long before they impact on daily functioning, and there have been various attempts to categorise these early changes. Diagnostic criteria for mild cognitive impairment (MCI), the currently accepted schema, were defined in 1995, and this has since been called amnestic MCI, to discriminate it from MCI which affects cognitive domains other than memory\textsuperscript{13} (such as executive function, language, behaviour, attention and visuo-spatial).

**Diagnostic criteria for amnestic MCI** (after Petersen et al. 1995\textsuperscript{13})

<table>
<thead>
<tr>
<th>Memory complaint</th>
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<tbody>
<tr>
<td>Normal general tests of cognitive function (Mini-mental state examination; Wechsler Adult Intelligence Scale - Revised; Dementia rating scale)</td>
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<tr>
<td>Specific memory tests/test components scored more than 1.5 s.d. below age-matched mean</td>
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<tr>
<td>Intact activities of daily living</td>
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MCI is thus defined by the clinical picture. Its usefulness as a category depends on how well it corresponds to any underlying disease and whether it has a distinct prognostic significance compared to early Alzheimer’s disease or the extreme of normal age-related cognitive decline.

Follow up studies of patients diagnosed with MCI have produced very variable results, as a consequence of differences in study population and diagnostic thresholds.\textsuperscript{14} Samples drawn from memory clinics have approximately double the annual rate of conversion to dementia of samples drawn from the community. Presumably memory clinics attract those patients with more concerning symptoms which reflect more severe underlying pathology. There also appears to be a change in the conversion rate with time from diagnosis of MCI, whereby in the first few years after diagnosis the rate may be over 10%: Petersen et al. found a 12% annual conversion rate for the first 4 years after diagnosis in their clinic population,\textsuperscript{15} whereas Geslani et al. found rates of 41% in the first year after diagnosis and 23% in the second year,\textsuperscript{16} and Schmidtke et al. found 44% had converted to Alzheimer’s 19 months after diagnosis of MCI.\textsuperscript{17} In contrast to these short follow ups, the annual conversion rate in long-term follow up is between 3-7%, and the conversion rate is inversely related to follow up duration.\textsuperscript{14} At 10 years after MCI is diagnosed, over 50% of patients have not converted to Alzheimer’s disease,\textsuperscript{18} and although with time longer follow up studies will appear, it seems fairly
probable that some patients with MCI do not ever progress to Alzheimer’s. Certainly some studies have found that a large minority of patients with MCI regress to normal within a few years. As the diagnosis of MCI relies heavily on psychological assessment, and there are no strict international criteria, there is likely to be a lot of variability in how the diagnosis is made. This may compound the variation introduced by different study populations and lengths of follow-up. In any case, notwithstanding the variation between studies, conversion to Alzheimer’s is clearly far from complete, and this must be accounted for. The two simplest explanations are that the MCI population may consist of a mix of early Alzheimer’s (with a small proportion of other dementia syndromes) and normal ageing, or it may represent an early stage of Alzheimer’s type pathology which has the potential to spontaneously resolve in some individuals.

To definitively correlate MCI with neuropathology requires patients diagnosed with MCI to undergo post-mortem studies, and these populations are only beginning to die in sufficient numbers to provide a clear picture. However, such studies as have been undertaken confirm that, when brains are assessed for neuritic amyloid plaque- and neurofibrillary tangle-burdens, MCI patients are on average intermediate between normal controls and Alzheimer’s patients. However there is considerable variation within MCI groups as to degree of pathology, as well as quite a high incidence of vascular lesions. Thus both prognostic and pathological studies point towards heterogeneity in the MCI group. This heterogeneity is problematic for studies which seek to correlate MCI in life with surrogate markers of Alzheimer’s-type pathology, as the biomarkers and imaging changes currently used are insufficiently sensitive and specific. However, longitudinal imaging studies allow individual patients to be re-tested to demonstrate changes over time as clinical problems develop, and these have found progressive cortical atrophy starts early, in individuals who go on to develop Alzheimer’s after a few years. However, these patients were from families with a positive history of early onset Alzheimer’s (by necessity, to ensure a high a priori chance of developing disease), and may not be fully representative of sporadic Alzheimer’s. They also were not tested formally for MCI, but only one patient had any symptoms at the first scan, so the imaging series probably spans the period of MCI. This study highlights the pathological changes which begin before any sort of subjective or objective cognitive problem develops, and this may be the biggest weakness of MCI as a tool for the early recognition of Alzheimer’s: it simply is not early enough.

