Alzheimer’s disease (AD) is diagnosed on clinical grounds, implying the presence of senile plaques and neurofibrillary tangles in the hippocampus and cerebral cortex. Brain autopsy studies indicate that these lesions are present in about 90% of patients clinically diagnosed by experienced physicians. The utility of biomarkers for enhancing diagnostic sensitivity for AD may consequently be relatively limited since clinical methods are generally reliable. Although biomarkers are also unlikely to alter treatment decisions about the currently available therapies (i.e., cholinesterase inhibitors and NMDA antagonists), biomarkers may serve as indices of disease activities (e.g., inflammation, oxidative damage, nitrosative damage, astrocitic activation, and neuronal damage or death) which are targets of future therapeutic intervention. Amyloid plaque and neurofibrillary tangle formation are other mechanisms in this category, and measurement of these mechanisms has been attempted with assays for beta amyloid 1-42, tau, and phospho-tau, which have been described in numerous publications and reviews. The present manuscript will focus on AD biomarkers other than beta amyloid and tau.

Mechanism-specific biomarkers may permit assessment of what might be called the "proximate" efficacy of therapeutic interventions (in contrast to "ultimate" efficacy based on clinical endpoints). For example, does a given anti-inflammatory intervention effectively suppress CNS inflammation? Does a putative astrocyte modulator actually suppress astrocyte activation in the patient? In the absence of mechanism-specific biomarkers, clinical trial design and analysis are dependent on extrapolation from animal models and on other inferences to ascertain the adequacy of dosing. When the intended clinical effects are not achieved, it is impossible to know whether the failure is due to an inadequate dose or a hopeless strategy. If markers of the target mechanism are included, however, it becomes possible to determine if the intervention adequately treated the mechanism of interest, and more definitively determine if the overall strategy is viable. For example, if inflammation or oxidative damage is dramatically suppressed by an intervention in patients with AD, but the clinical progression is not affected, then the strategy itself, rather than the specific agent and dose, can be judged as a failure.

Biomarkers also have a potential role as markers of neuroprotection, especially in very early or even "preclinical" phases of disease, when rates of clinical decline are so slow that treatment effects are not detectable on clinical grounds within the time frame of clinical trials. Just as transaminases are used to monitor hepatic cell death, and CPK is used to monitor myocyte cell death, CSF markers of neuronal death and damage may have sensitivities beyond clinical outcome measures in AD, especially at the very early stages. Biomarkers may therefore be rationally used in combination with mechanistic markers assessing the "proximate" efficacy of therapeutic intervention, and neuronal damage markers that assess "ultimate" neuroprotective effects.

Potential sources of biomarkers of AD include CSF, plasma and urine. While the advantage of the accessibility of peripheral body fluids is undeniable, the degree to which peripheral fluids reflect biochemical changes in the brain is hotly debated. Calculations to determine the CNS concentration of a theoretical analyte in brain tissue necessary to impact plasma levels in a measurable fashion provide theoretical evidence against the utility of plasma markers [1], and clinical experience shows that there are no plasma markers of CNS disease or injury which are currently in clinical use. In contrast, numerous studies have validated CSF markers of AD.

Inflammation

The remarkably reproducible epidemiological observation that users of non-steroidal anti-inflammatory drugs (NSAIDs) have a reduced incidence of AD is the foundation of the hypothesis that inflammatory mechanisms are causally involved in the pathogenesis of AD. However, two clinical trials of anti-inflammatory agents have failed to show any effect on the progression of established AD, raising some doubts about the "inflammatory hypothesis". It remains possible, however, that inflammatory events underly the initiation (but not the later progression) of AD, such that the failure of anti-inflammatory trials was due to the timing, rather than the nature, of the intervention.

Candidate biomarkers for monitoring inflammatory activity in AD prevention trials include inflammatory cytokines and chemokines, many of which are expressed in brain tissue. Most cytokines, however, are barely detectable in CSF, and chemokines do not consistently distinguish patients from control subjects, and are not known to be sensitive to NSAIDs. In contrast, the inflammatory prostaglandins are measurable in CSF and are increased in AD compared to control subjects [2]. Since prostaglandin synthesis is dependent on the enzyme inhibited by NSAIDs (i.e., cyclooxygenase) the prostaglandins are also sensitive to NSAIDs. At least one clinical trial of NSAIDs for the prevention of AD is using CSF prostaglandin levels as a means of assessing anti-inflammatory effects in the CNS.

Oxidative damage

Oxidative damage to proteins, nucleic acids, and lipids is an established feature of brain ageing and neurodegeneration, and measurement of each in CSF has been attempted in subjects with AD. While the utility of protein carboxyls and oxidised nucleic acids remains to be established, the measurement of oxidative damage to lipids in CSF has been quite fruitful. F2-isoprostanes are stable oxidative metabolites of arachidonic acid which have been shown to correlate with the degree of neurodegeneration in brains of AD patients. CSF levels of F2-isoprostanes have been reproducibly shown to be increased in AD compared to control subjects [3]. In one series of 40 subjects with mild AD, CSF F2-isoprostane levels were inversely correlated with total brain volume [4], replicating the post-mortem finding of the highest levels in the most atrophic brains. Serial levels of CSF F2-isoprostanes also increased over time in the same group of mild AD subjects, with the longitudinal increase attenuated by the use of antioxidant vitamins E and C [5]. CSF F2-isoprostanes therefore show great potential as an assay of antioxidant efficacy in CNS disease. F2-isoprostanes serve as markers of oxidative damage in all CNS cell types, since the precursor, arachidonic acid, is present in all cell

In addition to beta amyloid and tau, a number of CSF biomarkers of Alzheimer’s disease (AD) have potential as mechanism-specific markers suitable for inclusion in clinical trials of AD prevention or treatment. This article discusses some of these markers, their mechanisms of action and their clinical utility.