**CSF biomarkers**

Biomarkers are endogenous molecules which indicate the presence of underlying pathology, and they are of particular interest in Alzheimer’s because of the near-impossibility of getting tissue samples for direct pathological study ante-mortem. Cerebrospinal fluid is the obvious focus for investigation of a
neurodegenerative condition, and there are several proteins which have been extensively studied for their relationship to Alzheimer’s disease and prodromal states. Investigators have consistently found a reduced concentration of soluble \(\beta\)-amyloid_{1-42} in the CSF of autopsy-confirmed Alzheimer’s patients, and increased concentrations of total tau and phosphorylated tau (p-tau). \(^{24,25,26}\) The counter-intuitive reduction in \(\beta\)-amyloid_{1-42} has been ascribed to the increase in plaque formation, which uses up the soluble monomers, but this theory has not been confirmed. Total tau levels also increase in other conditions which cause neuronal degeneration, such as (commonly) stroke and trauma, and particularly high levels are found in Creutzfeldt-Jakob disease which is characterised by very rapid neurodegeneration. Fortunately, p-tau levels are more specific than total tau and can be used to discriminate between Alzheimer’s and other causes of dementia. \(^{27}\)

Having identified a CSF profile that distinguishes active Alzheimer’s disease from cognitively normal controls, the challenge is to see what biomarkers best identify those individuals who go on to develop Alzheimer’s disease later in life. Early studies were quite promising, but larger multi-centre studies have not replicated these early successes. One studied CSF \(\beta\)-amyloid_{1-42}, total tau and p-tau in 750 patients from 12 centres, and found a positive predictive value of only 62% and a negative predictive value of 88% for conversion to Alzheimer’s over 2 years. \(^{28}\) They also found considerable intersite variability in the assays used, which is an increasingly prevalent theme in the search for Alzheimer’s biomarkers. The Alzheimer’s Disease Neuroimaging Initiative (ADNI) is a large cohort from across the US which has made strenuous efforts to ensure uniformity in sample collection and assays, in an attempt to establish the most useful biomarkers. \(^{29}\) Early reports confirm that CSF \(\beta\)-amyloid_{1-42} and tau (total and phosphorylated) correspond to cognitive status, although the tests are not sensitive or specific enough for routine use in predicting progression of MCI patients. In conclusion, while CSF biomarkers will continue to be a focus of research for earlier diagnosis, and may well play a role in clinical trials of new treatments which seek to intervene earlier than symptom onset, they are unlikely to be sufficient alone. Combining CSF profiles with apolipoprotein E allele typing increases their utility, \(^{25}\) and combining several modalities of investigation will probably be necessary to achieve clinically useful sensitivity and specificity.

**Plasma Biomarkers**

The biggest advantage to plasma biomarkers is the ease with which plasma can be sampled, and any tests which depend on it can be easily used for the whole population for diagnosis. Plasma biomarkers in particular may solve another problem for clinical trials in Alzheimer’s, namely the variable progression of disease after diagnosis. Some patients do not progress predictably, and this makes it harder for trials to show a beneficial effect of the treatment. Additionally, other coincident
causes of dementia such as vascular disease and synucleinopathies may confuse the relationship between Alzheimer’s pathology and cognitive symptoms. Plasma biomarkers may help by representing ongoing neurodegeneration better than cognitive symptoms do, and thereby reducing the sample size needed and the expense of the trial. CSF biomarkers and imaging studies could in principle serve a similar function, but the cheapness and convenience of blood tests renders them particularly well suited to ongoing surveillance.