CSF biomarkers of AD: monitoring therapeutic targets

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types. In order to develop an oxidative marker more specific to neuronal cells, oxidative metabolites of docosahexanoic acid (DHA), which is specific to neurons, have been pursued. These markers, termed F₂-isoprostanes, are also increased in CSF of AD patients compared to control subjects. Serial measurement and the effects of antioxidant intervention upon CSF neuroprostanes are under investigation.

Nitrosative damage
Several lines of evidence have suggested that nitrogen-mediated damage may also play a role in the pathogenesis of AD. Peroxynitrite, a toxic intermediate resulting from the reaction of nitric oxide and superoxide, is increased in the brains of AD patients compared to control subjects. The “footprint” of peroxynitrite-mediated toxicity, nitrotyrosine, has also been localised to the pathologic lesions of AD. CSF nitrotyrosine is increased in AD compared to control subjects in most reports [6], and may serve as a biomarker in trials employing a “peroxynitrite-scavenging” strategy.

Astrocyte activation
Beta amyloid plaques are surrounded by activated astrocytes which produce IL-1 and other potentially toxic metabolites, leading to the hypothesis that astrocyte activation plays a pathogenic role in AD. “Astrocyte modulators” such as pentoxifylline and arundic acid have been proposed as therapeutic agents. Glial fibrillary acidic protein (GFAP), a marker of astrocyte activation, is increased in CSF of AD patients compared to control subjects. In our experience, however, CSF GFAP has not been reproducible within subjects upon repeated sampling, so its utility as a marker of astrocyte modulation appears doubtful.

Another astrocyte specific marker, the cytokine S100β, may be a more viable marker for this purpose. One study found that CSF S100β was increased in mild AD compared to control subjects, with an inverse correlation between dementia severity and S100β in AD subjects [7]. A separate study found an inverse correlation between CSF S100β and brain volume in AD [8], supporting the impression that this marker of astrocyte activation is most dramatically elevated in the very early stages of the disease, when preventive strategies may be most effectively employed.

Neuronal damage and death
Cytoplasmic neuron-specific proteins may serve as markers of neuronal death or damage when they appear in CSF. For example, CSF neurofilament and CSF neuron-specific enolase are increased in AD compared to control subjects. Some investigators consider that CSF tau plays a similar role, as a marker of neuronal damage which is not specific to a particular type of pathology. This may be a reasonable conclusion, as CSF tau is rarely shown to be correlated with neurofibrillary tangle burden.

The ability to quantify neuronal death may be of limited value in clinical trials, however, since the death of the neuron is a late and probably irreversible step in the pathogenesis of dementia. The emerging view that neurodegeneration in AD begins as “synaptotoxicity” suggests that markers of synaptic degeneration might serve as markers of a reversible phase of neurodegeneration which precedes outright neuronal death. Synaptophysin has been reported as unmeasurable in CSF, but synaptatin and chromogranin A have been reported to be reduced in the CSF of AD patients compared to control subjects [9].

The future of biomarkers
While the preceding discussion focuses on the validation of targeted mechanisms, it is clear that the biology of AD is not completely understood. The judicious application of proteomics technology to CSF may yield not only new biomarkers, but also new mechanisms. Publications describing initial results in this arena have just begun to appear [10, 11].

Conclusions
A number of CSF biomarkers of AD, in addition to beta amyloid and tau, hold promise as mechanism-specific markers suitable for inclusion in clinical trials of AD prevention or treatment. Trials currently under way are expected to shed additional light on the utility of specific markers, so that it may be possible in the near future to conduct small “proof of concept” clinical trials of candidate AD treatments using a validated panel of biomarker outcome measures. We anticipate that such biomarker-based demonstration trials will become a necessary precursor to large randomised clinical trials.

References

Table 1. A summary of CSF biomarkers and their utility.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Marker</th>
<th>Distinguishes AD from control</th>
<th>Correlation with disease severity</th>
<th>Reproducible on serial measurement</th>
<th>Sensitive to intervention</th>
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<tbody>
<tr>
<td>Inflammation</td>
<td>cytokines</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>?</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Prostaglandins</td>
<td>yes</td>
<td>?</td>
<td>?</td>
<td>Very likely sensitive to NSAIDs</td>
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<tr>
<td>Oxidative damage</td>
<td>F₂-isoprostanes</td>
<td>yes</td>
<td>yes</td>
<td>Yes increases over time</td>
<td>Yes</td>
</tr>
<tr>
<td>Oxidative damage</td>
<td>F₂-neuroprostanes</td>
<td>yes</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Nitrosative damage</td>
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<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
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<td>GFAP</td>
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<td>yes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Astrocyte activation</td>
<td>S100β</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>neurofilament</td>
<td>yes</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
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<td>Neuro-specific enolase</td>
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<tr>
<td>Neuronal damage</td>
<td>tau</td>
<td>yes</td>
<td>?</td>
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</table>

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