Unfortunately no such plasma biomarker has been identified. The blood brain barrier limits the extent to which plasma represents the extracellular milieu of the brain, but many biomarkers have been identified which show weak associations, such as factor H binding protein, alpha-2 macroglobulin and clusterin. Analysis of plasma proteomes and transcriptomes by several groups have yielded arrays of proteins, which when taken as a group provide good discrimination between Alzheimer’s disease and normal controls, but these have not been validated in other centres and inter-site assay variability may become problematic. One study extended their array to MCI patients, and found that their tests had a sensitivity for progression to Alzheimer’s over 2-6 years of 91%, with a specificity of 72%. It remains to be seen whether these promising results can be replicated in other centres. All of these studies suffer from a lack of histopathological confirmation of the Alzheimer’s diagnosis, so some probably include cases of non-Alzheimer’s dementia. They also do not include a control group of non-Alzheimer’s dementia, so the specificity and sensitivity values of their tests in a true clinical population would probably be lower than the published values.

**Neuroimaging**

Of the techniques discussed here, neuroimaging is the closest to routine clinical practice for the diagnosis of Alzheimer’s-type pathology. Structural MRI scans on Alzheimer’s patients show frontal and temporal cortex atrophy which corresponds to cognitive decline, in contrast to age-matched controls. Hippocampal atrophy, particularly in the subiculum and CA1 area, can be detected on diffusion-weighted MRI early in Alzheimer’s disease, although this may also be caused by strokes or trauma. Hippocampal atrophy in MCI is midway between cognitively normal controls and Alzheimer’s disease, but the difference is too variable for clinical utility. However, annual change in total brain volume and ventricular volume can predict, to some extent, which patients with MCI will convert to Alzheimer’s. In a similar vein, cortical thickness in various temporal lobe regions can distinguish between Alzheimer’s disease and controls, and predict progression to Alzheimer’s of amnestic MCI 76% of the time.
Positron emission tomography (PET) scanning measures the uptake of a labelled compound in the area scanned, so PET with fluorodeoxyglucose (FDG) measures metabolism. Reduced metabolism in the brain (especially parietotemporal cortex and posterior cingulate) is a common finding in Alzheimer’s disease, and this technique is approved for diagnosis in the US. A meta-analysis found that it had both sensitivity and specificity of 86% for diagnosing Alzheimer’s, although the meta-analysis included studies which used both healthy controls and other causes of dementia for comparison populations, and only two of the studies used autopsy-confirmed cases of Alzheimer’s. Whether FDG-PET can usefully predict progression in MCI is not well established. A large study of normal, MCI and Alzheimer’s subjects from the ADNI cohort found that FDG-PET could predict conversion to Alzheimer’s in the MCI cohort, although its predictions were not as reliable as those from baseline measurements on the Auditory Verbal Learning Test (AVLT). FDG-PET was also not a useful predictor of the extent of cognitive decline, after normalizing for the predictive value of the AVLT.

While FDG-PET measures metabolism, the use of the $^{11}$C-labelled Pittsburgh compound B (PIB) ligand enables amyloid plaque burden to be imaged directly, and Alzheimer’s patients show significantly higher $^{11}$C-PIB retention in cerebral cortex than healthy controls. $^{11}$C-PIB-PET can predict conversion of MCI, and higher amyloid loads correlate with speed of conversion. $^{11}$C-PIB also correlates with CSF levels of $\beta$-amyloid, tau and p-tau in both MCI and Alzheimer’s patients, and more studies of the relationships between the various imaging modalities and biomarkers will probably be crucial for future progress.

Single-proton emission CT scanning, SPECT, has been used to investigate Alzheimer’s disease, but its ability to distinguish between this and healthy controls is only 74%, which does not bode well for its usefulness in detecting pre-clinical disease states. It is more useful in diagnosing frontotemporal dementia and Dementia with Lewy Bodies.

Establishing a consensus

There is a wealth of potential investigations which might diagnose Alzheimer’s pathology in the very early stages of symptoms, or even pre-symptomatically. Those discussed here are only the best validated, and more are constantly being assessed. However, there are many sources of variation in these studies, from the investigators (differences in clinical assessment, laboratory assays and imaging interpretation) and the patients (demographics, co-incidence of other causes of dementia, community or clinic-derived population) which confound attempts to determine meaningful differences in experimental parameters which mostly have a high level of noise. To resolve this, large multicentre
co-ordinated projects, such as the ADNI and AddNeuroMed public-private initiatives are needed to undertake sufficiently large studies comparing these investigative modalities, to establish which are independently predictive and what the most useful threshold values are, and also to establish uniform laboratory analysis of the samples and images.

These studies will also, in time, be the first to follow up cognitively normal individuals until they develop MCI and subsequently dementia. Hitherto studies of biomarkers and imaging have used MCI cohorts as the group of interest, but with sufficiently long follow up of large enough samples, data will emerge on the biomarker and neuroimaging profile of individuals who eventually go on to develop Alzheimer’s disease. The only other way to achieve this is to study individuals from families with single gene defects in APP or the pre-senilins, as undertaken by Fox et al.23 but this approach runs the risk of studying a disease which is, by reason of its aetiology, different from sporadic Alzheimer’s disease.

Another limitation of most studies undertaken hitherto, particularly in biomarkers, is the exclusion of subjects with significant other comorbidities. This is a common practice in clinical research, and much lamented, but particularly problematic in studies of age related conditions like Alzheimer’s, when comorbidities are the norm rather than the exception. The large cohorts now being developed will help mitigate this problem, but it is likely to persist until the techniques are used outside the experimental settings and surveillance data become available.

It will also be profitable to undertake analyses of subgroups determined by risk factor genes. This is common practice for the apoE genotype, but many other risk genes have already been identified (GSK3β, DYRK1a, Tau, TOMM40, CLU) and more will no doubt follow. Environmental risk factors may also be a helpful way to stratify study populations, although apart from the cardiovascular risk factors, most mooted environmental risk factors have not stood up to close scrutiny.44

**New horizons**

In the search for biomarkers, it is natural to focus on serum and CSF, two tissue samples commonly used in clinical practice. However, there may be other more profitable tissues to investigate. Although Alzheimer’s disease appears to be exclusively a disease of the brain, amyloid formation occurs in a variety of ways, all round the body in some forms of systemic amyloidosis. The APP protein is not unique to neural tissue, but expressed widely, and although its function remains unclear, it has been implicated in various signalling cascades and may well serve a function in non-neural tissue.45 Deposition of amyloid plaques around cerebral blood vessels has been well described, and it
is possible that extracranial blood vessels may also show an amyloid vasculopathy. It may be profitable to study biopsies of peripheral connective tissue from Alzheimer’s disease patients for such deposits, or more diffuse amyloidosis.

Age-related macular degeneration has several remarkable similarities with Alzheimer’s disease, and there may be ocular signs or tests which would be helpful as part of the array of investigations used to diagnose early Alzheimer’s. β-amyloid is a component of drusen deposits, which are found in affected retinas; this β-amyloid takes the form of toxic oligomers, and seems to increase VEGF production. ApoE alleles are associated with macular degeneration as well as Alzheimer’s disease, and a mutation in complement factor H is strongly associated with macular degeneration Factor H is one of the most promising plasma biomarkers for Alzheimer’s disease.

Conclusions

Although our understanding of the key steps underlying neurodegeneration in Alzheimer’s disease is incomplete, it is clear that it begins long before symptoms are noticed by the patient. Any disease-modifying treatments which are developed are most likely to be successful if initiated early in the process, and this requires that we develop reliable, validated and economical ways to diagnose Alzheimer’s-type pathology. Psychological assessments of pre-dementia syndromes are helpful, particularly in guiding further discovery of biological markers in serum, CSF and neuroimaging which can be extrapolated back to the pre-symptomatic phase of the disease. However, despite comprehensive searches, no single test has shown adequate sensitivity or specificity, and it is likely that a combination will be needed. As the complexity of this diagnostic process increases, so does the vital importance of ensuring consistent and accurate laboratory assessment. The large cohorts being studied by multicentre teams will hopefully provided the solution to these problems in the next few years, paving the way to find effective treatments for this devastating disease.
References


